CHAPTER 4

Thermoreversible Periodontal Gel

4.1 INTRODUCTION

Thermoreversible gels of pluronic F127 could be one of the potential drug carriers for the intra periodontal drug delivery system, for the treatment of infectious periodontal diseases due to their inherent property of gel formation at body temperature along with mucoadhesive property to the mucous membrane, which would enhance the residence time of the formulation in the periodontal cavity and there by increases the local bioavailability of the active drug at the target site. Addition of the mucoadhesive polymer to the formulation can increase the mucoadhesive property of the formulations which results in increase in the residence time of the formulation at the local target site of the periodontal cavity. The periodontal mucoadhesive thermoreversible gel might be used to provide an enhanced bioavailability compared to the other conventional oral delivery systems for the treatment of periodontal diseases. The local delivery of minocycline hydrochloride/ clindamycin phosphate to the target site will not only result in rapid onset of action but also increases the residence time of the formulation in the periodontal cavity which results in effective periodontal therapy. Effect of addition of the mucoadhesive polymer on rheological behavior, mucoadhesive strength, gel strength, syringeability, ex-vivo permeation study through oral mucous membrane of periodontal thermoreversible gels of pluronic F127 have never been studied. Moreover, the combination of non-ionic polymeric surfactant pluronic F127 as thermoreversible polymer and different cationic/ anionic/ nonionic polymers such as carbopol 934P, polycarbophil, poly acrylic acid, HPMC, HEC, PVA as mucoadhesive polymer and absorption enhancing materials have never been explored as a potential drug delivery system with numerous advantages for the periodontal delivery system.

This chapter deals with the development of effective intra periodontal delivery system containing minocycline hydrochloride/ clindamycin phosphate using thermoreversible polymer pluronic F127 along with various mucoadhesive polymer carbopol 934P, polycarbophil, poly acrylic acid, HPMC, HEC, Polyvinyl alcohol and other additives. The formulations were evaluated for its gelation temperature, rheological characteristics, mucoadhesive strength, gel strength, syringeability and ex vivo permeation study across oral

skin mucous membrane and in vitro effectiveness of the formulations was done by gamma scintigraphy.

4.2 PREPARATION METHODS FOR VARIOUS PERIODONTAL THERMOREVERSIBLE GEL FORMULATIONS

4.2.1 Preparation of plain pluronic F127 periodontal gel

Different concentrations of pluronic F127 gels were prepared by cold method technique (Schmolka et al 1972, Choi et al. 1998). The compositions of the formulations are cited in Table No4.01. Accurately weighed amount of pluronic F127 was slowly added to half the volume of water to be taken and maintained at 10°C in a beaker with continuous stirring. Aqueous pluronic F127 mixture was kept overnight at 4 °C until a clear solution was obtained. Weight was adjusted with distilled water.

4.2.2 Preparation of medicated pluronic F127 periodontal gels

Formulations containing minocycline hydrochloride (1%)/ clindamycin phosphate (1%) were prepared by adopting the same cold method technique. The compositions of the formulations containing minocycline hydrochloride/ clindamycin phosphate are cited in Table No4.02 and 4.03 respectively. Minocycline hydrochloride/ clindamycin phosphate was added to half the volume of water to be taken and maintained at 10°C in a beaker until a clear solution was obtained. In case of minocycline hydrochloride formulations, to the above solution sodium meta-bisulphite was added as an antioxidant. After formation of a clear solution, weighed amount of pluronic F127 was added and kept overnight at 4°C until a clear transparent solution was obtained. Weight was adjusted to with distilled water. The pH of all the formulations was measured.

4.2.3 Preparation of pluronic F127 periodontal gels containing PEG 1000

Thermoreversible gels containing pluronic F127 along with 5.0, 7.5, 10 and 15 % (w/w) of PEG 1000 as the gelation temperature enhancing agent were prepared by cold method technique. Different concentrations of PEG 1000 were dissolved initially to half the volume of water to be taken and sonicated to get a clear solution. To this clear solution pluronic F127 was added and kept overnight at 4°C until a clear solution was obtained. Weight was adjusted

to with distilled water. The compositions of all the formulations are cited in the table no. 4.04- 4.07.

4.2.4 Preparation of pluronic F127 periodontal thermoreversible gels containing mucoadhesive polymers

Thermoreversible periodontal gels containing pluronic F127 and mucoadhesive polymers (Polycarbophil, HPMC, HEC, PVP, Carbopol 934P, PVA, and Poly acrylic acid) were prepared by cold method (Schmolka et al 1972, Choi et al. 1998) technique. Thermoreversible periodontal gels using mucoadhesive polymers were prepared by dispersing the polymer in water prior to addition of pluronic F127 and kept overnight at 4°C until a clear solution was obtained. Weight was adjusted to with distilled water. The compositions of the formulations are cited in Table No4.08-4.11 for polycarbophil, 4.12-4.14 for HPMC, 4.15-4.17 for HEC, 4.18- 4.20 for PVP, 4.21- 4.24 for carbopol 934P, 4.25- 4.27 for PVA, 4.28- 4.30 for poly acrylic acid.

4.2.5 Preparation of pluronic F127 periodontal gels containing sodium hydroxide

Thermoreversible gels containing pluronic F127 along with 0.25, 0.5, 0.75 and 1.0 % (w/w) sodium hydroxide as pH adjusting agent were prepared by cold method. Different concentration of sodium hydroxide was dissolved in half the volume of water. To this clear solution pluronic F127 was added and kept overnight at 4°C until a clear solution was obtained. Weight was adjusted to with distilled water. The compositions of the formulations are cited in table 4.31- 4.34.

4.3 CHARACTERISATION OF PLAIN PERIODONTAL FORMULATIONS

4.3.1 Measurement of gelation temperature by visual inspection

A 20 ml transparent vial containing a magnetic bar and 10 g of pluronic F127 gel was placed in thermostat water bath maintained at 4 °C. A digital thermosensor connected to a thermistor was immersed in the pluronic F127 gel. The gel was heated at the rate of 1°C/ min with

continuous stirring at 30 rpm. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was recorded as the gelation temperature (Choi et al. 1998, Miyazaki et al. 1991). Each preparation was tested thrice to control the repeatability of the measurement.

4.3.2 Enthalpy of gelation

The enthalpy of gelation (ΔH^0 gel) for plain pluronic F127 gels and for gels with various formulation additives were calculated using the method described by Eldridge (Eldridge et al. 1954) and Pandit (Pandit et al. 1996). The enthalpy of the gelation process will depend on the type and extent of interaction it favors. Enthalpy of transition was obtained from the semi-log plot of pluronic F127 concentration (lm C) versus reciprocal of transition temperature using following equation;

$$\text{lm } C = H^0 \text{ gel} / RT + \text{constant}$$

Where, H^0 gel is the enthalpy of gelation, T represents the gelation temperature in degree centigrade.

4.3.3 Viscosity studies

Viscosity of plain pluronic F127 gels containing different concentrations of PEG 1000 and gels containing different concentrations of mucoadhesive polymers were measured using the Brookfield's LVDV II+ model. At low temperature, the gel sample (about 10 ml) was placed in small sample adapter. The temperature of the sample was raised above 40°C using circulation bath (Neolab P125 model). The sample was allowed to cool and the viscosity at various temperatures was recorded using suitable spindle.

4.3.4 Statistical analysis

Data were expressed as mean with standard deviation (SD); statistical analysis of data was performed using ANOVA. A p-value of less than 0.001 was considered as significant.

4.3.5 Conclusion

The suitable range of gel transition temperature would be 28–37°C. Gelation temperature of liquid pluronic F127 solutions less than 28°C would lead to gel formation during storage at

room temperature, hence offers difficulty in administration. While at gelation temperature higher than 37°C, the formulation would stay as a liquid in the periodontal pocket. The temperature-dependent gelation of pluronic F127 solutions could be explained by configuration change (Kramaric et al 1992). Gel transition temperatures for plain pluronic F127 gels were observed for the concentrations of 19, 20, 23, 25, 27, 29 and 30% w/w. The decrease in gelation temperature was dependent on the concentration of pluronic F127. The concentration profile of plain pluronic F127 and pluronic F127 along with minocycline hydrochloride/ clindamycin phosphate (1% w/w) was prepared with varying concentrations of pluronic F127. The presence of both minocycline hydrochloride or clindamycin phosphate decreases the gelation temperature of pluronic F127 solutions. Thus, the gelation range narrowed with the addition of the polymer and in presence of minocycline hydrochloride/ clindamycin phosphate (Figure 4.01).

The gelation temperature of thermoreversible periodontal gels containing PEG 1000, used as a gel point enhancing agent, increases with the increase in concentration of PEG 1000 at constant pluronic F127 concentration. The same trend in the change in gelation process was observed at all the four concentrations of pluronic F127.

The effect of concentration of various mucoadhesive polymers (polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA, and poly acrylic acid) on the gelation temperature of pluronic F127 solution was investigated with their varying concentrations. Gelation temperature increased with increased concentrations of PEG 1000 (Figure 4.02). However, the decrease in gelation temperature profile was observed with the increase in polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA and poly acrylic acid concentration (Figure 4.03 to 4.09).

The effect of concentration of sodium hydroxide on the gelation temperature of pluronic F127 solution was investigated in the range of 0.25-1.0% (w/w). Addition of sodium hydroxide caused decrease in sol-gel transition temperature, in contrast to the effect of increased pluronic F127 concentration in plain gels. The decrease in gelation temperature was observed with the sodium hydroxide concentration (Figure 4.10). Therefore, basing on the gelation temperature of various plain and drug loaded mucoadhesive periodontal

thermoreversible gel formulations, formulations prepared using 19, 20, 23, and 25% w/w of pluronic F127 were optimized for further study.

The enthalpy of the transitions, at gelation, obtained for plain pluronic F127 gels (20, 23, and 25% w/w) and gels containing minocycline hydrochloride/ clindamycin phosphate, PEG 1000, polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA, poly acrylic acid and sodium hydroxide from the semi-log plot of polymer concentration versus reciprocal of transition temperature were calculated (Figure 4.11-4.20). The enthalpy of gelation for various gels under investigation is reported in table no. 4.35. Though the presence of minocycline hydrochloride/ clindamycin phosphate decreases the gelation range, the semi-logarithmic plot of the transition temperature in presence of minocycline hydrochloride/ clindamycin phosphate was parallel to that of the plain pluronic F127 (Figure 4.11). This implies that enthalpy of the transition remain nearly similar in presence of minocycline hydrochloride and clindamycin phosphate. In presence of PEG 1000, polycarbophil, and HPMC (Figure 4.12 to 4.14) and carbopol 934P, PVA, poly acrylic acid, sodium hydroxide (Figure 4.17 to 4.20) a linear relationship was not observed between log of polymer concentration and reciprocal of gelation temperature. However, linearity was observed in the semi-log plot of pluronic F127 concentration and reciprocal of gelling temperature for HEC and PVP concentrations (Figure 4.15 to 4.16).

Table no: 4.01: Composition and Characteristics of PluronicF127 Thermoreversible Periodontal Gel Formulations

Composition/ Characteristics	Formulation Code						
	GF1	GF2	GF3	GF4	GF5	GF6	GF7
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00	27.00	29.00	30.00
Purified water	qs	qs	qs	qs	qs	qs	qs
Gel Temperature (°C)	24.10	22.20	20.20	19.20	15.60	12.80	11.30
pH (Sol)	6.71	6.59	6.53	6.60	6.46	6.53	6.66
pH (Gel)	6.72	6.60	6.55	6.65	6.49	6.58	6.68
Sol viscosity (mPas)	6.27	8.73	9.25	10.26	11.32	17.82	19.29
Gel viscosity (mPas)	1028	1562	1674	1729	1831	1904	1967

Table no: 4.02: Composition and Characteristics of PluronicF127 Thermoreversible Periodontal Gels containing minocycline hydrochloride

Composition/ Characteristics	Formulation Code			
	GF8	GF9	GF10	GF11
PluronicF127(%w/w)	19.00	20.00	23.00	25.00
MnHcl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	22.09	20.2	18.3	17.1
pH (Sol)	6.51	6.59	6.53	6.60
pH (Gel)	6.54	6.60	6.55	6.65
Sol viscosity (mPas)	6.39	8.83	9.67	10.19
Gel viscosity (mPas)	1032	1547	1663	1719

Table No: 4.03: Composition and Characteristics of PluronicF127 Thermoreversible Periodontal Gels containing clindamycin phosphate

Composition/ Characteristics	Formulation Code			
	GF12	GF13	GF14	GF15
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Clindamycin phosphate (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temp. (°C)	23.08	21.1	19.3	18.1
pH (Sol)	6.52	6.60	6.54	6.61
pH (Gel)	6.55	6.61	6.56	6.66
Sol viscosity (mPas)	6.25	8.34	9.75	10.28
Gel viscosity (mPas)	1037	1553	1671	1741

Table No: 4.04: Composition and Characteristics of PluronicF127 Thermoreversible Periodontal Gels containing Polyethylene glycol 1000

Composition/ Characteristics	Formulation Code			
	GF16	GF17	GF18	GF19
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PEG 1000 (%w/w)	5.00	5.00	5.00	5.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	34.7	32.5	30.3	28.5
pH (Sol)	6.53	6.58	6.52	6.59
pH (Gel)	6.56	6.59	6.54	6.64
Sol viscosity (mPas)	5.94	7.59	8.92	9.05
Gel viscosity (mPas)	998	1487	1546	1679

Table No: 4.05: Composition and Characteristics of pluronicF127 Thermoreversible Periodontal Gels containing Polyethylene glycol 1000

Composition/ Characteristics	Formulation Code			
	GF20	GF21	GF22	GF23
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PEG 1000 (%w/w)	7.50	7.50	7.50	7.50
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	39.2	35.7	32.8	30.2
pH (Sol)	6.51	6.59	6.53	6.60
pH (Gel)	6.54	6.60	6.55	6.65
Sol viscosity (mPas)	5.32	7.18	8.26	8.93
Gel viscosity (mPas)	995	1449	1529	1632

Table No: 4.06: Composition and Characteristics of pluronicF127 Thermoreversible Periodontal Gels containing Polyethylene glycol 1000

Composition/ Characteristics	Formulation Code			
	GF24	GF25	GF26	GF27
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PEG1000 (%w/w)	10.00	10.00	10.00	10.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	42.6	38.5	35.6	33.9
pH (Sol)	6.52	6.60	6.54	6.61
pH (Gel)	6.55	6.61	6.56	6.66
Sol viscosity (mPas)	5.19	7.03	8.07	8.49
Gel viscosity (mPas)	989	1436	1517	1622

Table No: 4.07: Composition and Characteristics of PluronicF127 Thermoreversible Periodontal Gels containing Polyethylene glycol 1000

Composition/ Characteristics	Formulation Code			
	GF28	GF29	GF30	GF31
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PEG 1000 (%w/w)	15.00	15.00	15.00	15.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	46.8	43.8	40.9	37.5
pH (Sol)	6.54	6.61	6.55	6.62
pH (Gel)	6.57	6.62	6.57	6.67
Sol viscosity (mPas)	4.91	6.82	7.95	8.27
Gel viscosity (mPas)	980	1415	1503	1606

Table No: 4.08: Composition and Characteristics of Polycarbophil - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF32	GF33	GF34	GF35
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.2	0.2	0.2	0.2
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	22.1	20.2	18.3	17.3
pH (Sol)	5.64	6.01	6.05	6.18
pH (Gel)	5.67	6.04	6.07	6.22
Sol viscosity (mPas)	7.92	10.48	12.67	14.53
Gel viscosity (mPas)	1117	1641	1754	1865

Table No: 4.09: Composition and Characteristics of Polycarbophil - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF36	GF37	GF38	GF39
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.3	0.3	0.3	0.3
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.9	19.3	17.5	16.4
pH (Sol)	6.01	6.22	6.41	6.48
pH (Gel)	6.05	6.25	6.47	6.51
Sol viscosity (mPas)	8.26	11.03	13.29	15.17
Gel viscosity (mPas)	1156	1692	1806	1939

Table No: 4.10: Composition and Characteristics of Polycarbophil - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF40	GF41	GF42	GF43
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.4	0.4	0.4	0.4
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.6	18.2	16.4	15.4
pH (Sol)	6.11	6.32	6.48	6.36
pH (Gel)	6.15	6.35	6.51	6.39
Sol viscosity (mPas)	8.95	11.35	13.73	15.82
Gel viscosity (mPas)	1203	1747	1879	1991

Table No: 4.11: Composition and Characteristics of Polycarbophil - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF44	GF45	GF46	GF47
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	17.6	16.5	14.9	13.9
pH (Sol)	6.14	6.35	6.45	6.51
pH (Gel)	6.18	6.38	6.50	6.53
Sol viscosity (mPas)	9.13	11.81	14.36	16.24
Gel viscosity (mPas)	1285	1796	1949	2009

Table No: 4.12: Composition and Characteristics of HPMC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF48	GF49	GF50	GF51
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.25	0.25	0.25	0.25
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	22.5	20.6	18.7	17.6
pH (Sol)	5.85	5.91	6.08	6.12
pH (Gel)	5.87	5.94	6.10	6.15
Sol viscosity (mPas)	8.31	11.16	13.21	15.57
Gel viscosity (mPas)	1142	1673	1868	1922

Table No: 4.13: Composition and Characteristics of HPMC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF52	GF53	GF54	GF55
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.3	19.4	17.6	16.5
pH (Sol)	5.88	5.96	6.09	6.14
pH (Gel)	5.90	6.01	6.12	6.18
Sol viscosity (mPas)	8.86	11.94	13.73	16.36
Gel viscosity (mPas)	1153	1644	1835	1971

Table No: 4.14: Composition and Characteristics of HPMC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF56	GF57	GF58	GF59
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.75	0.75	0.75	0.75
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	18.9	17.7	16.2	15.3
pH (Sol)	5.93	6.09	6.18	6.19
pH (Gel)	5.94	6.11	6.21	6.24
Sol viscosity (mPas)	9.16	12.07	14.13	17.02
Gel viscosity (mPas)	1214	1702	1890	2012

Table No: 4.15: Composition and Characteristics of HEC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF60	GF61	GF62	GF63
Pluronic F127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.25	0.25	0.25	0.25
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	23.10	21.00	19.20	18.00
pH (Sol)	6.01	6.21	6.26	6.29
pH (Gel)	6.05	6.25	6.28	6.32
Sol viscosity (mPas)	8.21	11.38	13.74	15.69
Gel viscosity (mPas)	1138	1626	1892	1934

Table No: 4.16: Composition and Characteristics of HEC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF64	GF65	GF66	GF67
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.30	19.80	18.00	17.00
pH (Sol)	5.96	6.19	6.25	6.28
pH (Gel)	6.02	6.24	6.27	6.31
Sol viscosity (mPas)	8.49	11.76	14.04	15.86
Gel viscosity (mPas)	1172	1720	1973	2031

Table No: 4.17: Composition and Characteristics of HEC - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF68	GF69	GF70	GF71
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.75	0.75	0.75	0.75
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.90	18.20	16.70	15.70
pH (Sol)	5.96	6.18	6.22	6.25
pH (Gel)	6.02	6.23	6.26	6.28
Sol viscosity (mPas)	9.11	12.36	14.47	16.21
Gel viscosity (mPas)	1208	1812	2014	2100

Table No: 4.18: Composition and Characteristics of PVP - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF72	GF73	GF74	GF75
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP K30 (%w/w)	0.50	0.50	0.50	0.50
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.40	20.80	19.50	18.50
pH (Sol)	5.56	5.62	5.79	5.82
pH (Gel)	5.53	5.65	5.83	5.84
Sol viscosity (mPas)	9.21	12.23	14.35	17.15
Gel viscosity (mPas)	1223	1685	1829	1983

Table No: 4.19: Composition and Characteristics of PVP - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF76	GF77	GF78	GF79
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP K30 (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.40	19.30	18.60	17.60
pH (Sol)	5.59	5.78	5.80	5.86
pH (Gel)	5.62	5.82	5.82	5.89
Sol viscosity (mPas)	9.78	13.02	14.87	17.55
Gel viscosity (mPas)	1239	1701	1886	2011

Table No: 4.20: Composition and Characteristics of PVP - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF80	GF81	GF82	GF83
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP K30 (%w/w)	2.00	2.00	2.00	2.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	19.80	18.90	17.30	16.30
pH (Sol)	5.62	5.92	6.01	5.99
pH (Gel)	5.65	5.94	6.03	6.02
Sol viscosity (mPas)	10.13	13.27	15.13	18.02
Gel viscosity (mPas)	1288	1746	1902	2065

Table No: 4.21: Composition and Characteristics of Carbopol 934P - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF84	GF85	GF86	GF87
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol 934P (%w/w)	0.2	0.2	0.2	0.2
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.90	19.80	18.60	17.40
pH (Sol)	5.92	6.07	6.19	6.23
pH (Gel)	5.95	6.10	6.21	6.28
Sol viscosity (mPas)	9.89	12.63	14.72	17.46
Gel viscosity (mPas)	1321	1705	1793	1991

Table No: 4.22: Composition and Characteristics of Carbopol 934P - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF88	GF89	GF90	GF91
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol 934P (%w/w)	0.3	0.3	0.3	0.3
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	19.80	18.6	17.8	16.7
pH (Sol)	6.01	6.18	6.27	6.47
pH (Gel)	6.05	6.21	6.29	6.50
Sol viscosity (mPas)	10.25	13.31	15.27	18.19
Gel viscosity (mPas)	1387	1779	1843	2019

Table No: 4.23: Composition and Characteristics of Carbopol 934P - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF88	GF89	GF90	GF91
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol 934P (%w/w)	0.4	0.4	0.4	0.4
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	18.90	17.8	16.6	15.7
pH (Sol)	6.21	6.32	6.47	6.51
pH (Gel)	6.23	6.35	6.51	6.53
Sol viscosity (mPas)	11.37	14.16	16.34	19.33
Gel viscosity (mPas)	1437	1813	1921	2084

Table No: 4.24: Composition and Characteristics of Carbopol 934P - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF92	GF93	GF94	GF95
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol 934P (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	16.50	15.6	14.4	13.5
pH (Sol)	6.33	6.48	6.51	6.55
pH (Gel)	6.37	6.53	6.54	6.58
Sol viscosity (mPas)	12.19	14.85	17.29	20.22
Gel viscosity (mPas)	1551	1902	1995	2143

Table No: 4.25: Composition and Characteristics of PVA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF96	GF97	GF98	GF99
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.20	20.30	19.20	18.20
pH (Sol)	5.53	5.62	5.64	5.67
pH (Gel)	5.55	5.64	5.67	5.69
Sol viscosity (mPas)	8.76	11.74	13.83	17.27
Gel viscosity (mPas)	1195	1673	1733	1942

Table No: 4.26: Composition and Characteristics of PVA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF100	GF101	GF102	GF103
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.10	19.40	18.30	17.30
pH (Sol)	5.58	5.69	5.78	5.81
pH (Gel)	5.61	5.71	5.81	5.83
Sol viscosity (mPas)	9.05	11.93	14.07	17.39
Gel viscosity (mPas)	1212	1692	1781	1964

Table No: 4.27: Composition and Characteristics of PVA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF104	GF105	GF106	GF107
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	2.00	2.00	2.00	2.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	19.30	18.60	17.00	16.00
pH (Sol)	5.63	5.72	5.81	5.86
pH (Gel)	5.65	5.74	5.83	5.89
Sol viscosity (mPas)	9.41	12.29	14.81	18.14
Gel viscosity (mPas)	1239	1731	1802	2013

Table No: 4.28: Composition and Characteristics of PAA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF108	GF109	GF110	GF111
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PAA (%w/w)	0.5	0.5	0.5	0.5
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	20.70	19.30	18.90	17.90
pH (Sol)	6.01	6.11	6.21	6.38
pH (Gel)	6.04	6.13	6.22	6.40
Sol viscosity (mPas)	8.25	10.39	11.67	16.92
Gel viscosity (mPas)	1083	1564	1683	1839

Table No: 4.29: Composition and Characteristics of PAA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF112	GF113	GF114	GF115
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PAA (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	19.30	18.50	17.30	16.80
pH (Sol)	5.97	6.05	6.11	6.27
pH (Gel)	5.99	6.07	6.13	6.31
Sol viscosity (mPas)	8.92	11.12	12.01	17.81
Gel viscosity (mPas)	1143	1524	1579	1902

Table No: 4.30: Composition and Characteristics of PAA - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF116	GF117	GF118	GF119
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PAA (%w/w)	2.00	2.00	2.00	2.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	18.40	17.70	16.40	15.50
pH (Sol)	5.83	5.95	6.03	6.15
pH (Gel)	5.86	5.97	6.05	6.17
Sol viscosity (mPas)	9.03	11.79	12.59	18.29
Gel viscosity (mPas)	1202	1589	1708	1984

Table No: 4.31: Composition and Characteristics of 0.5%w/w NaOH - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF120	GF121	GF122	GF123
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
0.5% NaOH (%w/w)	0.25	0.25	0.25	0.25
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	22.40	21.3	19.4	18.2
pH (Sol)	5.94	6.15	6.21	6.32
pH (Gel)	5.97	6.17	6.28	6.38
Sol viscosity (mPas)	7.19	9.36	10.44	15.76
Gel viscosity (mPas)	995	1423	1538	1726

Table No: 4.32: Composition and Characteristics of 0.5%w/w NaOH - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF124	GF125	GF126	GF127
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
0.5% NaOH (%w/w)	0.50	0.50	0.50	0.50
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	22.40	20.30	18.2	17.1
pH (Sol)	5.96	6.21	6.33	6.42
pH (Gel)	5.99	6.28	6.36	6.48
Sol viscosity (mPas)	8.05	9.89	11.17	16.21
Gel viscosity (mPas)	1015	1487	1627	1801

Table No: 4.33: Composition and Characteristics of 0.5%w/w NaOH - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF128	GF129	GF130	GF131
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
0.5% NaOH (%w/w)	0.75	0.75	0.75	0.75
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	21.30	18.3	16.6	15.4
pH (Sol)	5.99	6.27	6.38	6.49
pH (Gel)	6.02	6.30	6.41	6.52
Sol viscosity (mPas)	8.47	10.15	11.39	16.83
Gel viscosity (mPas)	1078	1492	1641	1846

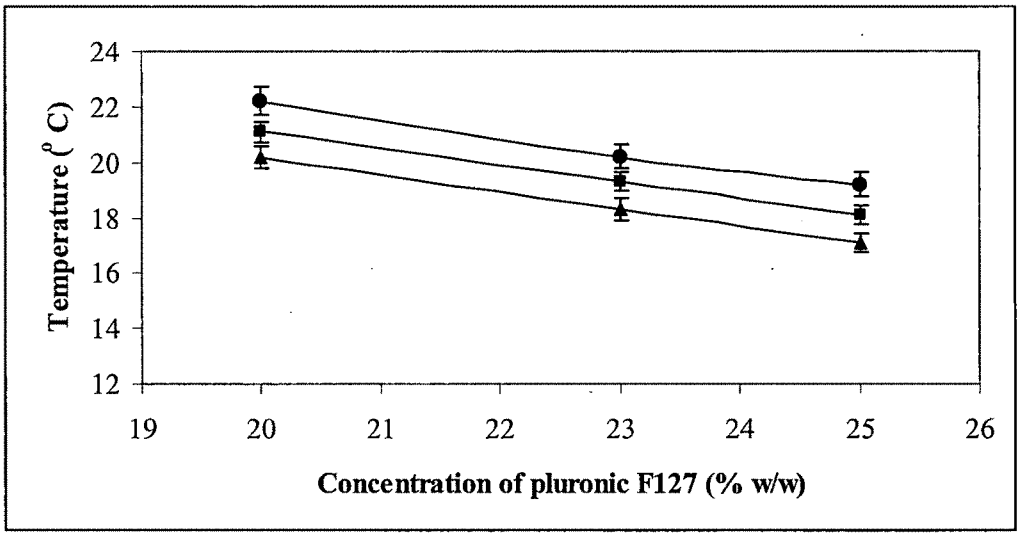
Table No: 4.34: Composition and Characteristics of 0.5%w/w NaOH - PluronicF127 Thermoreversible Periodontal Gels

Composition/ Characteristics	Formulation Code			
	GF132	GF133	GF134	GF135
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
0.5% NaOH (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Gel Temperature (°C)	19.40	17.2	15.5	14.3
pH (Sol)	6.03	6.29	6.40	6.48
pH (Gel)	6.07	6.31	6.43	6.51
Sol viscosity (mPas)	9.12	11.29	12.21	17.31
Gel viscosity (mPas)	1138	1517	1705	1895

Table No: 4.35: Effect of various additives on enthalpy of pluronic F127 gels

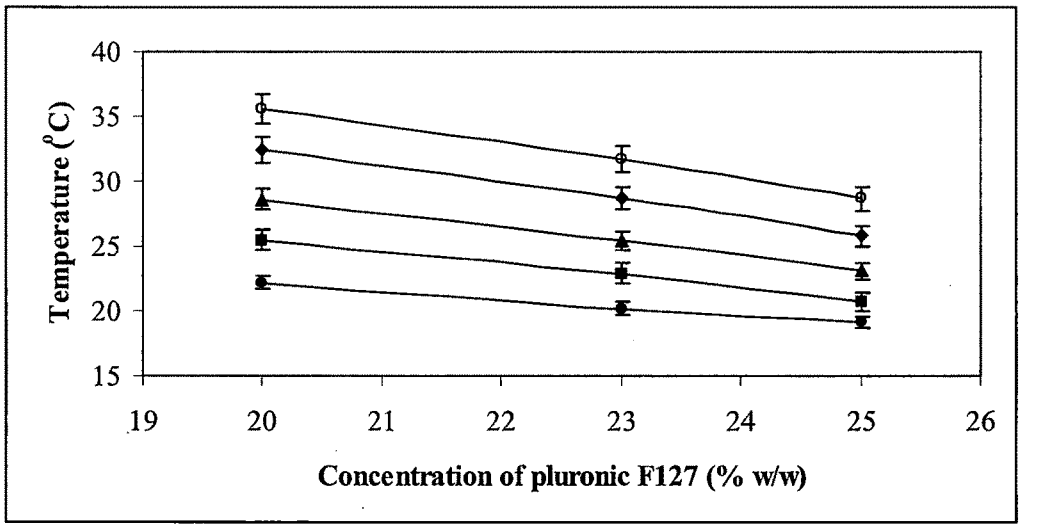
Sl. No	Formulation additives	H^0 gel (kcal)
1	Pluronic F127	22.330
2	Polycarbophil 0.2%	23.262
3	Polycarbophil 0.3%	23.438
4	Polycarbophil 0.4%	23.511
5	Polycarbophil 0.5%	26.672
5	HPMC 0.25%	22.790
6	HPMC 0.50%	23.943
7	HPMC 0.75%	26.085
8	HEC 0.25%	23.178
9	HEC 0.50%	24.220
10	HEC 0.75%	26.15
11	PVP 0.5%	22.857
12	PVP 1.00%	23.380
13	PVP 2.00%	24.073
14	Carbopol 934P 0.2%	23.858
15	Carbopol 934P 0.3%	24.211
16	Carbopol 934P 0.4%	25.119
17	Carbopol 934P 0.5%	32.424
18	PVA 0.5%	23.059
19	PVA 1.00%	23.722
20	PVA 2.00%	24.552
21	Poly acrylic acid 0.5%	24.147
22	Poly acrylic acid 1.00%	25.207
23	Poly acrylic acid 2.00%	28.641
24	Sodium hydroxide 0.25%	22.823
25	Sodium hydroxide 0.5%	22.657
26	Sodium hydroxide 0.75%	22.542
27	Sodium hydroxide 1.0%	22.616
28	PEG 1000 5.0%	12.151
29	PEG 1000 7.5%	09.862
30	PEG 1000 10.0 %	07.662
31	PEG 1000 15.0 %	06.451
32	Minocycline hydrochloride	21.283
33	Clindamycin phosphate	21.941

Figure: 4.01



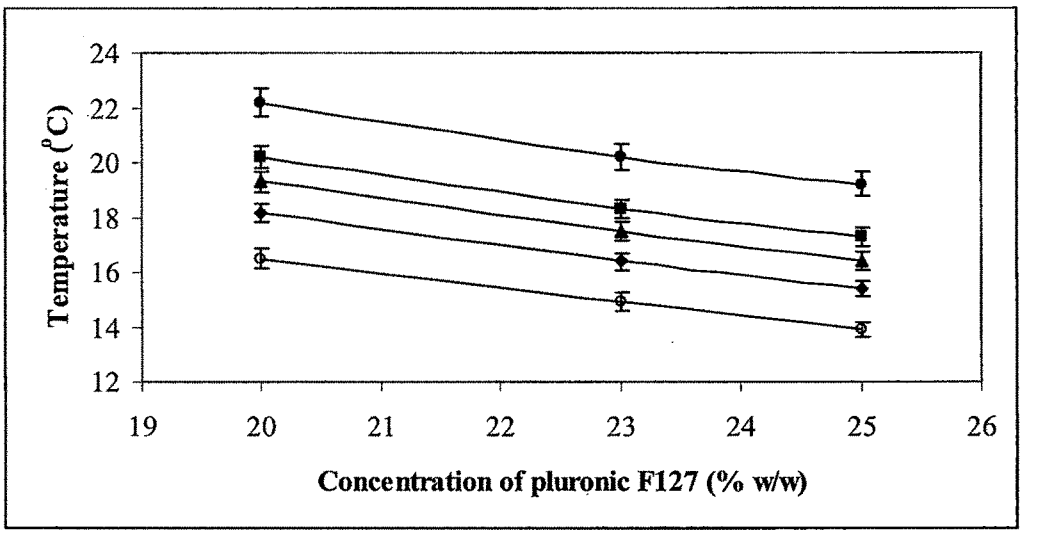
Gelation range of pluronic F127 and effect of clindamycin phosphate and minocycline hydrochloride concentration [-● - pluronic F127, - ■ – clindamycin phosphate, - ▲ – minocycline hydrochloride].
n=3

Figure: 4.02



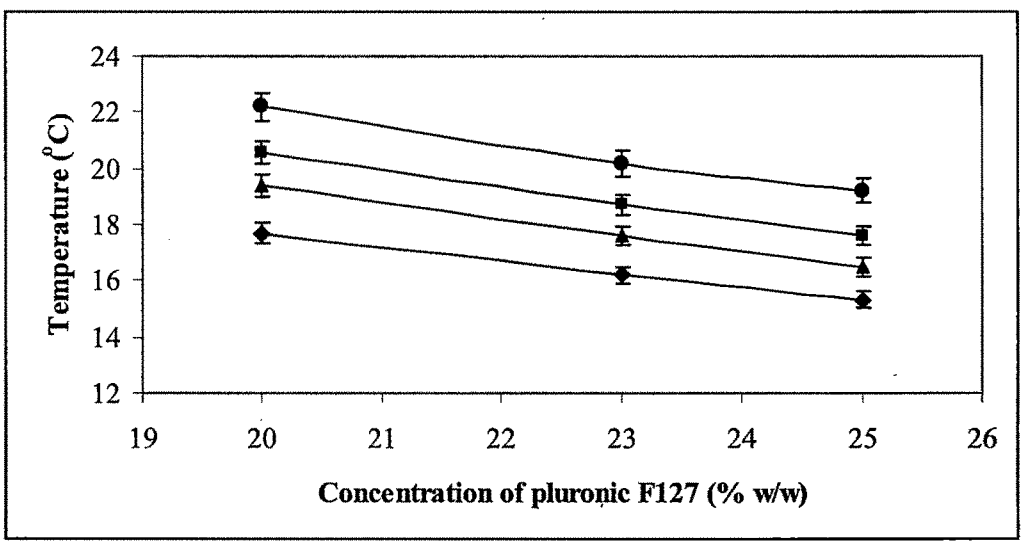
Gelation range of pluronic F127 and effect of different concentration of PEG 1000 [-● - pluronic F127, - ■ – 5.00 % w/w, - ▲ – 7.50 % w/w, - ♦ -10.00 % w/w, - ○ – 15.00 % w/w].
n=3

Figure: 4.03



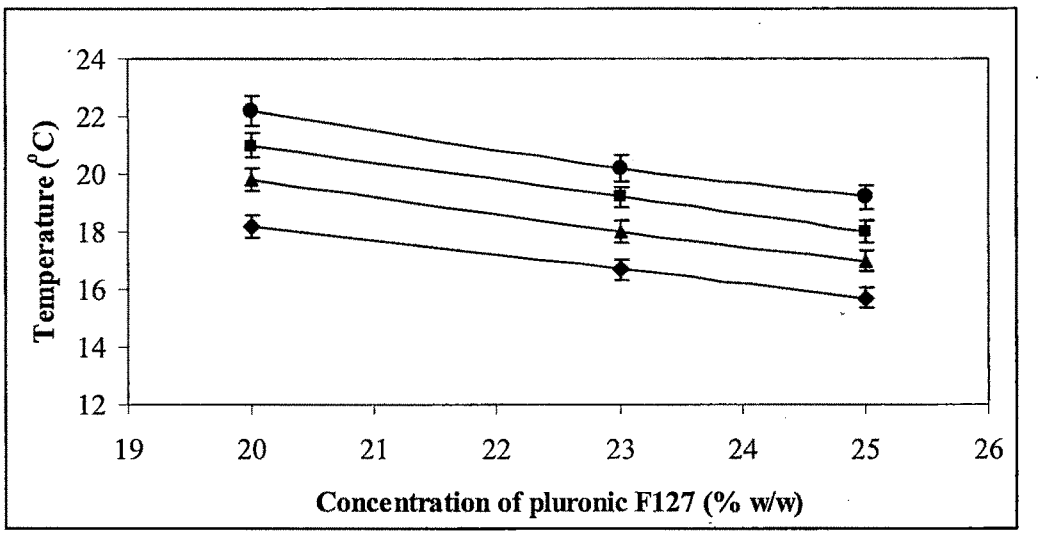
Gelation range of pluronic F127 and effect of different concentration of polycarbophil [- ● - pluronic F127, - ■ – 0.20 % w/w, - ▲ – 0.30 % w/w, - ♦ - 0.40 % w/w, - ○ – 0.50 % w/w].
n=3

Figure: 4.04



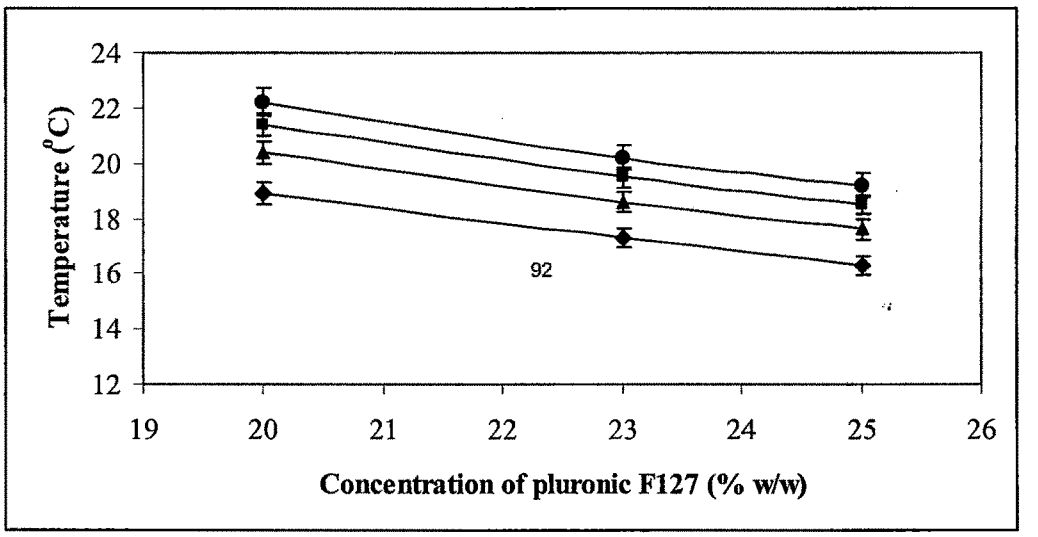
Gelation range of pluronic F127 and effect of different concentration of HPMC [-● - pluronic F127, - ♦ - 0.75 % w/w, - ▲ – 0.50 % w/w, - ■ – 0.25 % w/w].
n=3

Figure: 4.05



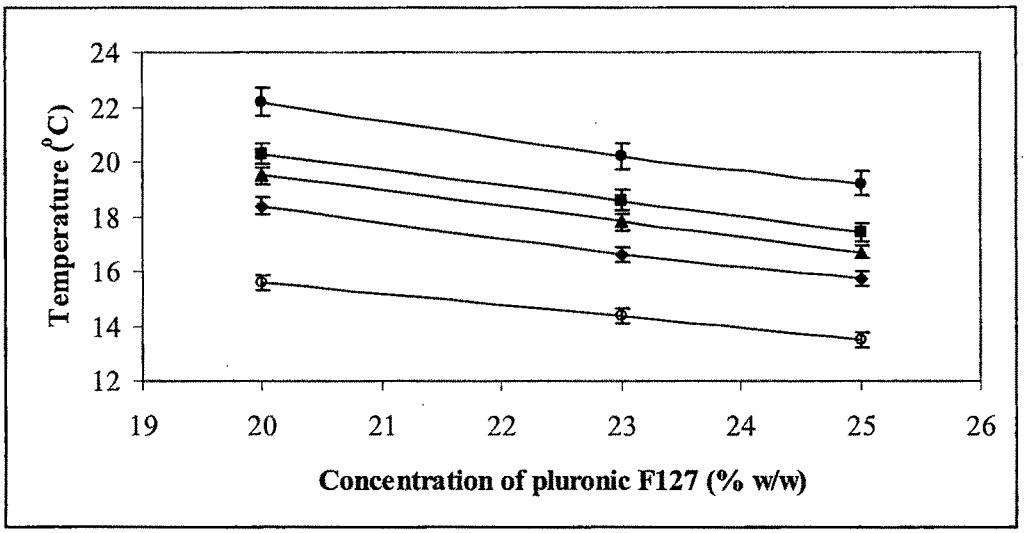
Gelation range of pluronic F127 and effect of different concentration of HEC [-● - pluronic F127, - ♦ - 0.75 % w/w, - ▲ – 0.50 % w/w, - ■ – 0.25 % w/w].
n=3

Figure: 4.06



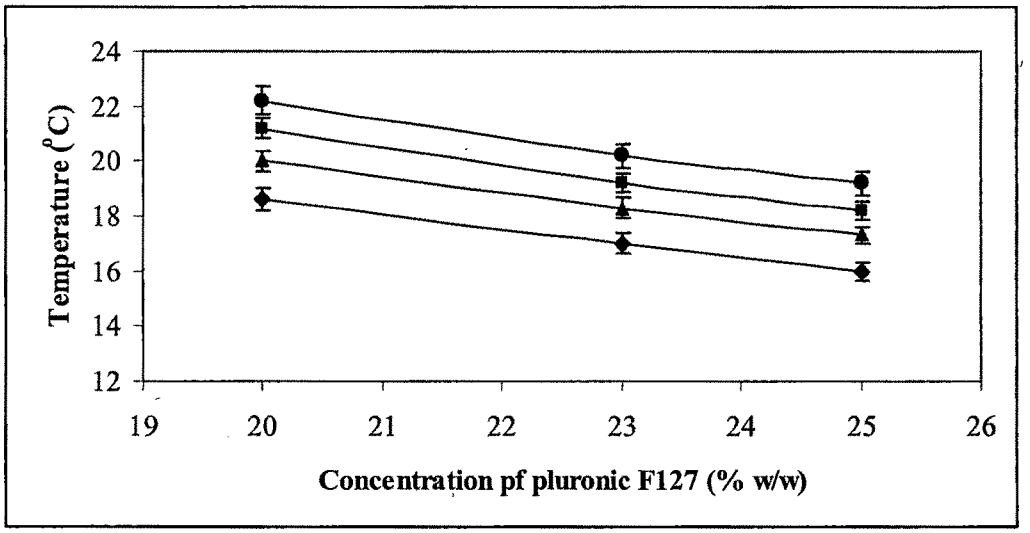
Gelation range of pluronic F127 and effect of different concentration of PVP [-● - pluronic F127, - ♦ - 0.50 % w/w, - ▲ – 1.00 % w/w, - ■ – 2.00 % w/w].
n=3

Figure: 4.07



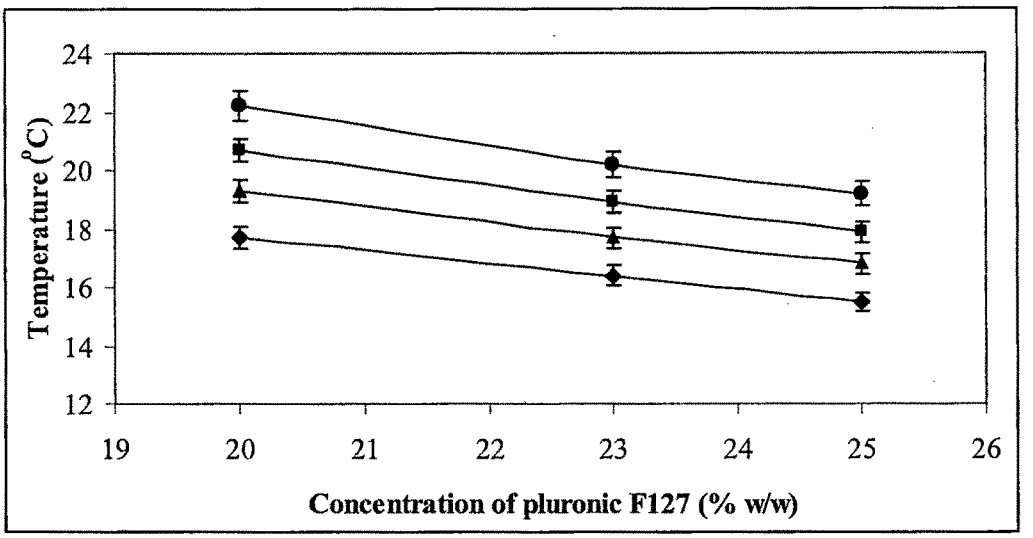
Gelation range of pluronic F127 and effect of different concentration of carbopol 934P [-● - pluronic F127, - ■ – 0.20 % w/w, - ▲ – 0.30 % w/w, - ♦ - 0.40 % w/w, - ○ – 0.50 % w/w].
n=3

Figure: 4.08



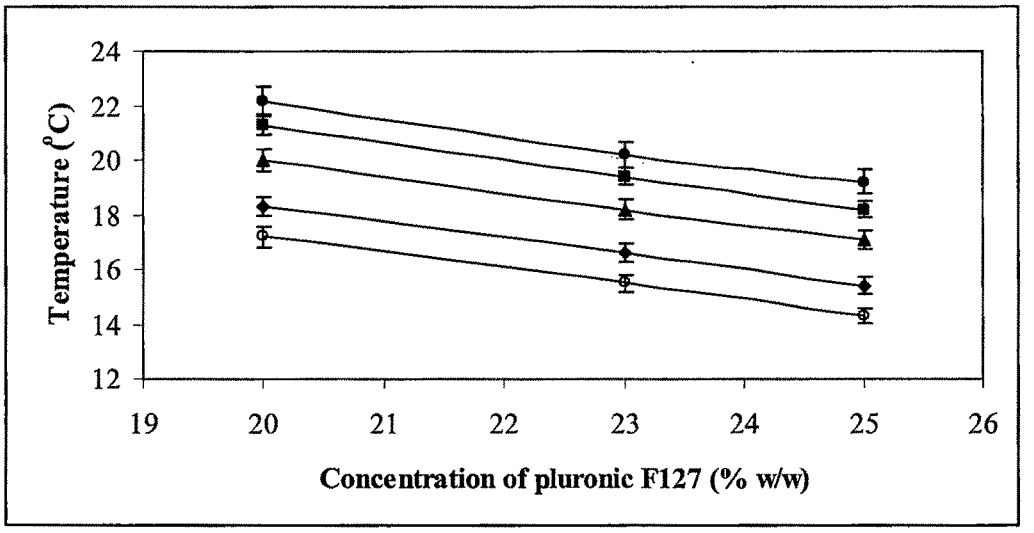
Gelation range of pluronic F127 and effect of different concentration of PVA [-● - pluronic F127, - ■ - 0.50 % w/w, - ▲ – 1.00 % w/w, - ♦ – 2.00 % w/w].
n=3

Figure: 4.09



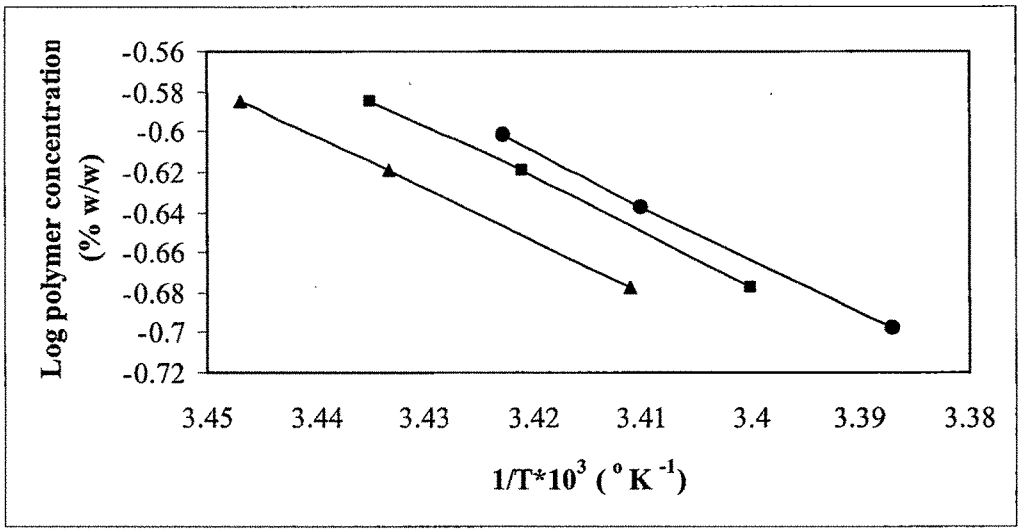
Gelation range of pluronic F127 and effect of different concentration of poly acrylic acid [-● - pluronic F127, - ■ - 0.50 % w/w, - ▲ – 1.00 % w/w, - ♦ – 2.00 % w/w].
n=3

Figure: 4.10



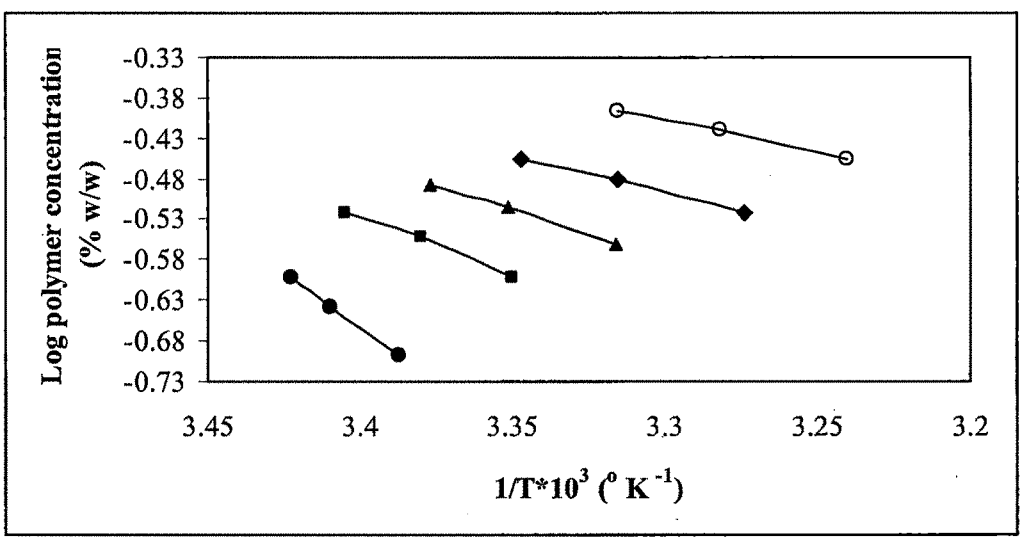
Gelation range of pluronic F127 and effect of different concentration of 0.5% NaOH [-● - pluronic F127, - ■ – 0.25 % w/w, - ▲ – 0.50 % w/w, - ♦ - 0.75 % w/w, - ○ – 1.00 % w/w].
n=3

Figure: 4.11



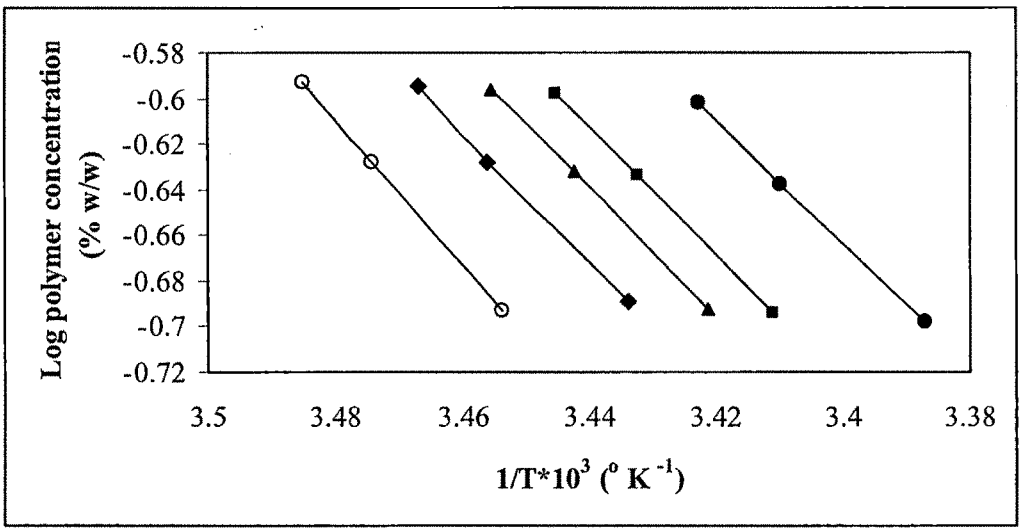
Enthalpy of gelation of pluronic F127 and effect of minocycline hydrochloride [-● - pluronic F127 (22.330), - ■ – clindamycin phosphate (21.941), - ▲ - minocycline hydrochloride (21.283)].
n=3

Figure: 4.12



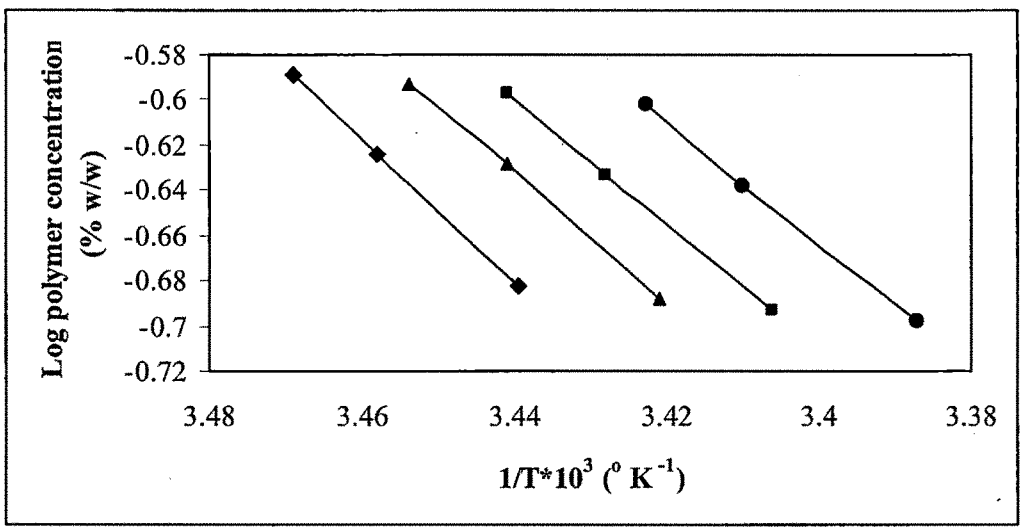
Enthalpy of gelation of pluronic F127 and PEG 1000 [- ○ – 15 % w/w (06.451) , - ♦ -10 % w/w (07.662) , - ▲- 7.5 % w/w (09.862) , - ■ - 5% w/w (12.151) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.13



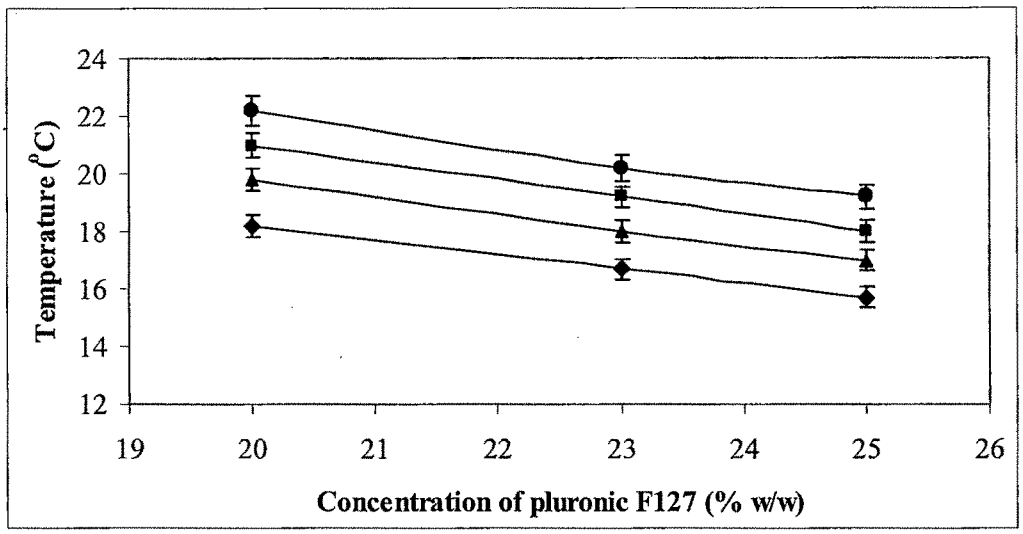
Enthalpy of gelation of pluronic F127 and effect of polycarbophil [- ○ – 0.50 % w/w (26.672) , - ♦ -0.40 % w/w (23.511) , - ▲- 0.30 % w/w (23.438) , - ■ – 0.20 % w/w (23.262) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.14



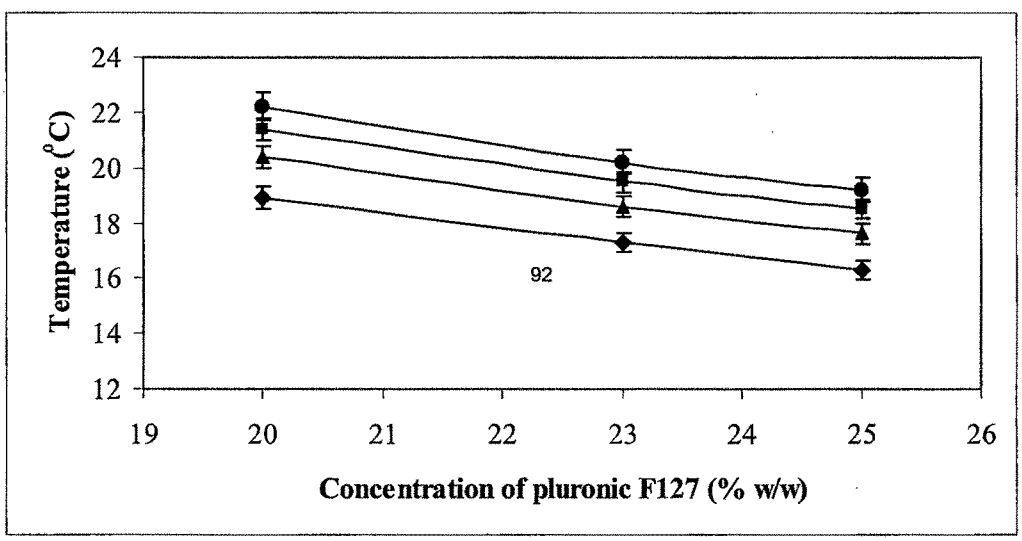
Enthalpy of gelation of pluronic F127 and effect of HPMC [- ♦ -0.75 % w/w (26.085) , - ▲- 0.50 % w/w (23.943) , - ■ – 0.25 % w/w (22.790) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.15



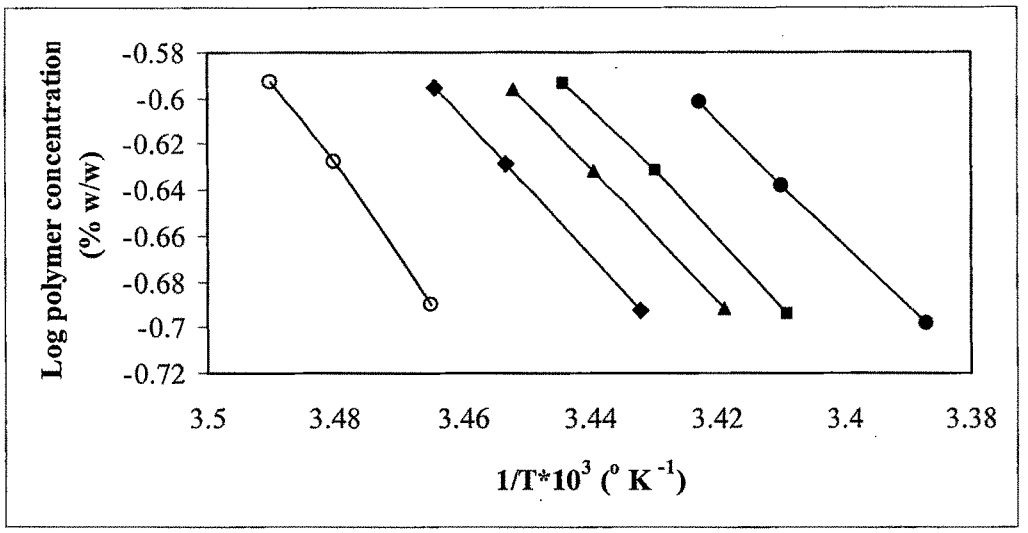
Enthalpy of gelation of pluronic F127 and effect of HEC [- ♦ -0.75 % w/w (26.15) , - ▲- 0.50 % w/w (24.220) , - ■ – 0.25 % w/w (23.178) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.16



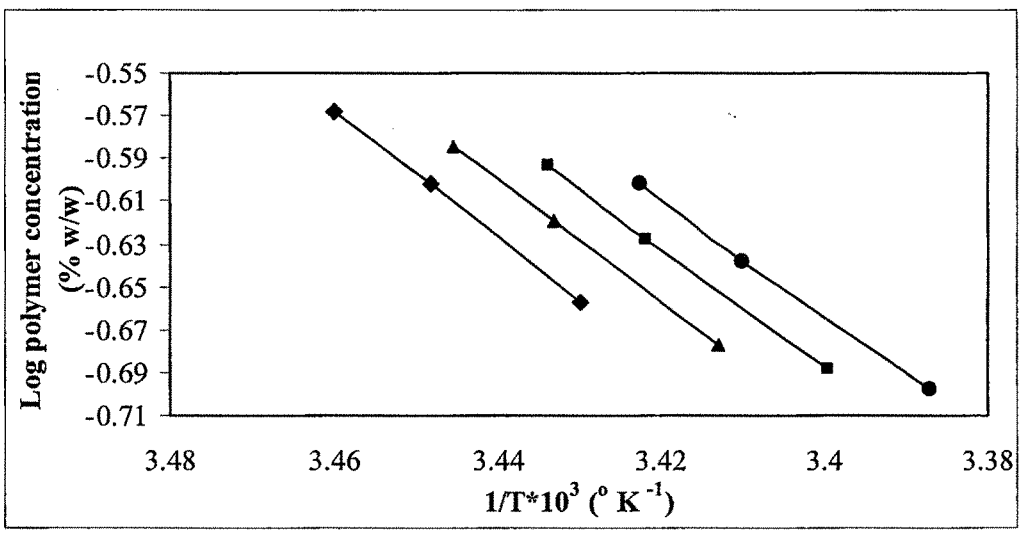
Enthalpy of gelation of pluronic F127 and effect of PVP [- ♦ - 2.00 % w/w (24.073) , - ▲- 1.00 % w/w (23.380) , - ■ – 0.50 % w/w (22.857) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.17



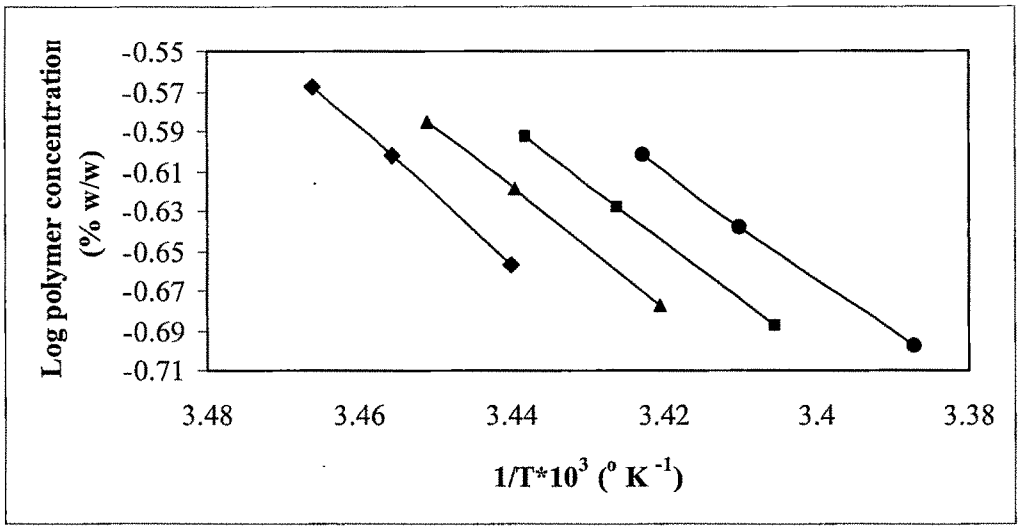
Enthalpy of gelation of pluronic F127 and effect of carbopol 934P [- ○ - 0.50 % w/w (32.424), - ♦ -0.40 % w/w (25.119) , - ▲- 0.30 % w/w (24.211) , - ■ – 0.20 % w/w (23.858),- ● - pluronic F127 (22.330)].
n=3

Figure: 4.18



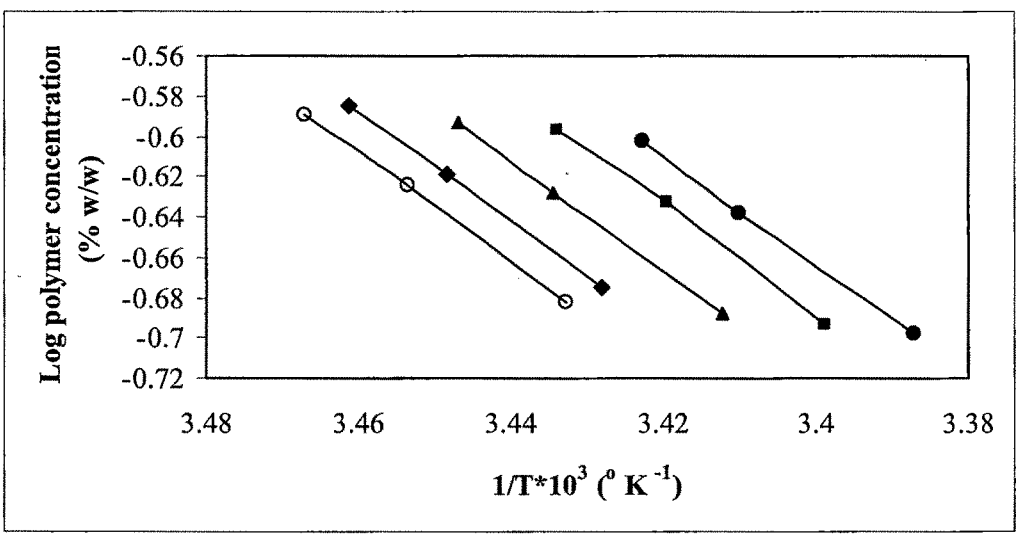
Enthalpy of gelation of pluronic F127 and effect of PVA [- ♦ - 2.00 % w/w (24.552) , - ▲- 1.00 % w/w (23.722) , - ■ – 0.50 % w/w (23.059) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.19



Enthalpy of gelation of pluronic F127 and effect of poly acrylic acid [- ♦ - 2.00 % w/w (28.641) , - ▲- 1.00 % w/w (25.207) , - ■ – 0.50 % w/w (24.147) ,- ● - pluronic F127 (22.330)].
n=3

Figure: 4.20



Enthalpy of gelation of pluronic F127 and effect of 0.5% NaOH [- ○ – 1.00 % w/w (22.616) , - ♦ -0.75 % w/w (22.542) , - ▲- 0.50 % w/w (22.657) , - ■ – 0.25 % w/w (22.823) ,- ● - pluronic F127 (22.330)].
n=3

4.4 SUMMARIZATION

The feasibility study of pluronic F127 thermoreversible gel formulations for periodontal administration was prepared by incorporating the antibiotics; minocycline hydrochloride and clindamycin phosphate. Formulations containing 19, 20, 23 and 25% w/w pluronic F127 exhibited thermoreversible property well below the physiological temperature in the periodontal cavity and they also exhibited the intended mucoadhesive properties. The results of the study reveal that the addition of minocycline hydrochloride/ clindamycin phosphate alone or the addition of the other formulation additives alters the gelation temperatures and its property. The thermodynamic property of the pluronic F127 gels is dependent on the concentration of the polymers and water soluble additives. In the presence of PEG 1000 the gelation temperature broadens while in presence of mucoadhesive polymers; polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA and poly acrylic acid the gelation temperature decreases. The enthalpy changes were significant in presence of PEG 1000, whereas the enthalpy changes were non-significant in presence of other formulation additives. Addition of PEG 1000 affords the advantages of desired gelation characteristics for use of desired formulation additives. Thus the thermoreversible periodontal gel of minocycline hydrochloride/ clindamycin phosphate with PEG 1000 and mucoadhesive polymers like; polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA, poly acrylic acid and pluronic F127 is more stable and suitable for periodontal administration. Therefore this can be concluded from the study that the use of in situ gelling vehicle could effectively and safely improve the periodontal residence time and there by the absorption of the minocycline hydrochloride/ clindamycin phosphate to the target site.

4.5 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND POLYCARBOPHIL

In order to fortify the adhesion of administrated drugs onto the mucosal surfaces, mucoadhesive polymers have been added to the in situ gelling vehicles of pluronic F127 (Chu et al., 1991). Enhancement of the absorption of drugs loaded into pluronic F127 gels through the epithelial cell monolayer of the upper small intestine has been reported by one of the research group (Bromberg et al., 2003). However, combination of thermoreversible

polymer pluronic F127 and mucoadhesive polymer polycarbophil, which has the property of increasing the residence time in the periodontal cavity, increase in the absorption of minocycline hydrochloride/ clindamycin phosphate across the periodontal cavity with enhanced drug delivery into the periodontal cavity has never been screened as effective periodontal mucoadhesive drug delivery system.

This chapter deals with the development of a periodontal system containing effective amount of antibiotics; minocycline hydrochloride/ clindamycin phosphate along with thermoreversible polymer pluronic F127 and mucoadhesive polymer; polycarbophil at varying concentrations to give the maximum therapeutic activity at the target site. The effect of various concentrations of pluronic F127 and polycarbophil on gelling temperature, viscosity, mucoadhesive property, gel strength, syringeability and in vitro permeation were studied. The pH of all the formulations ranged within 5.5 to 6.5, which is suitable for the periodontal administration.

4.5.1 Preparation of mixed periodontal gels of polycarbophil - pluronic F127

Formulations containing the minocycline hydrochloride (1%)/ clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998). The compositions of the formulations are cited in Table No4.36 to 4.43. Minocycline hydrochloride/ clindamycin phosphate was added to half the volume of water maintained at 10°C in a beaker until a clear solution was obtained. To the above solution sodium meta-bisulphite was added as an antioxidant in case of minocycline hydrochloride loaded periodontal formulations. To this clear solution weighed amount of polycarbophil was added and dispersed prior to the addition of pluronic F127 and kept overnight at 4°C until a clear solution was obtained. The pH of all the formulations was adjusted using 0.5% NaOH. Weight was adjusted to with distilled water.

Table No: 4.36: Composition and Characteristics of MnHCl loaded mixed periodontal gels of polycarbophil (0.20%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF01	MGF02	MGF03	MGF04
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.20	0.20	0.20	0.20
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	41	32	30	28
Visual gel Temp. (°C)	41.7	32.8	30.9	29.2
Drug content	97.8 ± 0.92	98.1 ± 1.12	99.25 ± 1.31	98.6 ± 0.97
Mucoadhesion (gf/mm)	12.72 ± 0.22	13.63 ± 1.25	16.70 ± 1.26	18.58 ± 1.08
Gel strength (N/m)	8128.12 ± 78.25	9157.10 ± 79.65	12644.85 ± 117.45	14759.63 ± 100.25
pH (Sol)	5.81	6.01	5.98	6.05
pH (Gel)	5.84	6.04	6.01	6.07
Sol Viscosity mPas	10.89	17.33	19.12	23.38
Gel Viscosity mPas	1820	2870	3110	3340

Table No: 4.37: Composition and Characteristics of MnHCl loaded mixed periodontal gels of polycarbophil (0.30%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF05	MGF06	MGF07	MGF08
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.30	0.30	0.30	0.30
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	38	30	27	24
Visual gel Temp. (°C)	38.5	30.6	27.8	24.9
Drug content	98.2 ± 1.17	99.3 ± 1.14	97.9 ± 0.34	98.4 ± 0.21
Mucoadhesion (gf/mm)	13.12 ± 0.21	14.53 ± 1.26	17.50 ± 1.25	19.18 ± 0.32
Gel strength (N/m)	8936.62 ± 117.45	9893.70 ± 100.25	13283.85± 78.25	15164.91± 79.65
pH (Sol)	5.95	6.22	6.53	6.41
pH (Gel)	6.02	6.25	6.54	6.47
Sol Viscosity mPas	13.51	17.89	19.4	26.0
Gel Viscosity mPas	2170	3110	3450	3780

Table No: 4.38: Composition and Characteristics of MnHCl loaded mixed periodontal gels of polycarbophil (0.40%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF09	MGF10	MGF11	MGF12
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.40	0.40	0.40	0.40
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	36	27	25	22
Visual gel Temp. (°C)	36.5	27.8	25.6	23.1
Drug content	99.3 ± 1.32	97.6 ± 1.19	99.56 ± 1.29	98.37 ± 1.75
Mucoadhesion (gf/mm)	13.91 ± 0.28	15.17 ± 0.22	18.26 ± 0.20	19.87 ± 0.32
Gel strength (N/m)	9163.19 ± 106.25	10193.70 ± 98.04	13436.29 ±73.82	15429.37 ±86.78
pH (Sol)	6.43	6.32	6.28	6.48
pH (Gel)	6.45	6.35	6.31	6.51
Sol Viscosity mPas	15.32	18.52	20.35	26.00
Gel Viscosity mPas	2390	3450	3650	4020

Table No: 4.39: Composition and Characteristics of MnHCl loaded mixed periodontal gels of polycarbophil (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF13	MGF14	MGF15	MGF16
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	34	25	23	20
Visual gel Temp. (°C)	34.8	25.7	23.9	21.1
Drug content	98.17 ± 2.01	99.38 ± 1.78	97.48 ± 1.85	99.05 ± 2.12
Mucoadhesion (gf/mm)	14.63 ± 0.21	16.02 ± 0.17	19.31 ± 0.26	20.47 ± 0.41
Gel strength (N/m)	9163.19 ± 106.25	10193.70 ± 98.04	13436.29 ± 73.82	15429.37 ± 86.78
pH (Sol)	6.08	6.35	6.42	6.45
pH (Gel)	6.11	6.38	6.45	6.50
Sol Viscosity mPas	16.69	18.92	23.11	28.94
Gel Viscosity mPas	2655	3674	3925	4285

Table No: 4.40: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of polycarbophil (0.20%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF01	CGF02	CGF03	CGF04
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.20	0.20	0.20	0.20
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	40	31	29	27
Visual gel Temp. (°C)	40.9	32.0	30.1	27.9
Drug content	97.63 ± 1.11	98.31 ± 1.76	99.36 ± 2.57	98.67 ± 3.21
Mucoadhesion (gf/mm)	12.72 ± 0.22	13.63 ± 1.25	16.70 ± 1.26	18.58 ± 1.08
Gel strength (N/m)	8128.12 ± 78.25	9157.10 ± 79.65	12644.85 ± 117.45	14759.63 ± 100.25
pH (Sol)	5.87	6.12	6.14	6.16
pH (Gel)	5.81	6.02	6.03	6.09
Sol Viscosity mPas	11.05	18. 22	19.56	23.68
Gel Viscosity mPas	1920	2990	3220	3450

Table No: 4.41: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of polycarbophil (0.30%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF05	CGF06	CGF07	CGF08
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.30	0.30	0.30	0.30
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	37	29	26	23
Visual gel Temp. (°C)	38.1	30.1	27.5	24.2
Drug content	97.29 ± 0.97	99.28 ± 1.05	98.38 ± 1.19	98.82 ± 1.28
Mucoadhesion (gf/mm)	13.43 ± 0.19	14.37 ± 0.63	17.42 ± 0.35	19.75 ± 1.19
Gel strength (N/m)	9014.41 ± 108.13	9967.36 ± 106.03	13225.57 ± 79.39	14938.91 ± 115.36
pH (Sol)	6.05	6.23	6.32	6.38
pH (Gel)	6.04	6.27	6.37	6.42
Sol Viscosity mPas	13.98	18.25	19.98	26.85
Gel Viscosity mPas	2280	3220	3540	3910

Table No: 4.42: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of polycarbophil (0.40%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF09	CGF10	CGF11	CGF12
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.40	0.40	0.40	0.40
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	35	26	24	21
Visual gel Temp. (°C)	36.1	27.2	25.3	22.1
Drug content	99.25 ± 0.34	98.19 ± 1.19	98.69 ± 1.76	97.84 ± 0.93
Mucoadhesion (gf/mm)	14.12 ± 0.38	16.11 ± 0.20	19.05 ± 0.57	20.19 ± 0.85
Gel strength (N/m)	9189.64 ± 111.05	10165.23 ± 103.5	13349.26 ± 87.36	1520.76 ± 69.73
pH (Sol)	6.23	6.18	6.29	6.37
pH (Gel)	6.25	6.21	6.33	6.41
Sol Viscosity mPas	16.25	19.25	21.17	27.65
Gel Viscosity mPas	2550	3540	3780	4150

Table No: 4.43: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of polycarbophil (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF13	CGF14	CGF15	CGF16
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Polycarbophil (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	33	24	22	18
Visual gel Temp. (°C)	34.1	25.1	22.8	19.2
Drug content	99.21 ± 0.78	98.69 ± 0.57	97.64 ± 0.38	98.74 ± 0.93
Mucoadhesion (gf/mm)	15.73 ± 0.191	16.76 ± 0.25	20.05 ± 0.41	21.19 ± 0.34
Gel strength (N/m)	9345.16 ± 99.63	10021.68 ± 101.02	13429.18 ± 67.54	15396.44 ± 93.46
pH (Sol)	6.15	6.32	6.40	6.29
pH (Gel)	6.11	6.35	6.45	6.32
Sol Viscosity mPas	17.85	20.28	23.65	29.65
Gel Viscosity mPas	2785	3792	4036	4563

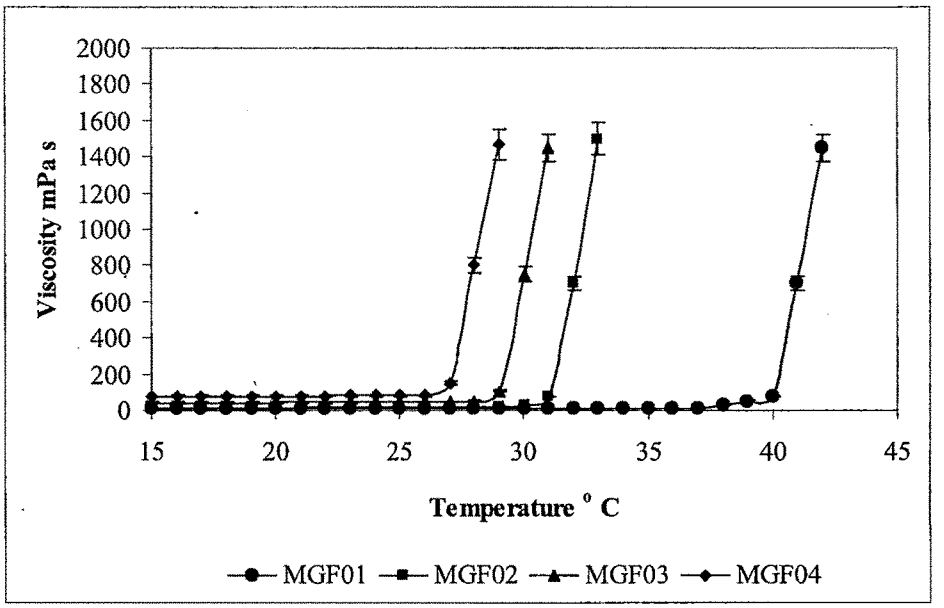
4.5.2 Measurement of gelation temperature by visual inspection

A 20 ml transparent vial containing a magnetic bar and 10 g of pluronic F127 gel was placed in thermostat water bath maintained at 4 °C. A digital thermosensor connected to a thermistor was immersed in the pluronic F127 gel. The gel was heated at the rate of 1°C/min with continuous stirring at 30 rpm. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was recorded as the gelation temperature (Choi et al. 1998, Miyazaki et al. 1991). Each preparation was tested thrice to control the repeatability of the measurement.

4.5.3 Determination of gelling temperature by rheological method

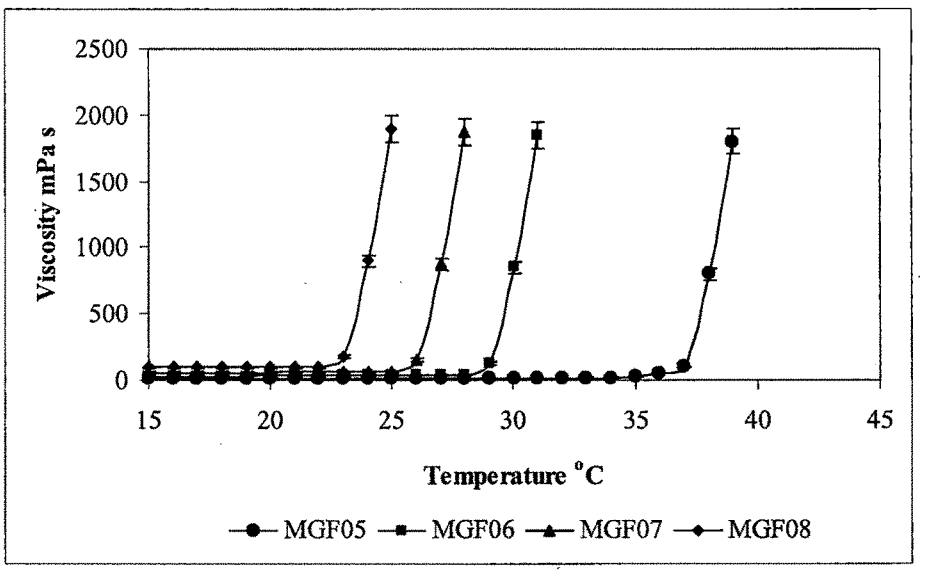
Rheological studies were performed with a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LDDV III+) fitted with CP-52 spindle using the cone/ plate geometry. The cone had 1.2 cm radius and an angle of 3°C. Pluronic F127 thermoreversible periodontal gel formulations were evaluated for rheological measurement of gelation temperature by Brookfield viscometer at 1.66 s^{-1} and 10 s^{-1} shear rate. This value was chosen to allow precise determination of the gelling temperature. The temperature was increased in step of 1°C /min, from 10°C to 35°C to locate the sol/gel transition point. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (mPas) as a function of the temperature (°C). Each preparation was tested trice to control the repeatability.

Figure 4.21: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.2 % w/w polycarbophil and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



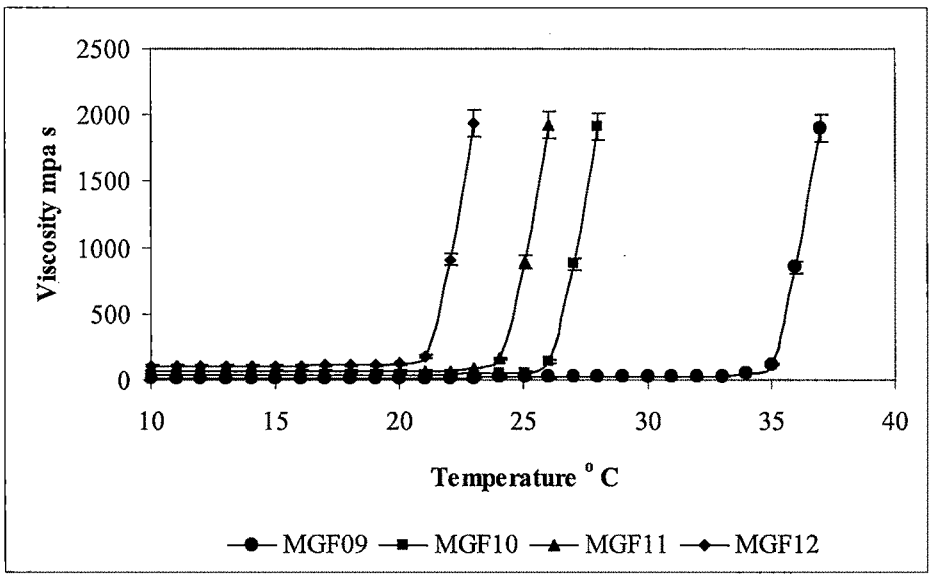
Values are expressed as mean ± SD (n =3)

Figure 4.22: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.3 % w/w polycarbophil and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



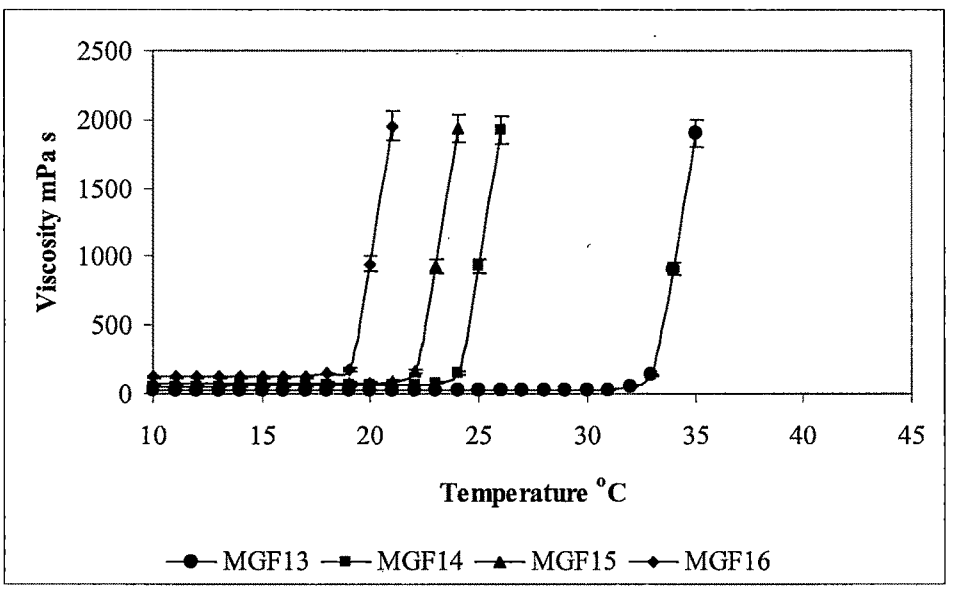
Values are expressed as mean ± SD (n =3)

Figure 4.23: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.4 % w/w polycarbophil and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



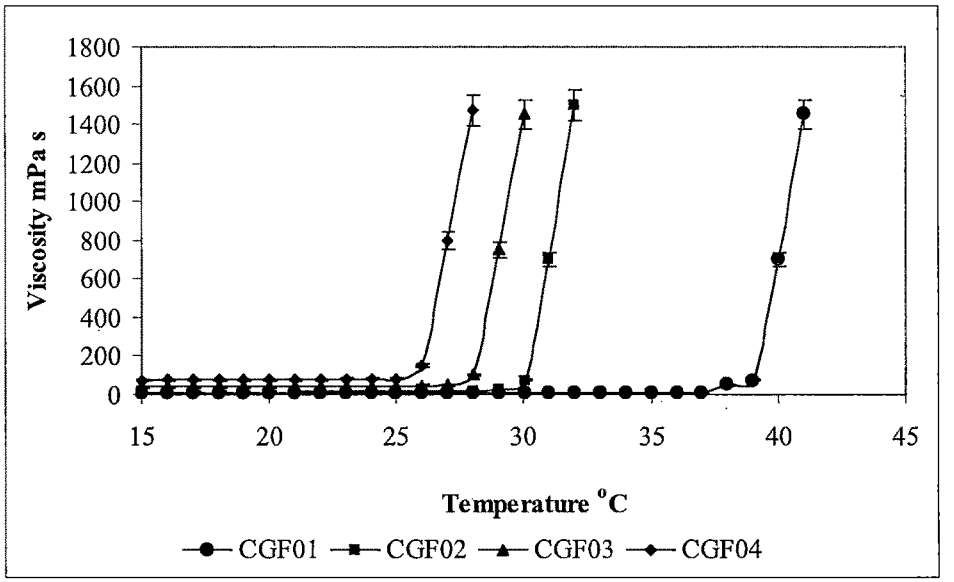
Values are expressed as mean ± SD (n =3)

Figure 4.24: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.5 % w/w polycarbophil and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



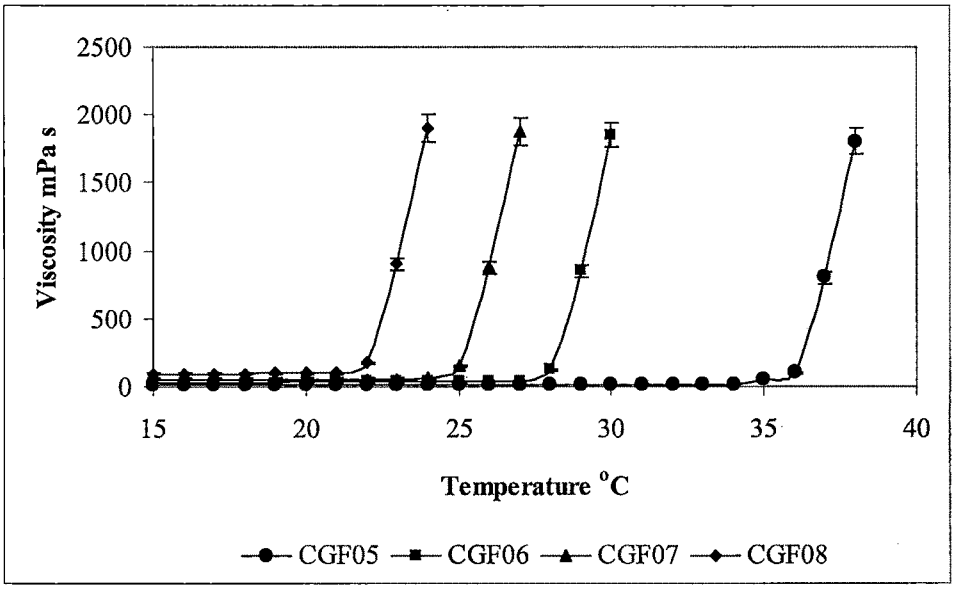
Values are expressed as mean ± SD (n =3)

Figure 4.25: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.2 % w/w polycarbophil and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



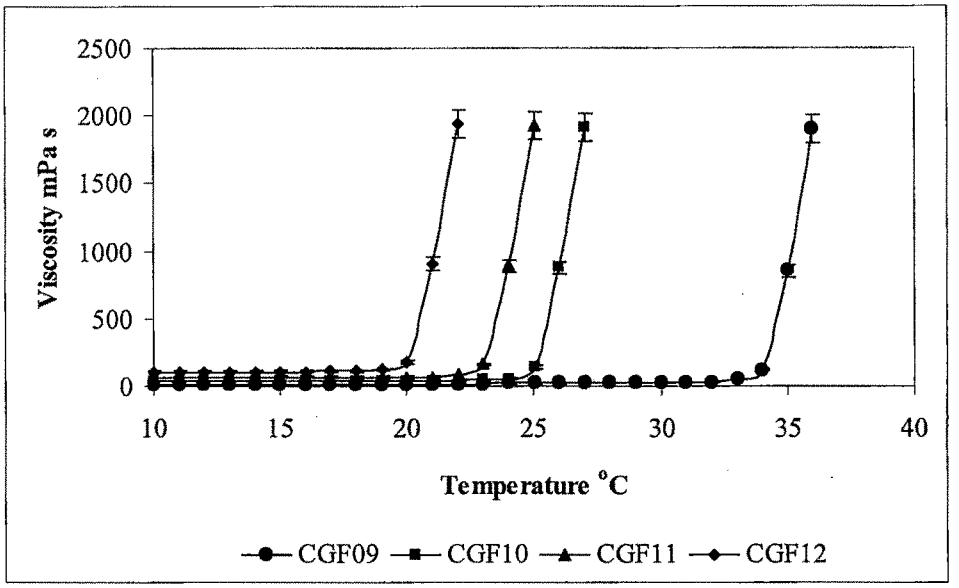
Values are expressed as mean ± SD (n =3)

Figure 4.26: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.3 % w/w polycarbophil and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



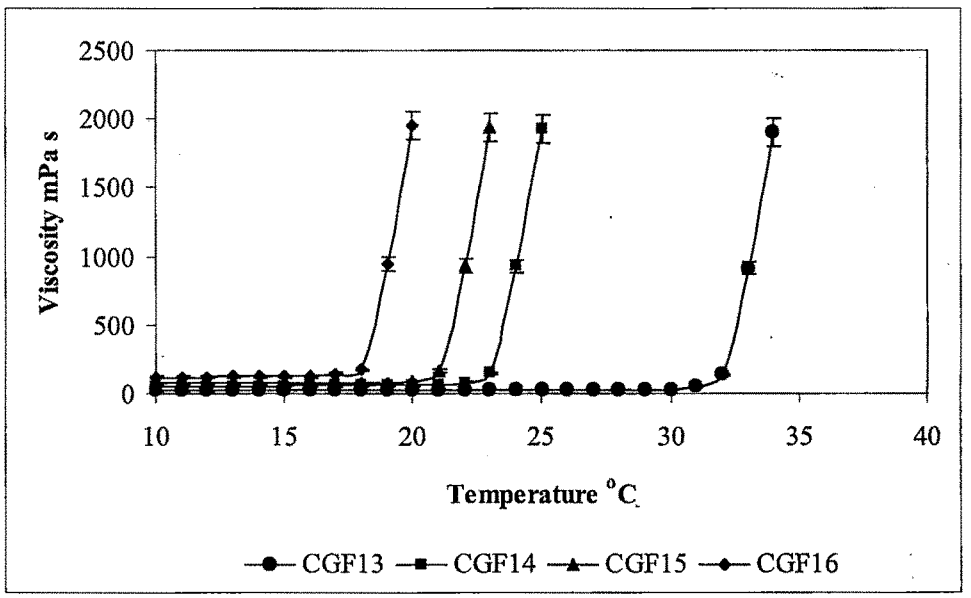
Values are expressed as mean ± SD (n =3)

Figure 4.27: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.4 % w/w polycarbophil and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.

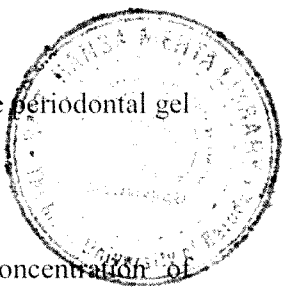


Values are expressed as mean ± SD (n =3)

Figure 4.28: Effect of temperature on the viscosity of various polycarbophil-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.5 % w/w polycarbophil and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



Values are expressed as mean ± SD (n =3)



4.5.4 Viscosity studies

Viscosity of plain pluronic F127 gel and gels containing different concentration of mucoadhesive polymer were measured using the Brookfield's LVDV III+ model. The gel sample (about 1 ml) at low temperature was placed in small sample adapter. The temperature of the sample was raised above 35°C using circulation bath (Neolab P125 model). The sample was allowed to cool and the viscosity at various temperatures was recorded using CP-52 spindle.

4.5.5 Measurement of gel strength

Gel strength of the formulations was measured using the Universal Testing Machine (UTM), as described by Lee (Lee et al. 1989) and Chung (Chung et al. 1972) with slight modification. Gel hardness was measured at room temperature using a 6 mm diameter probe, on the UTM (Model, LF Plus, Lloyd Instruments, U.K.). The compression test was done with a 150 N weight beam, utilized with a cross head and chart speed of 50 mm/min and 20 mm/min respectively, with a recovery period of 60s between the end of the first compression and start of the second compression. The test consisted of two compression cycles, and the results were used to calculate the texture profile parameter cohesiveness, as explained by Bourne (Bourne, 1978). Hardness was defined as the maximum load stress attained on gel compression, whereas cohesiveness was defined as the minimum load stress on probe withdrawal from the gel. These parameters were the measures of the resistance to penetration to withdrawal of the probe.

4.5.6 Determination of mucoadhesive force

Mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from the oral mucosal tissue using the modified method described by Jones et al. (Jones et al. 2000). A section of mucosal tissue, cut from the cheek mucous membrane of sheep, was washed thoroughly with distilled water followed by isotonic phosphate buffer pH 6.75 and kept in PBS pH 6.75 to remove all soluble components. After confirmation of the integrity of the mucosal tissue by microscopic investigation (Raykar et al.1998), it was secured with mucosal side out on to a glass plate using cyanoacrylate adhesives. The surface area of each exposed mucosal membrane was kept 2.0 cm^2. About

200 mg of gel formulations were added onto the mucosa and spread uniformly. The test was conducted at room temperature using a 6mm diameter probe, on the UTM (Model LF plus, Lloyd Instruments, U.K.). The probe was brought into contact with the mucosal surface. A preload of 20 g was placed over the gel surface for 2min as initial pressure, and then the probe was moved upward at a speed of 3.00 mm/min until the probe is detached from the mucosal membrane. Mucoadhesive force, the detachment stress, was determined from the minimal force required to detach the probe from the mucosal membrane. The mucosal membrane was changed for each measurement.

4.5.7 Syringeability

Syringeability of the formulations was measured using the Universal Testing Machine (UTM). Syringeability was measured at room temperature using a 6 mm diameter probe, on the UTM (Model, LF Plus, Lloyd Instruments, U.K.) by filling the sample in a syringe. Before filling the syringe, the opening of the syringe was sealed. Samples were filled in a 3ml glass syringe up to 2ml mark from the back of the syringe and stoppered by the help of forceps. To the rubber stopper the plunger of the syringe was attached. The sample syringe was placed in a holder for holding the syringe. The probe was attached to the load shell of the UTM. The compression test was done with a 150 N weight beam, utilized with a cross head and chart speed of 2.66 mm/min up to 40 mm. Force recorded was the mean of three readings.

4.5.8 In vitro release studies

The in vitro release study of gel formulations was performed by using sigma dialysis bag (MWCO 3500 and diameter 2.4 cm), which was filled with 500 mg of formulation. The bags were individually immersed in a beaker containing 25 ml of receiver phosphate buffer solution pH 6.75. The temperature was maintained at 37 ± 1°C and the receptor medium were constantly stirred at 100 rpm to maintain the sink condition. At appropriate time intervals, samples were withdrawn from the receiver solution and an equal volume of pre-warmed buffer was replaced and the samples were assayed spectroscopically after appropriate dilution to quantitate the amount of minocycline hydrochloride/ clindamycin phosphate release through the membrane.

4.5.9 In vitro permeation studies

Mucosal permeation studies of the prepared gels were carried out using a modified Franz Diffusion cell. Fresh sheep cheek mucosal membrane was fixed onto the Franz Diffusion cell. The 500 mg of gel was spread uniformly on to the mucosa previously fixed in between the donor and the receptor compartment of Franz Diffusion cell. The receptor compartment contained phosphate buffer, pH 6.75. The temperature of the elution medium was thermostatically controlled at 37±1°C by a surrounding water jacket and the medium was stirred with a bar magnet at 500 rpm, using a magnetic stirrer (Kakkar and Gupta, 1992). Aliquots withdrawn at predetermined intervals over 8 hr were spectroscopically estimated to quantitate the amount of minocycline hydrochloride/ clindamycin phosphate permeated through the membrane. The results were plotted, as cumulative amount released (Q) versus time (t).

4.5.10 Data analysis of permeation studies of drug loaded periodontal thermoreversible gel

The steady state permeation flux was determined from the slope of the linear portion of the cumulative amount permeated (Q) versus time (t) plot. The lag time (t_L) was determined by extrapolating the linear portion of Q versus t curve to the abscissa. The partition coefficient of minocycline hydrochloride/ clindamycin phosphate was calculated as described by equation (Saket et.al, 1984);

$$\text{Partition coefficient} = \frac{Cs - Ceg}{Ceg} \times \frac{1000}{We}$$

Where, Cs, Ceg and We are the initial concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer solution ($mg.ml^{-1}$), equilibrium concentration ($mg.ml^{-1}$) and weight (mg) of mucous membrane respectively. The dry weight of the mucous membrane was considered for calculating the partition coefficient.

The permeability coefficient (P) was calculated using the relation derived from fick's first law of diffusion (Aslani and Kennedy, 1996);

$$P = \frac{J.h}{C}$$

Where J is the steady state permeation flux, c is the initial concentration; h is the thickness of the mucous membrane.

Diffusion coefficient was calculated using the relation derived from fick's second law of diffusion (Pefile et.al,, 1998);

$$D = \frac{h^2}{6L}$$

Where h is the thickness of the mucous membrane and L is the lag time.

4.5.11 Stability studies

The optimized gel formulation samples were stored in well sealed glass vials for a period of 180 days at room temperature and at 4°C. After storage, samples were evaluated for their physical appearance and gelation temperature (visual method). Determination of Minocycline hydrochloride/ clindamycin phosphate content of the samples was done by the method described earlier.

4.6 CHARACTERIZATION OF MIXED MUCOADHESIVE PERIODONTAL GELS OF POLYCARBOPHIL- PLURONIC F127

Thermoreversible polymer based liquid formulations that provide in situ gelling property in periodontal cavity were designed to delay the clearance of the formulations from the periodontal cavity and enhance the retention and thereby increase the absorption of drug from the periodontal cavity. Usually, the gelation temperatures have been considered to be suitable if in the range of 25-37°C. If the gelation temperature of a thermoreversible formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling and administration. If the gelation temperature is higher than 37°C, liquid dosage form still exist at the body temperature, resulting in the oral clearance of the administered drug at an early stage. As the temperature of the periodontal cavity is 37°C, our study is aimed at preparing the thermoreversible liquid formulations of pluronic F127 that may convert into gel below or nearly equal to 37°C and possess suitable rheological and mucoadhesive potential. The pH of all the samples was made within 5.5-6.5, which is suitable for periodontal administration.

4.6.1 Viscosity and gelling temperature determination

Pluronic F127 formulations containing minocycline hydrochloride/ clindamycin phosphate studied, showed to be existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37°C), and return to the liquid state upon cooling below gelation temperature. For preparation of in situ gel that gels upon instillation into the periodontal cavity at body temperature, the sol gel transition temperature has to be lower than 37°C. The gelation temperature of pluronic F127 vehicle is reported to result from the changes in micelle number with temperature. With increasing temperature, number of micelles formed are increased which is consequence of the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel formed (Kabanov et al., 2002). Recently Cabana et al., (Cabana et al., 1997) suggested a mechanism of gelation based on micelles packing and entanglements. Also conformational changes in the orientation of methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon (Rassing et al, 1983). At 4°C all the formulations were at liquid state with viscosity ranging from 10.89 mPas to 29.65 mPas for 19 % w/w to 30 % w/w pluronic F127 with 0.2%w/w, 0.3 % w/w, 0.4 % w/w and 0.5 % w/w polycarbophil. Rheological behavior of all the formulations was measured. All the formulations exhibit Newtonian behavior at 4°C, all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. The formulations prepared with 19% w/w pluronic F127 remained fluid and showed a constant viscosity between 7.92 mPas to 16.24 mPas. When Newtonian viscosity values were investigated for formulations prepared using 19% w/w pluronic F127 resulted that the temperature does not dramatically affect the viscosity. Results are shown in Table No4.36- 4.43. However, formulations prepared using 19%w/w pluronic F127 become gel and showed a shear-thinning (pseudoplastic) behavior. The reason for this is that the formulation has a sol-gel transition temperature below 37°C. They formed semisolid gels at 37°C, which is due to the entanglement of molecular chains. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water

molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates the polymer chains approach closer and squeeze ordered water molecule.

The gelation temperature of the formulations prepared using pluronic F127 above 19% w/w was much below 37°C. The cup of the viscosity of each of the gels measured at a shear rate of $10s^{-1}$ as a function of temperature (°C) was composed of three phases. In the first part the viscosity was nearly constant, in the second part it increases dramatically and reached its maximum; in third part it again remained constant at plateau. Figure 4.21 to 4.28 represents the viscosity of each gel formulations measured at a shear rate of $10s^{-1}$, as a function of the temperature in degree centigrade. The gelling temperature determined graphically as the inflection point of the second part of the curve, decreases as the polymer concentration increases. The result of gelation temperature observed by visual inspection and rheological study did not vary more than ± 1.5°C.

It is evident from the data that the presence of mucoadhesive polymer polycarbophil, lowered the gelation temperature. It is also noted that adhesion of increasing concentration of polycarbophil from 0.2-0.5% w/w further lower the gelation temperature. The gelation temperature lowering effect of mucoadhesive polymer might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the polycarbophil is exposed to water the polymer begins to uncoil and generating an increase in viscosity and gel formation. The uncoiling and expansion of the molecule result in polymer swelling and elastic gel formation. The formulation containing higher concentration of polycarbophil i.e. 0.5% w/w was found to be of higher viscosity and hence was difficult to administer into the periodontal cavity. All the formulations prepared above 19% w/w with pluronic F127 gelled at temperature range from 32°C to 12.5°C. However formulations prepared with 25% concentration of the pluronic F127 and polycarbophil shows the gelling temperature below room temperature (25°C), hence are not suitable for administration into the periodontal cavity. Formulations showing the gelling temperature above 25°C seem to be proper for in

situ gelling of the formulations at periodontal cavity and minimizing the loss of administered drug due to clearance from the site of application.

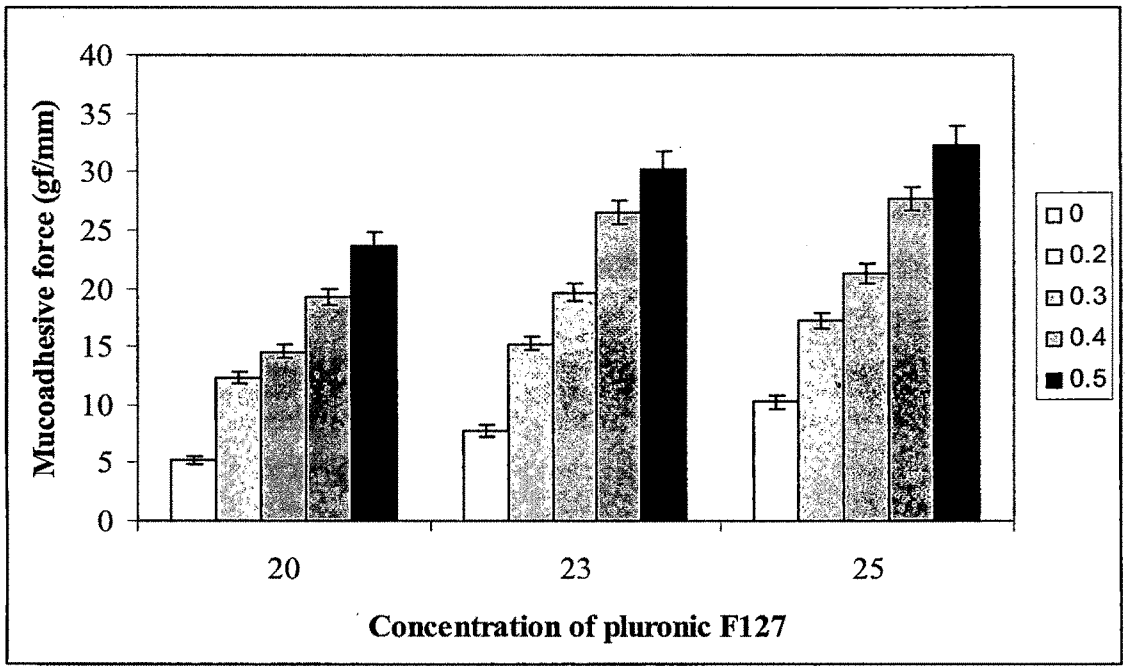
4.6.2 Gel strength

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of polycarbophil (table 4.36-4.43). All the formulations prepared using varying concentrations of pluronic F127 along with 0.2% w/w, 0.3% w/w, 0.4% w/w and 0.5% w/w polycarbophil concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. There is no significant difference in the formulations containing minocycline hydrochloride and clindamycin phosphate. Increase in the gel strength in presence of different concentration of polycarbophil may be due to bond formation between pluronic F127 and polycarbophil. Higher gel strength formulations possess the higher mucoadhesive property and increases the residence time at the application site thereby increasing the bioavailability of the drug.

4.6.3 Mucoadhesive strength

The assessment of the mucoadhesive strength showed that the pluronic F127 preparations possess adhesive properties that increase significantly ($P< 0.001$) with addition of polycarbophil (Figure 4.29) and their difference is non significant ($P>0.001$). From the results it was evidenced that the availability of the carboxyl groups determines the mucoadhesion. Thus polycarbophil having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. There is evidence that the higher mucoadhesive strength delivery systems possess prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.29: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of polycarbophil- pluronic F127



n=3

4.6.4 Syringeability

The assessment of the syringeability may be performed in terms of force required to syringe the formulation to the application site. Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.2% w/w, 0.3% w/w, 0.4% w/w and 0.5% w/w polycarbophil concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. The results of the syringeability are shown in Table No4.44.

Table No: 4.44: Determination of syringeability of various minocycline hydrochloride/ clindamycin phosphate loaded mixed mucoadhesive periodontal gels of polycarbophil-pluronic F127

Formulation Code	Syringeability (gf)
MGF02	85.41
MGF03	101.52
MGF04	122.81
CGF02	84.32
CGF03	100.32
CGF04	123.54

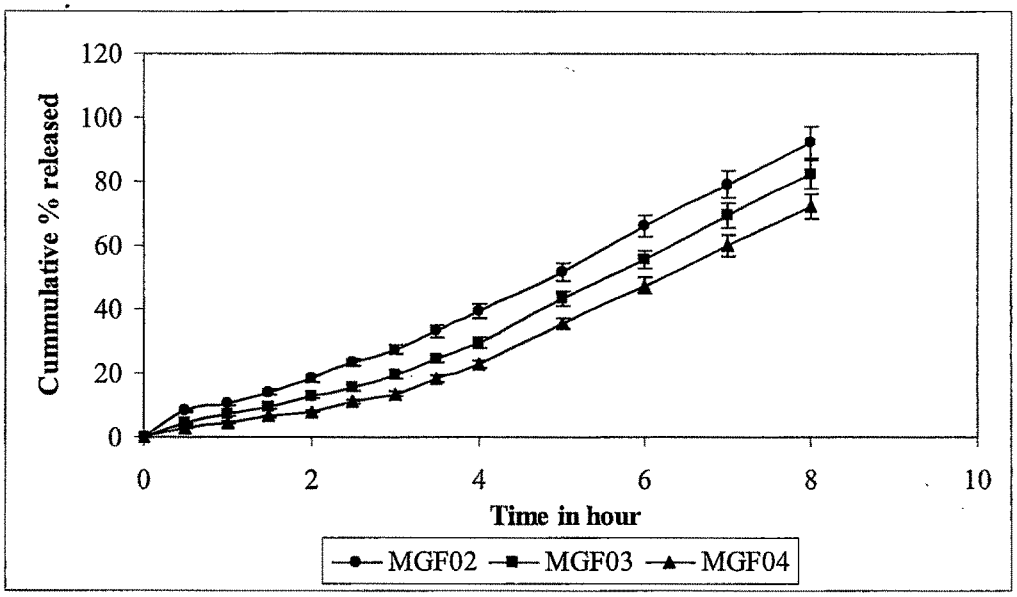
4.6.5 In vitro release study

The in vitro release profile of minocycline hydrochloride/ clindamycin phosphate is illustrated in Figure 4.30 and 4.31 respectively. The maximum release of minocycline hydrochloride from the thermoreversible gels was shown by the formulation MGF02 where as the least was shown by the formulation MGF04 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF02 where as the least was shown by the formulation CGF04 after 8 hours. The higher release of minocycline hydrochloride/ clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation MGF02 and CGF02 had low viscosity than MGF04 and CGF04. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF02 and CGF02. In addition, formulation MGF02 and CGF02 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF04 and CGF04 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No: 4.45: In vitro release profile of minocycline hydrochloride from polycarbophil - pluronic F127 thermoreversible periodontal gel

Time in Hour	% Minocycline hydrochloride released ± SD		
	MGF02	MGF03	MGF04
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	8.08 ± 0.042	4.22 ± 0.005	3.05 ± 0.022
1.00	10.58 ± 0.001	7.24 ± 0.011	4.51 ± 0.028
1.50	14.01 ± 0.057	9.37 ± 0.023	6.53 ± 0.043
2.00	18.22 ± 0.050	12.85 ± 0.030	7.98 ± 0.044
2.50	23.26 ± 0.028	15.46 ± 0.049	11.10 ± 0.071
3.00	27.46 ± 0.058	19.49 ± 0.058	13.52 ± 0.026
3.50	33.15 ± 0.057	24.49 ± 0.060	18.25 ± 0.043
4.00	39.29 ± 0.017	29.34 ± 0.034	22.59 ± 0.077
5.00	51.56 ± 0.019	43.22 ± 0.021	35.54 ± 0.029
6.00	66.15 ± 0.032	55.60 ± 0.055	47.47 ± 0.030
7.00	79.08 ± 0.025	69.47 ± 0.005	60.04 ± 0.040
8.00	92.34 ± 0.011	82.35 ± 0.067	72.20 ± 0.031

Figure 4.30: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from polycarbophil - pluronic F127 thermoreversible periodontal gel

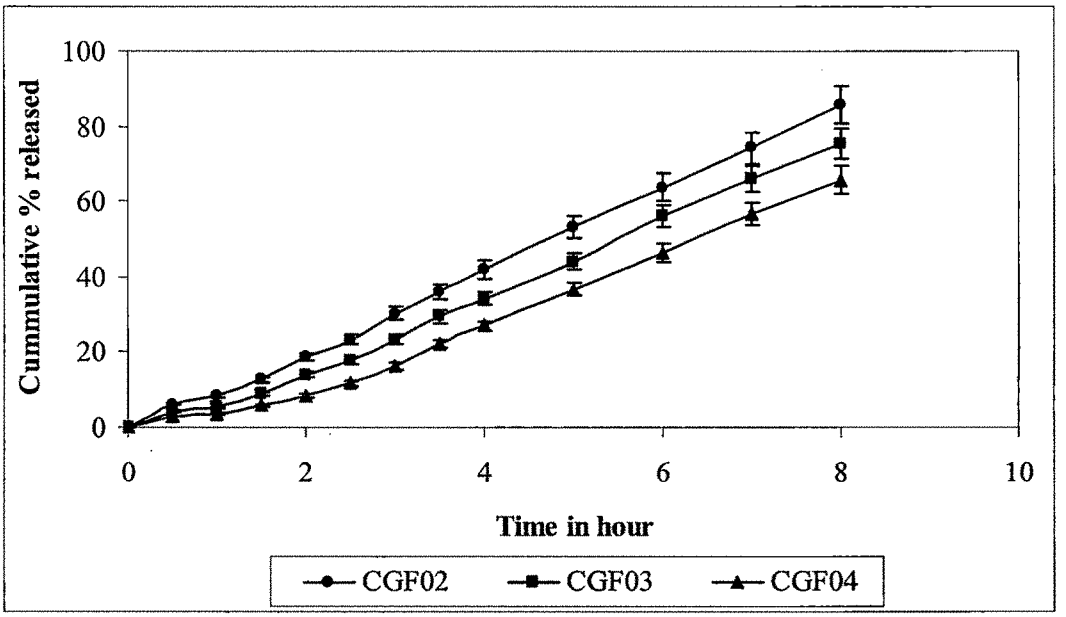


n =3

Table No: 4.46: In vitro release profile of clindamycin phosphate from polycarbophil - pluronic F127 thermoreversible periodontal gel

Time in Hour	% Clindamycin phosphate released ± SD		
	CGF02	CGF03	CGF04
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	6.09 ± 0.023	4.11 ± 0.056	2.88 ± 0.022
1.00	8.58 ± 0.024	5.29 ± 0.014	3.64 ± 0.016
1.50	12.76 ± 0.032	8.70 ± 0.043	5.71 ± 0.034
2.00	18.69 ± 0.026	13.84 ± 0.066	8.49 ± 0.064
2.50	23.24 ± 0.028	17.57 ± 0.029	11.69 ± 0.088
3.00	30.19 ± 0.029	23.36 ± 0.042	16.29 ± 0.057
3.50	36.10 ± 0.014	29.34 ± 0.030	22.13 ± 0.051
4.00	41.96 ± 0.032	34.09 ± 0.043	26.89 ± 0.038
5.00	53.05 ± 0.033	44.08 ± 0.025	36.70 ± 0.036
6.00	63.74 ± 0.021	55.95 ± 0.051	46.47 ± 0.024
7.00	74.27 ± 0.024	66.03 ± 0.084	56.48 ± 0.025
8.00	85.64 ± 0.055	75.41 ± 0.065	65.76 ± 0.010

Figure 4.31: Cumulative percentage release profile of Clindamycin phosphate in mcg/cm^2 from polycarbophil - pluronic F127 thermoreversible periodontal gel



n=3

Table no. 4.47: Release kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded polycarbophil - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				N (Release exponent)	K (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF02	0.9882	0.6972	0.8632	0.5195	11.372	2.355
MGF03	0.9712	0.6555	0.8170	0.1172	10.270	1.862
MGF04	0.9527	0.5651	0.7787	0.5100	9.069	1.172
CGF02	0.9978	0.7456	0.8967	0.5708	10.858	7.211
CGF03	0.9927	0.6706	0.8687	0.5703	9.781	6.039
CGF04	0.9800	0.5447	0.8299	0.5521	8.564	3.664

4.6.6 In Vitro permeation study

4.6.6.1 Determination of saturated drug concentration

A saturated minocycline hydrochloride/ clindamycin phosphate solution in phosphate buffer pH 6.75 was prepared separately by equilibrating the excess minocycline hydrochloride/ clindamycin phosphate with the vehicle for 2hours. The temperature of the solution was maintained at 25°C using a circulating water bath. The sample was filtered and appropriately diluted for estimation of saturation solubility of minocycline hydrochloride/ clindamycin phosphate. The saturated concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was found to be 106.994 mg ml^{-1} and 103.900 mg ml^{-1} respectively.

4.6.6.2 Preparation of mucosal tissue

The animal was sacrificed in the slaughter house and the sheep cheek pouch was excised. It was washed thoroughly with distilled water. The mucosal membrane so separated was cut into pieces of 3×3 cm. A piece of the mucosal membrane was washed with isotonic phosphate buffer pH 6.75 and kept in the phosphate buffer pH 6.75 in order to remove any soluble components. The integrity of the mucosal surface was tested microscopically (Raykar et.al, 1998) before to confirm the absence of any significant change.

4.6.6.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane was measured microscopically after staining with hematoxylin eosin. A wax block of skin was prepared by using steel molds. Molten wax was poured into the steel mold from a paraffin dispenser heated within 68 to 70°C. The skin was pushed down to the bottom of the mold so that it was positioned on the cutting surface. After cooling, the block was removed by slightly reheating the mold. The wax block was held on the holder of the microtome. The sections were cut 5 to 7μm thickness with a dispersible microtome blade. The section was transferred to a glass slide and affixed. The glass slide was put into a caplin jar containing hematoxylin eosin for 10 minutes, then rinsed with isopropyl alcohol, and kept under running water for 5 to 7 minutes. The glass slide was dipped 30 times in a caplin jar containing 1% eosin and then rinsed with isopropyl alcohol. The glass slide was then allowed to dry completely and kept in a caplin jar containing xylene for 7 to 10 minutes. Finally the glass slide was air dried and observed under a microscope and the thickness was measured using a micrometer. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ μm, which is the mean of 3 measurements.

Polycarbophil are reported to demonstrate permeation enhancing property as shown to express a high Ca^{++} binding ability. Effective permeability coefficient determined for minocycline hydrochloride/ clindamycin phosphate in each gel formulations are given in Table No4.50 and the cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various polycarbophil pluronic F127 gel formulations are given in the Figure 4.32 and 4.33. It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for polycarbophil pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug release may be due to diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the polycarbophil ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of polycarbophil results in very rapid dissolution of the drug due to swelling and

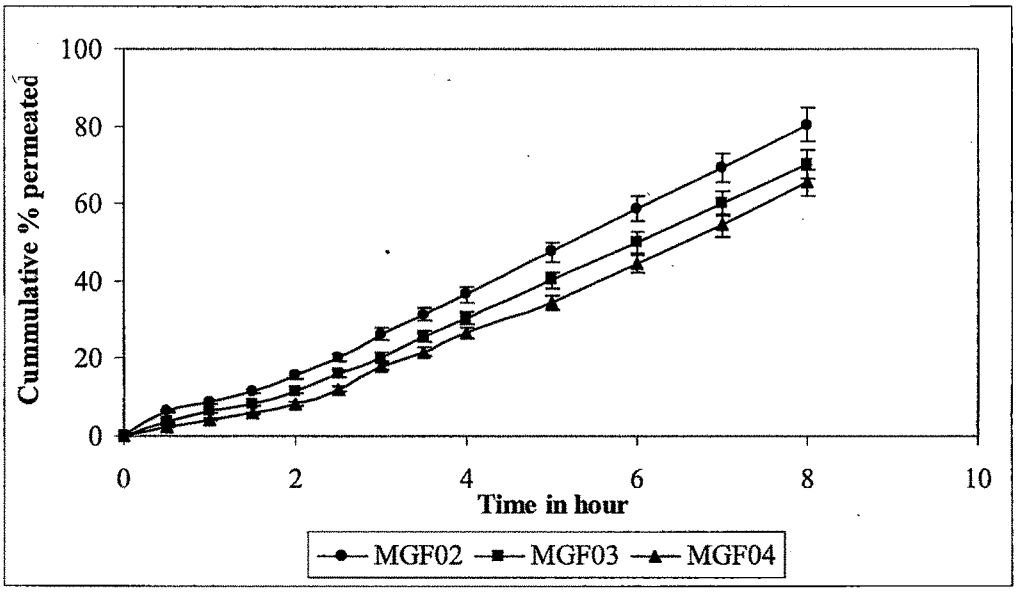
dissolution of polycarbophil at pH 6.75. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25%w/w pluronic F127 drug release is less compared to 20%w/w and 23%w/w pluronic F127 containing formulations, which may be due to the formation of larger concentrations of the micelles. Addition of the polycarbophil increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in concentrations of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network (Chen et al, 1997). At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized polycarbophil (Chen et al, 1997). Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of polycarbophil not only increase in the Ca^{++} binding site but also increase the inter accessibility of Ca^{++} binding sites due to relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability, thermoreversible periodontal gel formulations containing 0.2% polycarbophil along with 20% and 23 % w/w pluronic F127 were found to be the best. However formulations containing 0.2% w/w polycarbophil along with 25% pluronic F127 showed lower gelling temperature, low permeation profile and high syringeability which may make it difficult to administer the drug to the periodontal cavity. Formulations containing the higher concentrations of polycarbophil (0.3, 0.4 and 0.5% w/w) showed a high syringeability and blockage of the syringe which may be due to high viscous solution. Hence MGF02, CGF02, MGF03 and CGF03 were selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No: 4.48: In vitro permeation profile of minocycline hydrochloride from polycarbophil - pluronic F127 thermoreversible periodontal gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF 02	MGF 03	MGF 04
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	6.23 ± 0.026	3.55 ± 0.114	2.44 ± 0.039
1.00	8.49 ± 0.037	6.38 ± 0.033	4.32 ± 0.009
1.50	11.51 ± 0.025	8.30 ± 0.042	5.83 ± 0.020
2.00	15.63 ± 0.038	11.63 ± 0.048	8.06 ± 0.018
2.50	20.24 ± 0.059	16.09 ± 0.033	12.05 ± 0.041
3.00	26.34 ± 0.040	20.38 ± 0.023	18.03 ± 0.031
3.50	31.41 ± 0.049	25.67 ± 0.058	21.62 ± 0.036
4.00	36.57 ± 0.064	30.38 ± 0.011	26.44 ± 0.046
5.00	47.50 ± 0.043	40.21 ± 0.036	34.55 ± 0.055
6.00	58.68 ± 0.032	50.07 ± 0.044	44.55 ± 0.029
7.00	69.10 ± 0.017	60.20 ± 0.006	54.43 ± 0.064
8.00	80.33 ± 0.071	70.07 ± 0.058	65.44 ± 0.040

Figure 4.32: Cumulative permeation profile of minocycline hydrochloride from polycarbophil - pluronic F127 thermoreversible periodontal gel

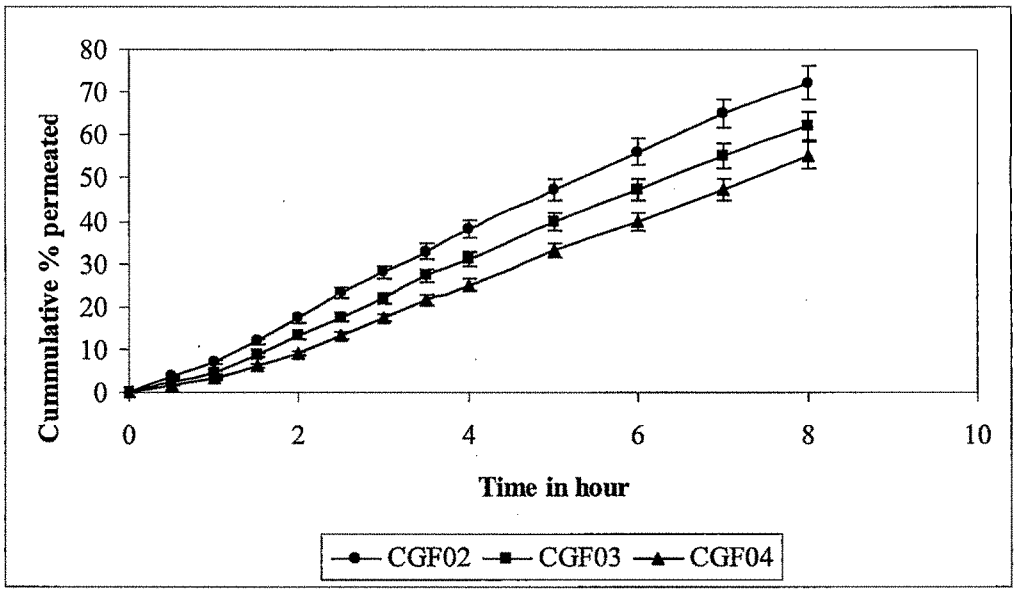


n=3

Table No: 4.49: In vitro permeation profile of clindamycin phosphate from polycarbophil - pluronic f127 thermoreversible periodontal gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF 02	CGF 03	CGF 04
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	3.53 ± 0.019	2.56 ± 0.109	1.60 ± 0.027
1.00	7.19 ± 0.032	4.59 ± 0.028	3.27 ± 0.007
1.50	11.96 ± 0.021	8.62 ± 0.036	6.28 ± 0.012
2.00	17.23 ± 0.029	13.12 ± 0.041	9.09 ± 0.011
2.50	23.33 ± 0.046	17.46 ± 0.028	13.28 ± 0.037
3.00	28.05 ± 0.031	21.96 ± 0.017	17.31 ± 0.024
3.50	32.92 ± 0.041	27.26 ± 0.049	21.47 ± 0.031
4.00	38.13 ± 0.058	31.03 ± 0.005	25.08 ± 0.039
5.00	47.21 ± 0.039	39.64 ± 0.029	33.22 ± 0.046
6.00	56.10 ± 0.026	47.13 ± 0.036	39.94 ± 0.018
7.00	65.12 ± 0.011	55.03 ± 0.010	47.10 ± 0.057
8.00	72.29 ± 0.063	62.08 ± 0.052	55.32 ± 0.038

Figure 4.33: Cumulative permeation profile of clindamycin phosphate from polycarbophil - pluronic F127 thermoreversible periodontal gel



n=3

Table no. 4.50: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gels

Formulations	Permeation flux $J(mcg.cm^{-2}.hr^{-1})$	Lag time ($t_L$hr)	Diffusion coefficient $(D\times10^{-8}cm^2.sec^{-1})$	Permeability coefficient $(P\times10^{-8}cm.sec^{-1})$
MGF02	10.50	0.45	2.376	2.726
MGF03	9.40	1.25	0.855	2.440
MGF04	8.94	1.50	0.713	2.321
CGF02	9.41	0.50	2.130	2.515
CGF03	8.83	0.75	1.430	2.360
CGF04	7.52	1.00	1.070	2.010

4.6.7 Stability Study

The minocycline hydrochloride/ clindamycin phosphate loaded periodontal thermoreversible gels prepared using 20%w/w and 23%w/w pluronic F127 along with 0.2 % w/w polycarbophil were studied for the stability of the formulation at Freeze condition (4°C) and at RT. All the four formulations showed good physical stability, as there was no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The results of the stability study of the optimized periodontal formulations at 4°C and at room temperature are given in table no. 4.51 and 4.52 respectively. The gel stability results were found to be similar to the published data (Katakam et al, 1997).

Table no.4.51: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF02	99.25 ± 0.95	5.89	32.4
MGF03	99.05 ± 0.69	5.93	30.5
CGF02	98.52 ± 0.75	6.01	32.1
CGF03	100.25 ± 0.77	5.94	30.2

Table no.4.52: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF02	101.22 ± 0.98	5.93	32.7
MGF03	99.26 ± 0.87	5.98	30.4
CGF02	98.75 ± 0.96	6.01	32.3
CGF03	99.25 ± 0.81	6.03	29.9

4.6.8 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration were prepared using different concentrations of pluronic F127 along with mucoadhesive polymer polycarbophil by incorporating the antibiotics minocycline hydrochloride/ clindamycin phosphate. Periodontal gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. At 4°C all the formulations were at liquid state with viscosity ranging from 10.89 mPas to 29.65 mPas for 19%w/w to 25 %w/w pluronic F127 with 0.2-0.5 w/w % polycarbophil. Rheological behavior of all the formulations was measured. All the formulations exhibited Newtonian behavior at 4°C; all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer polycarbophil lowered the gelation temperature. It is also noted that addition of increasing concentration of polycarbophil from 0.2-0.5% w/w further lowered the gelation temperature, which might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the polycarbophil is exposed to water the polymer begins to uncoil generating an increase in viscosity and gel formation. The uncoiling and expansion of the molecule result in polymer swelling and elastic gel formation.

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness property that increase with addition of polycarbophil. Increase in the gel strength in presence of different concentration of

polycarbophil may be due to bond formation between pluronic F127 and polycarbophil. Increase in gel strength shows that the addition of polycarbophil increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property and increases the residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of polycarbophil. From the study it was evidenced that the availability of the carboxyl groups determines the mucoadhesion. Presence of mucoadhesive polymer polycarbophil having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.2% w/w polycarbophil concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation study showed a sustain release of the drug for a period of 8 hours compared to plain drugs. A preliminary study shows that the formulation prepared with 20%w/w pluronic F127 along with 0.2% polycarbophil (MGF02 and CGF02) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.2% polycarbophil (MGF03 and CGF03). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF02 and CGF02. In addition, formulation MGF02 and CGF02 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF03 and CGF03 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for polycarbophil pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the

pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the polycarbophil ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of polycarbophil results in very rapid dissolution of the drug due to swelling and dissolution of polycarbophil. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 23% w/w and 20% w/w pluronic F127 containing formulations which may be due to the formation of larger concentrations of the micelles. Addition of the polycarbophil increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in concentrations of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized polycarbophil. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of polycarbophil not only increase in the Ca^{++} binding site but also increase the inter accessibility of Ca++ binding sites due to relaxation of polymer network.

The investigation of in vitro release and permeation data showed that the release mechanism of drug followed zero order release model. Hence, this can be concluded that the main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed desired stability during the storage period of 6 months, and their chemical and mechanical property does not change significantly.

4.7 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND HYDROXY PROPYL METHYL CELLULOSE

A combination of poly-oxy ethylene-poly-oxy-propylene block copolymers (pluronic F127) as thermoreversible polymer and cationic polymer hydroxy propyl methyl cellulose as mucoadhesive polymer and absorption enhancing material has never been tried as the potential drug delivery system. The research work is aimed at development of a delivery system containing effective amount of minocycline hydrochloride/ clindamycin phosphate, thermoreversible polymer pluronic F127 along with mucoadhesive polymer HPMC which has the property of increasing the residence time in enhanced delivery to periodontal cavity. Effect of concentration of HPMC on viscosity, gelling temperature, mucoadhesive potential and in vitro release and ex vivo permeation was also studied. The pH of all the formulations was maintained within 5.5 - 6.5, which is suitable for periodontal administration.

4.7.1 Preparation of mixed HPMC - pluronic F127 periodontal gels

Formulations containing the minocycline hydrochloride (1%)/ clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with HPMC. The compositions of the formulations are cited in Table No4.53 to 4.58.

Table No 4.53: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HPMC (0.25%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF17	MGF18	MGF19	MGF20
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.25	0.25	0.25	0.25
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temperature(°C)	42	35	31	27
Visual gel Temperature(°C)	42.8	36.1	32.2	28.5
Drug content	98.37 ± 0.25	99.31 ± 0.19	99.15 ± 0.48	97.67 ± 0.21
Mucoadhesion (gf/mm)	18.92 ± 1.34	22.39 ± 2.14	26.48 ± 1.93	29.76 ± 1.42
Gel strength (N/m)	10247.31 ± 134.86	17343.24 ± 119.86	19783.39 ± 121.67	21829.74 ± 125.66
pH (Sol)	5.87	5.96	6.10	6.21
pH (Gel)	5.91	6.01	6.11	6.23
Sol Viscosity mPas	17.23	24.16	26.91	28.37
Gel Viscosity mPas	2109	3098	3327	3508

Table No 4.54: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HPMC (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF21	MGF22	MGF23	MGF24
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	38	30	25	22
Visual gel Temp. (°C)	39.5	31.4	25.9	22.9
Drug content	99.3 ± 0.28	98.68 ± 0.34	99.72 ± 0.31	99.15 ± 0.62
Mucoadhesion (gf/mm)	19.34 ± 0.49	23.39 ± 1.11	27.09 ± 1.13	30.19 ± 1.92
Gel strength (N/m)	10983.23 ± 129.78	18102.63 ± 121.29	20389.65 ± 102.32	21876.43 ± 98.67
pH (Sol)	5.89	5.99	6.10	6.15
pH (Gel)	5.91	6.02	6.12	6.17
Sol Viscosity mPas	18.05	24.53	27.13	29.14
Gel Viscosity mPas	2172	3124	3412	3545

Table No 4.55: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HPMC (0.75%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF25	MGF26	MGF27	MGF28
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.75	0.75	0.75	0.75
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	30	24	22	18
Visual gel Temp. (°C)	31.1	25.1	23.1	19.2
Drug content	97.28 ± 0.48	98.7 ± 0.38	99.38 ± 0.47	07.39 ± 0.59
Mucoadhesion (gf/mm)	19.91 ± 1.34	24.13 ± 0.48	27.87 ± 1.21	30.94 ± 1.03
Gel strength (N/m)	11102.79 ± 173.26	18309.43 ± 105.27	20786.78 ± 119.37	22345.38 ± 139.20
pH (Sol)	5.95	6.11	6.15	6.19
pH (Gel)	5.98	6.10	6.13	6.18
Sol Viscosity mPas	18.36	24.97	27.69	29.76
Gel Viscosity mPas	2209	3176	3459	3602

Table No 4.56: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HPMC (0.25%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF17	CGF18	CGF19	CGF20
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.25	0.25	0.25	0.25
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	41	33	30	26
Visual gel Temp. (°C)	41.5	34.2	31.2	27.3
Drug content	99.25 ± 0.25	97.82 ± 0.28	98.64 ± 0.39	99.29 ± 0.29
Mucoadhesion (gf/mm)	18.76 ± 0.91	22.42 ± 1.36	26.73 ± 1.19	30.04 ± 0.79
Gel strength (N/m)	10380.19 ± 184.32	16879.37 ± 120.39	20209. 48 ± 148.79	21394.65 ± 181.25
pH (Sol)	5.75	5.87	5.89	5.92
pH (Gel)	5.77	5.92	5.93	5.98
Sol Viscosity mPas	17.63	24.59	27.34	29.21
Gel Viscosity mPas	2135	3049	3341	3519

Table No 4.57: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HPMC (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF21	CGF22	CGF23	CGF24
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.50	0.25	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	37	29	24	21
Visual gel Temp. (°C)	38.2	30.1	25.1	21.9
Drug content	97.85 ± 0.39	99.23 ± 0.81	98.79 ± 0.27	98.68 ± 0.26
Mucoadhesion (gf/mm)	19.92 ± 0.81	24.14 ± 0.68	27.36 ± 0.94	31.07 ± 0.83
Gel strength (N/m)	11137.84 ± 119.27	18739.28 ± 111.39	21008.67 ± 143.74	21943.26 ± 109.38
pH (Sol)	5.82	5.97	6.04	6.07
pH (Gel)	5.87	6.01	6.07	6.09
Sol Viscosity mPas	18.76	24.69	27.78	29.46
Gel Viscosity mPas	2204	3169	3449	3587

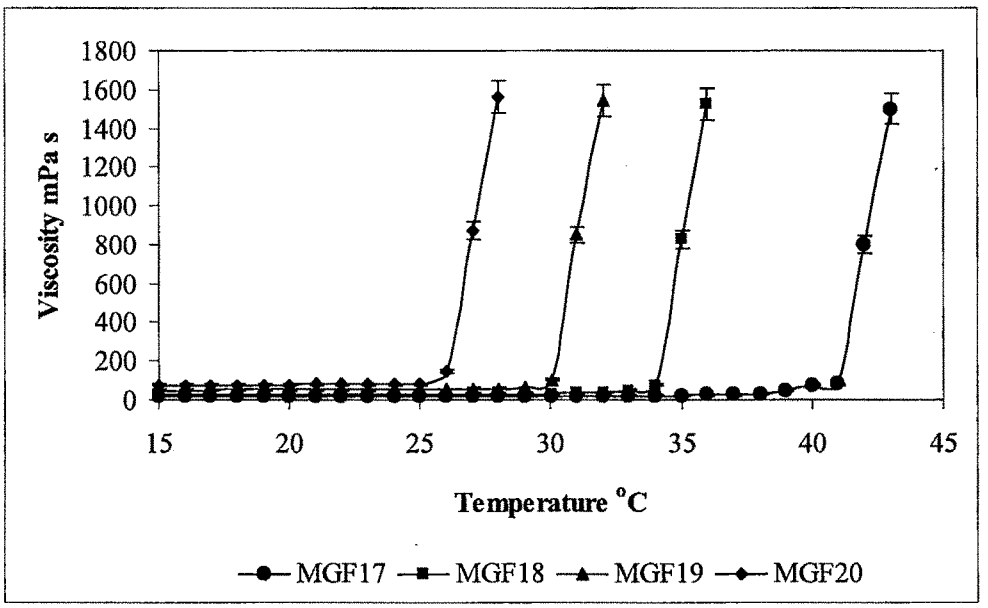
Table No 4.58: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HPMC (0.75%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF25	CGF26	CGF27	CGF28
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HPMC (%w/w)	0.75	0.75	0.75	0.75
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	29	23	21	17
Visual gel Temp. (°C)	30.2	24.2	21.8	18.1
Drug content	99.26 ± 0.39	99.1 ± 0.28	97.78 ± 0.47	98.2 ± 0.67
Mucoadhesion (gf/mm)	20.17 ± 0.97	24.95 ± 1.23	28.14 ± 1.09	31.27 ± 1.89
Gel strength (N/m)	12973.49 ± 119.83	19211.87 ± 185.06	21038.47 ± 104.72	22978.35 ± 185.06
pH (Sol)	5.91	5.94	5.97	6.05
pH (Gel)	5.93	5.96	5.95	6.03
Sol Viscosity mPas	19.1	25.42	28.14	30.52
Gel Viscosity mPas	2279	3189	3523	3671

4.7.2 Viscosity and gelling temperature determination

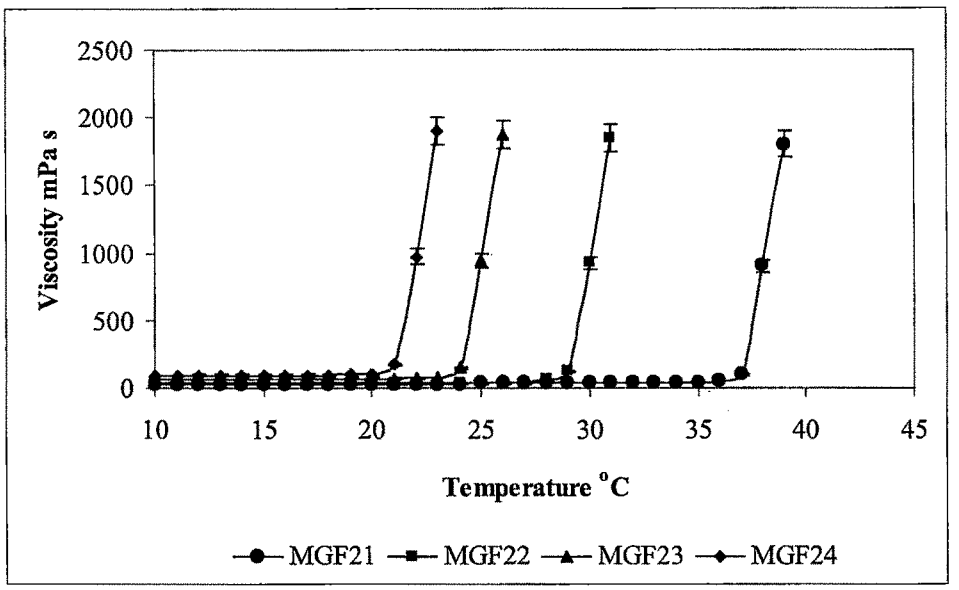
As evident from the results in table 4.53 to 4.58 the gelling temperature of pluronic F127 vehicle as determined by rheological method were lowered by the addition of increasing concentration of the mucoadhesive polymer HPMC i.e. 0.25 % w/w to 0.75% w/w. Figure 4.34 to 4.39 shows the viscosity of various pluronic gels with varying concentration of HPMC measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method did not vary more than ± 1.5°C. The decrease in the gelation temperature with increase in HPMC concentration may be due to the enhanced viscosity of the gel formulation. The formulations showing the gelling temperature between 25°C to 37°C seems to be suitable for in situ gelling of the various vehicles at the periodontal cavity, minimizing the loss of administered drug due to clearance from the site of application.

Figure 4.34: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.25 % w/w HPMC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



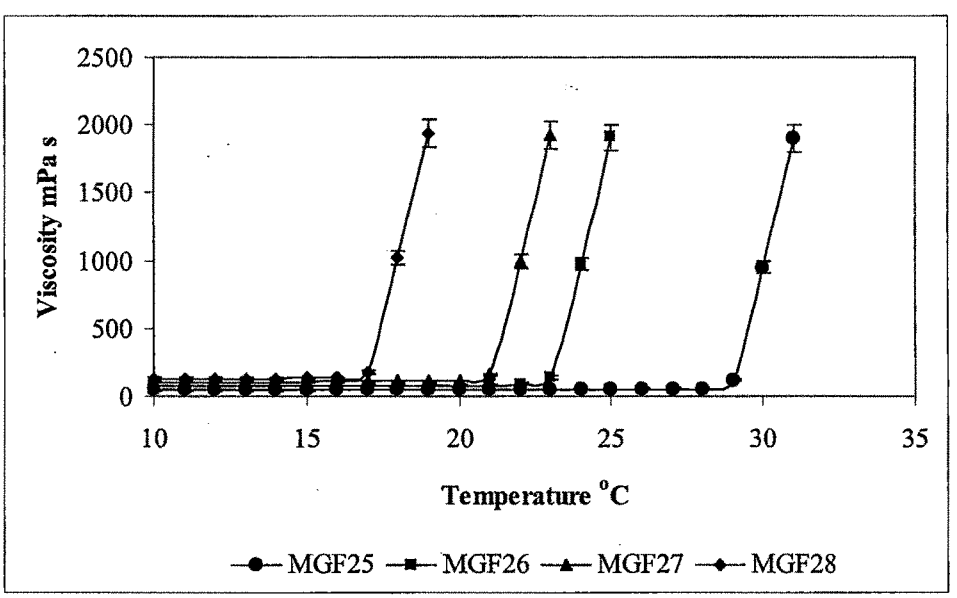
Values are expressed as mean ± SD (n =3)

Figure 4.35: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w HPMC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



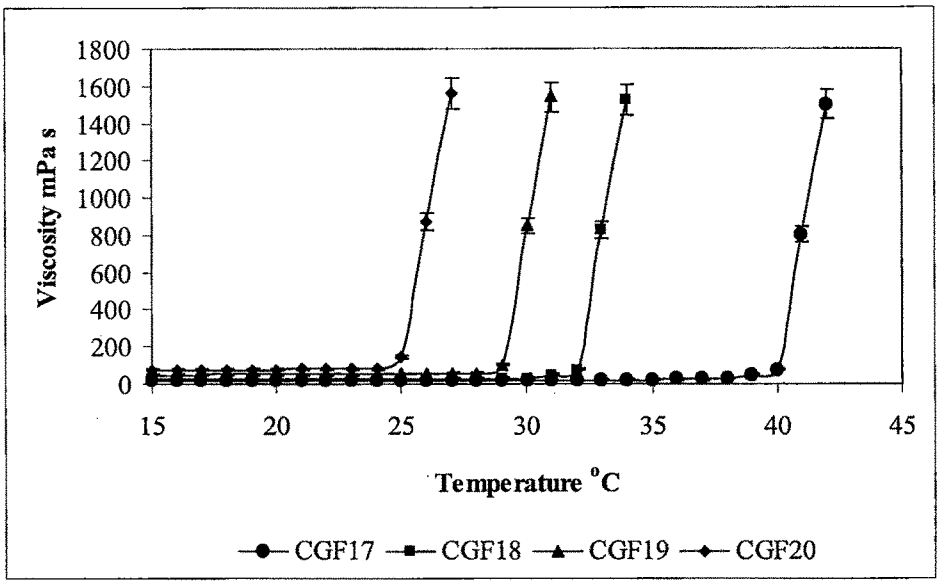
Values are expressed as mean ± SD (n =3)

Figure 4.36: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.75 % w/w HPMC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



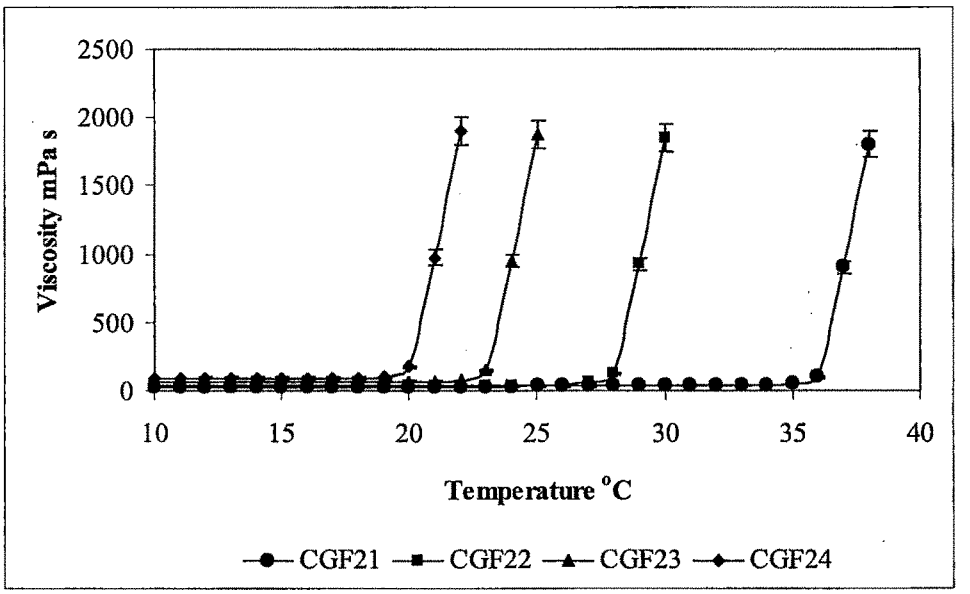
Values are expressed as mean ± SD (n =3)

Figure 4.37: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.25 % w/w HPMC and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



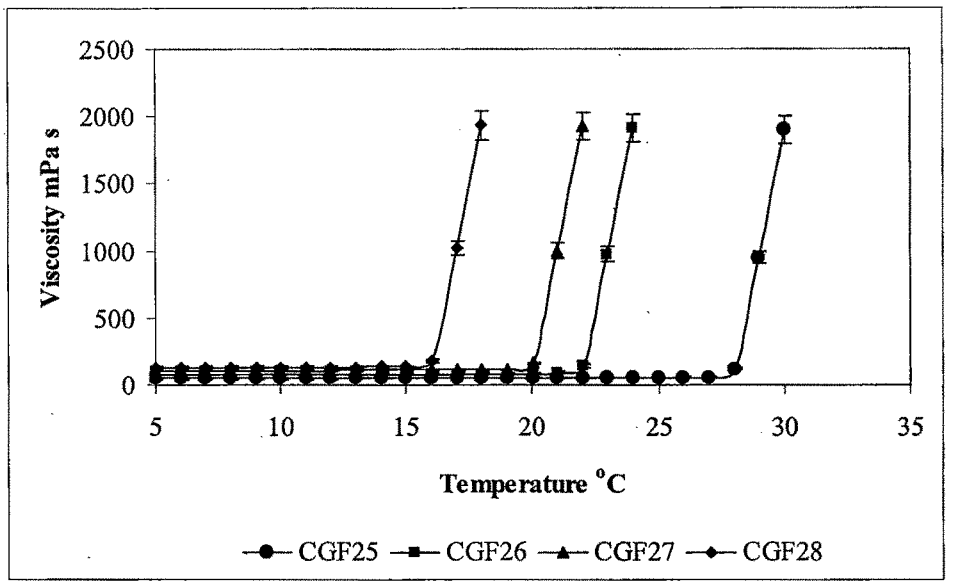
Values are expressed as mean ± SD (n =3)

Figure 4.38: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w HPMC and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



Values are expressed as mean ± SD (n =3)

Figure 4.39: Effect of temperature on the viscosity of various HPMC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.75 % w/w HPMC and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



Values are expressed as mean ± SD (n =3)

4.7.3 Measurement of Gel strength

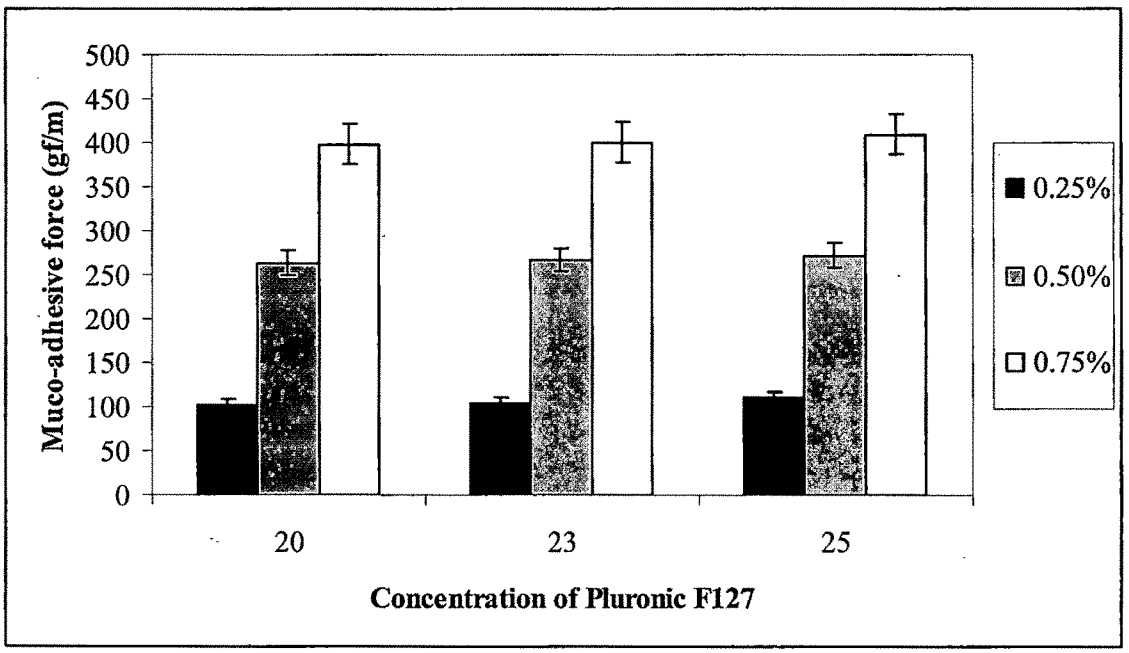
The gel strength of the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.25 % w/w, 0.50 % w/w, and 0.75 % w/w HPMC concentration increases significantly (P< 0.001) with respect to plain pluronic F127 gel. There is no significant difference in the formulations containing minocycline hydrochloride/ clindamycin phosphate. Increase in the gel strength in presence of different concentration of HPMC may be due to the bond formation between pluronic F127 and HPMC. Higher gel strength formulations possess the higher mucoadhesive property and increases the residence time at the application site thereby increasing the bioavailability of the drug.

4.7.4 Mucoadhesive strength

The assessment of the mucoadhesive strength in terms of detachment stress showed that the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.25% w/w, 0.50 % w/w, and 0.75% w/w HPMC concentration increases significantly (P< 0.001) with respect to plain pluronic F127 gel (Figure 4.40). Difference in the mucoadhesive

strength was found to be non significant (P>0.001). From the results it is evident that the availability of the hydroxyl groups determines the mucoadhesion. Thus HPMC having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoprotein. There is evidence that the higher mucoadhesive strength delivery system possesses prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.40: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of HPMC- pluronic F127



4.7.5 Syringeability

The assessment of the syringeability was done as described in section 4.6.4 which followed the similar manner as that for polycarbophil. The results of the syringeability are shown in Table No4.59.

Table No 4.59: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of HPMC- pluronic F127

Formulation Code	Syringeability
MGF18	153.39
MGF19	194.75
MGF20	225.63
CGF18	156.79
CGF19	189.93
CGF20	219.38

4.7.6 In vitro release study

The in vitro release profile of minocycline hydrochloride/ clindamycin phosphate loaded periodontal gels containing HPMC as mucoadhesive polymer is illustrated in Figure 4.41 and 4.42 respectively. The maximum release of minocycline hydrochloride from the thermoreversible gels was shown by the formulation MGF18 where as the least was shown by the formulation MGF20 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF18 where as the least was shown by the formulation CGF20 after 8 hours. The higher release of minocycline hydrochloride and clindamycin phosphate from HPMC containing periodontal gels can be explained by the viscosity of the polymer solution. A preliminary study showed that the formulation MGF18 and CGF18 had low viscosity than MGF20 and CGF20, which may lead to easy and quick release of the drug. In addition, formulation MGF18 and CGF18 due to low viscosity have more available waters to diffuse consequently showed more diffusion through the membrane, similarly formulation MGF20 and CGF20 showed high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.60: In vitro release profile of minocycline hydrochloride from HPMC - Pluronic F127 thermoreversible periodontal gel

Time in Hour	% Minocycline hydrochloride released ± SD		
	MGF18	MGF19	MGF20
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	2.71 ± 0.018	2.04 ± 0.024	1.27 ± 0.009
1.00	5.67 ± 0.022	4.30 ± 0.019	3.27 ± 0.010
1.50	8.91 ± 0.032	7.16 ± 0.020	5.74 ± 0.021
2.00	11.94 ± 0.045	10.45 ± 0.017	8.65 ± 0.008
2.50	15.07 ± 0.060	13.53 ± 0.034	11.66 ± 0.007
3.00	18.31 ± 0.025	16.71 ± 0.013	14.78 ± 0.012
3.50	22.55 ± 0.058	19.92 ± 0.058	18.09 ± 0.055
4.00	26.83 ± 0.024	23.82 ± 0.065	21.83 ± 0.031
5.00	34.15 ± 0.036	32.11 ± 0.046	29.43 ± 0.028
6.00	44.31 ± 0.022	40.92 ± 0.011	37.38 ± 0.034
7.00	54.40 ± 0.037	49.94 ± 0.022	46.51 ± 0.069
8.00	65.09 ± 0.060	60.18 ± 0.129	55.02 ± 0.072

Figure 4.41: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from HPMC - Pluronic F127 thermoreversible periodontal gel

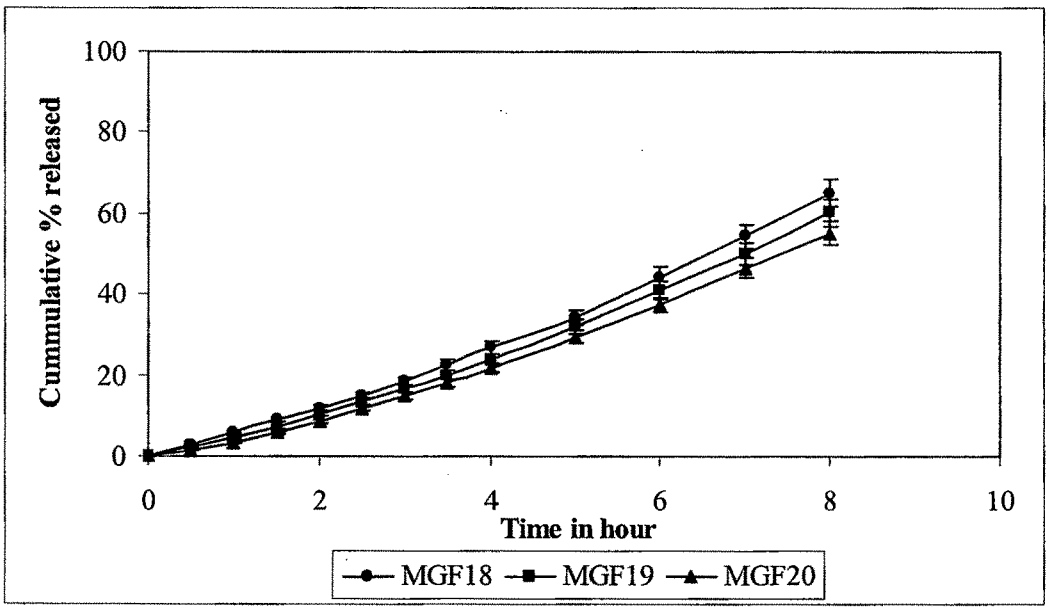


Table No 4.61: In Vitro Release Profile of Clindamycin phosphate from HPMC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate released ± SD		
	CGF18	CGF19	CGF20
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	2.24 ± 0.015	1.92 ± 0.021	1.44 ± 0.044
1.00	5.22 ± 0.026	4.24 ± 0.020	3.17 ± 0.030
1.50	7.35 ± 0.047	6.65 ± 0.056	5.31 ± 0.015
2.00	10.19 ± 0.026	9.15 ± 0.016	7.76 ± 0.016
2.50	12.82 ± 0.038	11.42 ± 0.052	10.46 ± 0.031
3.00	16.18 ± 0.018	14.41 ± 0.064	13.41 ± 0.040
3.50	19.33 ± 0.039	17.50 ± 0.052	15.99 ± 0.023
4.00	22.50 ± 0.040	20.94 ± 0.066	19.39 ± 0.017
5.00	30.14 ± 0.031	28.56 ± 0.071	25.73 ± 0.046
6.00	38.95 ± 0.036	36.06 ± 0.055	32.22 ± 0.044
7.00	48.29 ± 0.060	44.71 ± 0.051	39.49 ± 0.035
8.00	59.17 ± 0.115	54.22 ± 0.052	47.24 ± 0.037

Figure 4.42: Cumulative percentage release profile of clindamycin phosphate in mcg/cm^2 from HPMC - Pluronic F127 thermoreversible periodontal gel

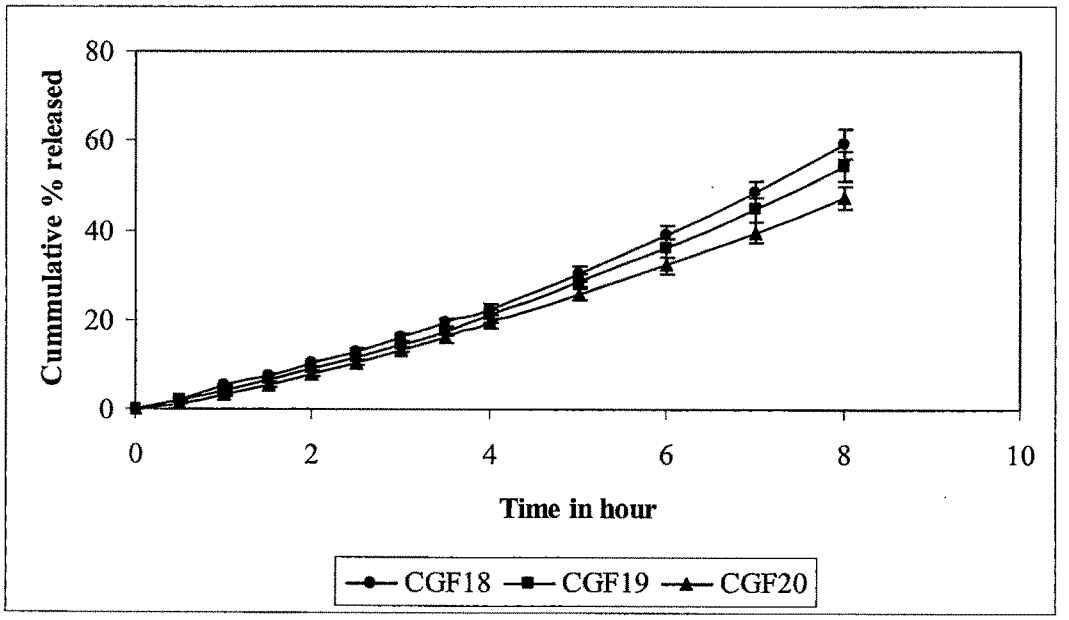


Table no. 4.62: Release kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded HPMC - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				N (Release exponent)	K (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF18	**0.9876**	0.5367	0.8555	0.5666	8.047	1.114
MGF19	**0.9867**	0.4728	0.8492	0.1542	7.514	3.266
MGF20	**0.9527**	0.4045	0.8429	0.5919	9.069	1.172
CGF18	**0.9819**	0.4257	0.8418	0.5589	7.228	1.690
CGF19	**0.9831**	0.3609	0.8414	0.566	6.713	5.164
CGF20	**0.9886**	0.264	0.8522	0.5843	5.961	9.141

4.7.7 In Vitro permeation study

4.7.7.1 Determination of saturated drug concentration

The saturated drug concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was prepared as described earlier in section 4.6.6.1 and was found to be 106.994 mg ml-1 and 103.900 mg ml^{-1} respectively.

4.7.7.2 Preparation of mucosal tissue

The sheep cheek mucosal tissue was prepared as described earlier in the section 4.6.6.2.

4.7.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane of sheep was measured as described earlier. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ µm, which is the mean of 3 measurements.

The cationic polymer HPMC is reported to demonstrate the permeation enhancing property. The cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various HPMC-pluronic F127 gels formulations are given in the Figure 4.43 and 4.44. It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for HPMC pluronic F127 thermoreversible gels, plain pluronic F127 thermoreversible gels compared to the pure drug solution. Permeation of the minocycline

hydrochloride and clindamycin phosphate significantly differ in formulations containing the HPMC ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of HPMC results in very rapid dissolution and release of drug due to the swelling and dissolution of HPMC at pH 6.75. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micelle structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations which may be due to the formation of larger micelle concentration. Addition of the HPMC increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in wetability and swelling of the polymers. The swelling of the polymers was also due to ionic strength and pH (Park and Robinson, 1985). At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the HPMC. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of ionized drug molecules helps in the formation of hydrogen binding site and relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability of the gel formulations, MGF18, MGF19, CGF18, and CGF19 were found to be the best. However formulations containing 0.25 % w/w HPMC along with 25 % pluronic F127 shows lower gelling temperature, low permeation profile and high syringeability which may make difficult to administer the drug to the periodontal cavity. Formulations containing the higher concentrations of HPMC (1.00 %w/w and 2.00% w/w) showed a high syringeability and blockage of the syringe which may be due to high viscous solution. Hence MGF18, CGF18, MGF19 and CGF19 was selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No 4.63: In vitro Permeation Profile of Minocycline hydrochloride from HPMC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF 18	MGF 19	MGF 20
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.88 ± 0.026	1.21 ± 0.114	0.87 ± 0.039
1.00	3.63 ± 0.037	2.93 ± 0.033	2.53 ± 0.009
1.50	6.79 ± 0.025	5.73 ± 0.042	4.36 ± 0.020
2.00	10.40 ± 0.038	8.63 ± 0.048	5.87 ± 0.018
2.50	13.48 ± 0.059	11.31 ± 0.033	8.44 ± 0.041
3.00	16.66 ± 0.040	14.08 ± 0.023	12.10 ± 0.031
3.50	19.55 ± 0.049	16.89 ± 0.058	14.53 ± 0.036
4.00	23.46 ± 0.064	20.40 ± 0.011	17.64 ± 0.046
5.00	31.41 ± 0.043	28.96 ± 0.036	25.82 ± 0.055
6.00	40.86 ± 0.032	38.06 ± 0.044	34.59 ± 0.029
7.00	49.33 ± 0.017	45.73 ± 0.006	42.24 ± 0.064
8.00	55.41 ± 0.071	51.90 ± 0.058	47.02 ± 0.040

Figure 4.43: Cumulative permeation profile of minocycline hydrochloride from HPMC - pluronic F127 thermoreversible periodontal gel

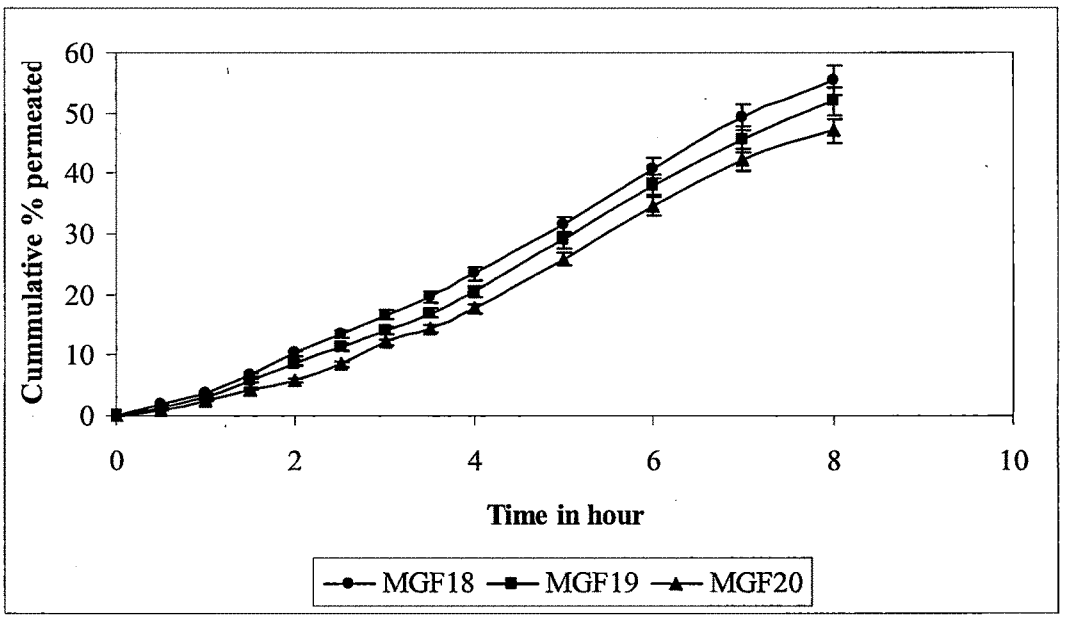


Table No: 4.64: In vitro Permeation Profile of Clindamycin phosphate from HPMC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF 18	CGF 19	CGF 20
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.60 ± 0.019	1.35 ± 0.109	0.96 ± 0.027
1.00	3.27 ± 0.032	2.62 ± 0.028	2.38 ± 0.007
1.50	5.64 ± 0.021	4.77 ± 0.036	3.98 ± 0.012
2.00	8.42 ± 0.029	7.55 ± 0.041	5.73 ± 0.011
2.50	11.31 ± 0.046	9.60 ± 0.028	8.20 ± 0.037
3.00	13.97 ± 0.031	11.89 ± 0.017	9.47 ± 0.024
3.50	16.41 ± 0.041	14.24 ± 0.049	12.06 ± 0.031
4.00	19.82 ± 0.058	17.27 ± 0.005	14.47 ± 0.039
5.00	28.09 ± 0.039	24.83 ± 0.029	21.53 ± 0.046
6.00	33.37 ± 0.026	30.35 ± 0.036	26.48 ± 0.018
7.00	39.69 ± 0.011	36.60 ± 0.010	32.51 ± 0.057
8.00	46.77 ± 0.063	43.64 ± 0.052	39.94 ± 0.038

Figure 4.44: Cumulative permeation profile of clindamycin phosphate from HPMC - Pluronic F127 thermoreversible periodontal gel

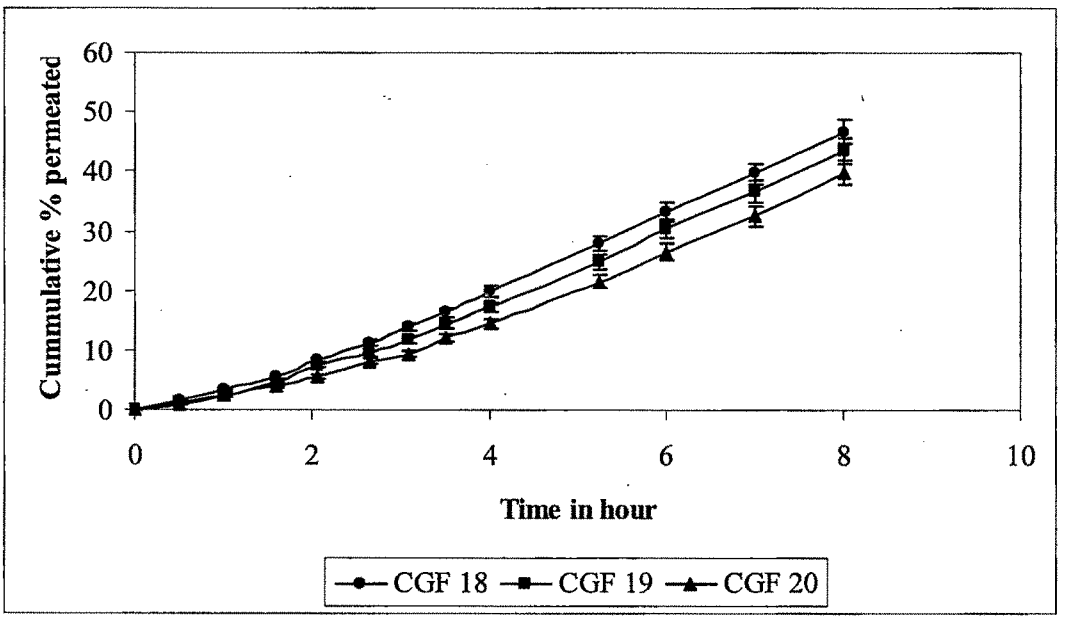


Table no. 4.65: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gels

Formulations	Permeation flux $J(mcg.cm^{-2}.hr^{-1})$	Lag time ($t_L$hr)	Diffusion coefficient $(D\times10^{-8}cm^2.sec^{-1})$	Permeability coefficient $(P\times10^{-8}cm.sec^{-1})$
MGF18	7.58	1.00	1.070	1.967
MGF19	7.38	1.50	0.713	1.915
MGF20	7.20	2.00	0.534	1.869
CGF18	6.28	1.00	1.070	1.678
CGF19	6.01	1.40	0.764	1.606
CGF20	5.69	1.90	0.562	1.521

4.7.8 Stability Study

The Minocycline hydrochloride/ clindamycin phosphate loaded periodontal thermoreversible gels were studied for their stability at Freeze condition (4°C) and at RT (25°C). All the four formulations showed good physical stability with no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of the formulations was within the range of 5.80 to 5.99 which is the neutral pH

Table no. 4.66: Drug Content, pH and gelling temperature of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF18	98.35 ± 1.25	5.95	36.2
MGF19	97.05 ± 0.89	5.99	31.9
CGF18	99.52 ± 0.79	5.84	34.1
CGF19	100.11 ± 0.87	5.82	32.3

Table no. 4.67: Drug Content, pH and gelling temperature of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF18	99.26 ± 0.78	5.92	35.8
MGF19	97.28 ± 0.85	5.95	32.7
CGF18	97.45 ± 0.98	5.79	33.9
CGF19	98.55 ± 0.71	5.80	32.1

4.7.9 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with mucoadhesive polymer HPMC by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate. Drug loaded periodontal gel formulations studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37°C), and return to the liquid state upon cooling below gelation temperature. At 4°C all the formulations were at liquid state with viscosity ranging from 17.23 mPas to 30.52 mPas for 19 % w/w to 25 % w/w pluronic F127 with 0.25-0.75 w/w % HPMC. Rheological behavior of all the formulations was measured. All the formulations exhibited Newtonian behavior at 4°C; all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of the formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer HPMC and addition of increasing concentration of HPMC from 0.5- 0.75 % w/w lowered the gelation temperature significantly, which might be due to the uncoiling and expansion of the mucoadhesive polymer resulted in polymer swelling and elastic gel formation.

The gel strength of the formulations in terms of force required to penetration shows that the pluronic F127 preparations possess stiffness property that increase with addition of HPMC. This may be due to bond formation between pluronic F127 and HPMC. Increase in gel strength shows that the addition of HPMC increases the strength thereby possessing high mucoadhesive property and increased residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of HPMC. From the study it was evidenced that the availability of the hydroxyl groups determines the mucoadhesion. Presence of mucoadhesive polymer HPMC having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Syringeability of the formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; that may be due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.50 % w/w HPMC concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation showed a sustain release of the drug for a period of 8 hours compared to plain drugs. A preliminary study shows that the formulation prepared with 20%w/w pluronicF127 along with 0.25 % HPMC (MGF18 and CGF18) had low viscosity than formulations prepared with 23% w/w pluronic F127 along with 0.25% HPMC (MGF19 and CGF19). In addition, formulation MGF18 and CGF18 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF19 and CGF19 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for HPMC-pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the HPMC ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of HPMC

results in very rapid dissolution of the drug due to swelling and dissolution of HPMC. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations which may be due to the formations of larger concentrations of the micelles. Addition of the HPMC increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in concentrations of ionized hydroxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized hydroxyl group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized HPMC.

The investigation of in vitro release and permeation data showed that the drug release followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stability during the storage period of six months, and their chemical and mechanical property does not change significantly.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.25% HPMC along with 20% and 23 % w/w pluronic F127 were found to be the best among the rest of the HPMC-pluronic F127 periodontal thermoreversible gel delivery systems.

4.8 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND HYDROXY ETHYL CELLULOSE

4.8.1 Preparation of mixed HEC - pluronic F127 periodontal gels

Formulations containing the minocycline hydrochloride (1%) and clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with HEC. The compositions of the formulations are cited in Table No4.68 to 4.73.

Table No 4.68: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HEC (0.25%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF29	MGF30	MGF31	MGF32
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.25	0.25	0.25	0.25
Sodium metabisulphite	0.50	0.50	0.50	0.50
PEG1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	38.0	32	29	27
Visual gel Temp. (°C)	39.2	32.7	29.8	28.5
Drug content	99.23 ± 0.36	99.19 ± 0.29	98.63 ± 0.34	98.94 ± 0.82
Mucoadhesion (gf/mm)	18.34 ± 0.39	21.87 ± 1.03	26.14 ± 1.11	29.36 ± 0.97
Gel strength (N/m)	10395.28 ± 141.28	17834.59 ± 132.69	20112.76 ± 134.68	22349.52 ± 114.87
pH (Sol)	5.87	5.93	5.96	5.99
pH (Gel)	5.89	5.90	5.92	5.97
Sol Viscosity mPas	17.89	25.21	27.38	29.39
Gel Viscosity mPas	2148	3142	3349	3563

Table No 4.69: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HEC (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF33	MGF34	MGF35	MGF36
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	33	28	24	21
Visual gel Temp. (°C)	33.9	28.7	25.1	22. 2
Drug content	97.82 ± 0.29	98.83 ± 0.67	99.18 ± 0.44	98.79 ± 0.54
Mucoadhesion (gf/mm)	20.35 ± 0.79	24.64 ± 1.32	28.58 ± 1.19	31.3 ± 0.97
Gel strength (N/m)	11231.67 ± 189.72	18781.33 ± 119.29	21197.93 ± 192.28	22981.49 ± 111.25
pH (Sol)	5.85	5.88	5.92	5.96
pH (Gel)	5.87	5.90	5.91	5.95
Sol Viscosity mPas	18.72	25.76	27.87	29.74
Gel Viscosity mPas	2183	3176	3392	3591

Table No 4.70: Composition and Characteristics of MnHCl loaded mixed periodontal gels of HEC (0.75%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF37	MGF38	MGF39	MGF40
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.75	0.75	0.75	0.75
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	30	25	23	21
Visual gel Temp. (°C)	31.1	26.2	24.1	22.3
Drug content	98.28 ± 0.49	99.23 ± 0.39	98.71 ± 0.69	97.81 ± 0.94
Mucoadhesion (gf/mm)	21.41 ± 1.17	25.21 ± 1.14	28.93 ± 0.97	32.46 ± 1.25
Gel strength (N/m)	11467.75 ± 134.79	19024.48 ± 109.69	21304.76 ± 129.67	23417.52 ± 182.39
pH (Sol)	5.91	5.94	5.96	5.98
pH (Gel)	5.89	5.92	5.99	6.01
Sol Viscosity mPas	19.04	26.32	28.12	29.91
Gel Viscosity mPas	2213	3247	3345	3527

Table No 4.71: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HEC (0.25%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF29	CGF30	CGF31	CGF32
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.25	0.25	0.25	0.25
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	37	31	28	26
Visual gel Temp. (°C)	37.8	31.9	29.2	27.3
Drug content	99.1 ± 0.38	98.79 ± 0.37	99.58 ± 0.67	97.51 ± 0.62
Mucoadhesion (gf/mm)	18.78 ± 0.46	21.38 ± 1.19	26.74 ± 0.97	29.54 ± 1.12
Gel strength (N/m)	10425.67 ± 139.57	17925.38 ± 129.76	20238.68 ± 120.63	22576.83 ± 116.79
pH (Sol)	5.90	5.91	5.94	5.97
pH (Gel)	5.91	5.93	5.92	5.94
Sol Viscosity mPas	17.34	24.83	27.12	28.91
Gel Viscosity mPas	2056	3112	3327	3567

Table No 4.72: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HEC (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF33	CGF34	CGF35	CGF36
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.50	0.50	0.50	0.50
PEG1000 %w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	32	27	23	20
Visual gel Temp. (°C)	32.5	27.6	23.6	20.8
Drug content	99.26 ± 0.94	99.1 ± 0.62	99.75 ± 0.37	98.6 ± 0.44
Mucoadhesion (gf/mm)	20.62 ± 0.81	25.21 ± 1.22	28.63 ± 0.78	31.42 ± 1.14
Gel strength (N/m)	11376.85 ± 128.29	19178.28 ± 129.36	21348.76 ± 111.97	23139.15 ± 138.79
pH (Sol)	5.92	5.94	5.96	5.98
pH (Gel)	5.91	5.92	5.94	5.95
Sol Viscosity mPas	18.91	26.14	28.24	29.85
Gel Viscosity mPas	2247	3214	3468	3682

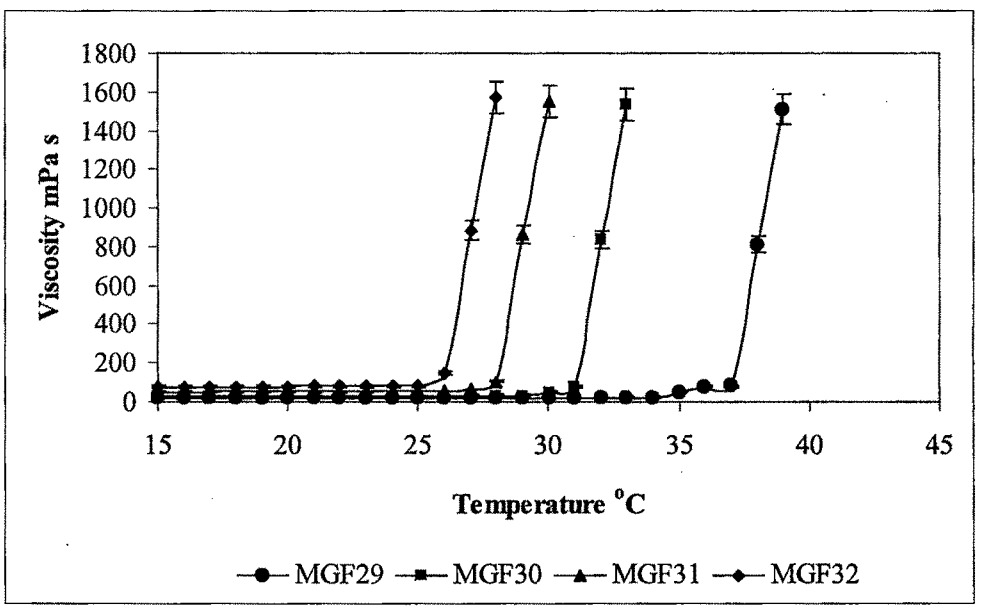
Table No 4.73: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of HEC (0.75%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF37	CGF38	CGF39	CGF40
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
HEC (%w/w)	0.75	0.75	0.75	0.75
PEG1000 (%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temperature (°C)	29	24	22	20
Visual gel Temperature (°C)	30.1	25.2	23.1	20.9
Drug content	98.27 ± 0.19	98.38 ± 0.37	98.82 ± 0.58	98.29 ± 0.48
Mucoadhesion (gf/mm)	22.04 ± 1.23	15.76 ± 0.98	29.26 ± 1.37	33.21 ± 1.73
Gel strength (N/m)	11983.27 ± 189.76	20342.67 ± 142.36	21789.39 ± 134.29	23869.93 ± 143.29
pH (Sol)	5.93	5.96	5.99	5.97
pH (Gel)	5.92	5.95	6.01	6.03
Sol Viscosity mPas	19.56	26.89	28.73	30.36
Gel Viscosity mPas	2238	3287	3393	3583

4.8.2 Viscosity and gelling temperature determination

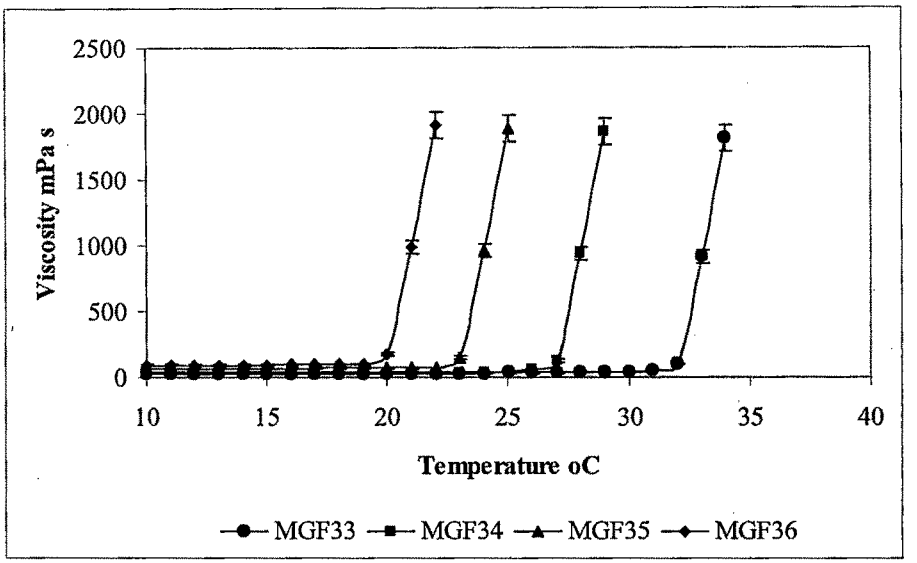
As evident from the results in table no 4.68 to 4.73, the gelling temperature of pluronic F127 vehicle as determined by rheological method was lowered by the addition of increasing concentration of the mucoadhesive polymer HEC i.e. 0.25 % w/w to 0.75% w/w. Figure 4.45 to 4.50 showed the viscosity of various pluronic F127 gels with varying concentration of HEC measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method did not vary more than ± 1.5°C. The decrease in the gelation temperature with increase in HEC concentration may be due to enhanced viscosity of the gel formulation. The formulations showing the gelling temperature between 25°C to 37°C seemed to be suitable for in situ gelling of the various vehicles at the periodontal cavity, minimizing the loss of administered drug due to clearance from the site of application.

Figure 4.45: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.25 % w/w HEC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



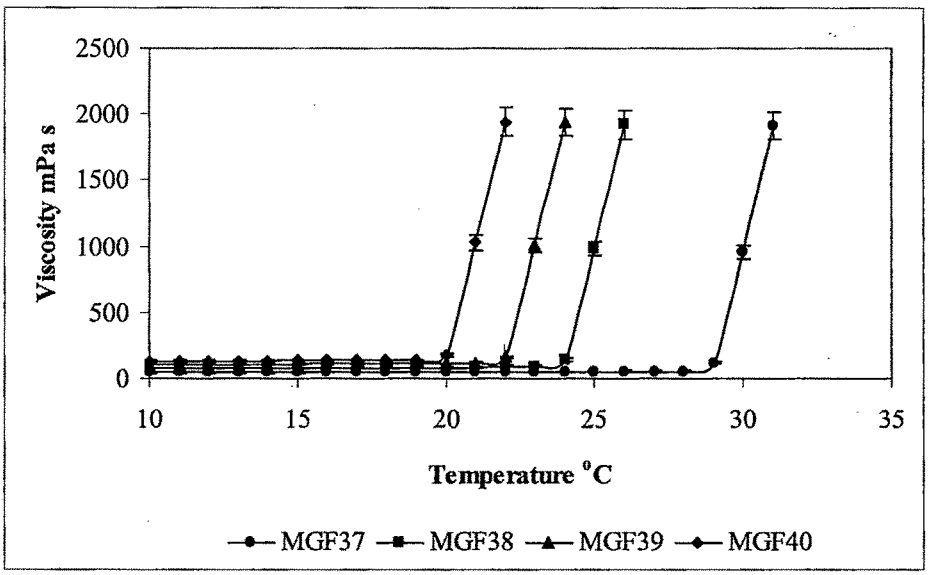
Values are expressed as mean ± SD (n =3).

Figure 4.46: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w HEC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate.



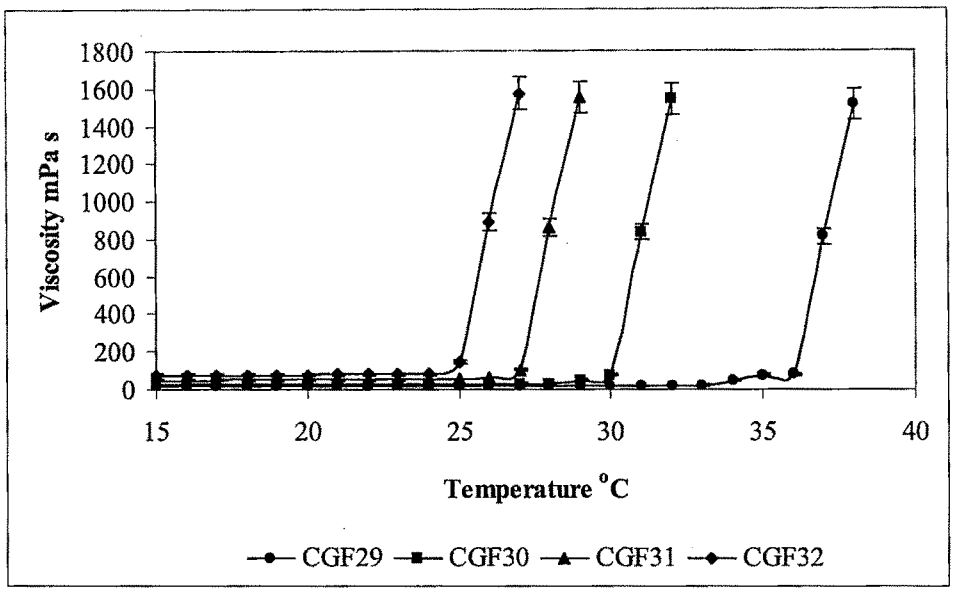
Values are expressed as mean ± SD (n =3).

Figure 4.47: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.75 % w/w HEC and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



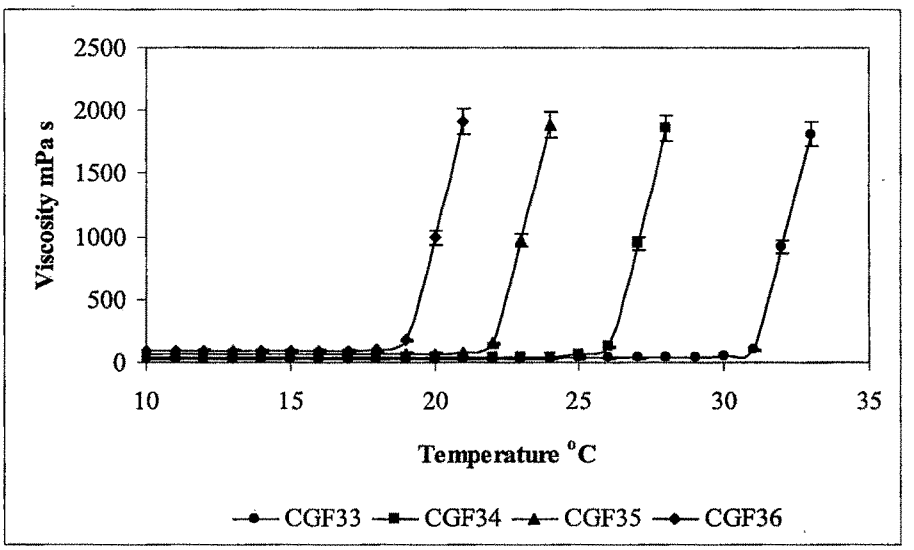
Values are expressed as mean ± SD (n =3).

Figure 4.48: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.25 % w/w HEC and 1% w/w phosphate measured at 10 s^{-1} shear rate



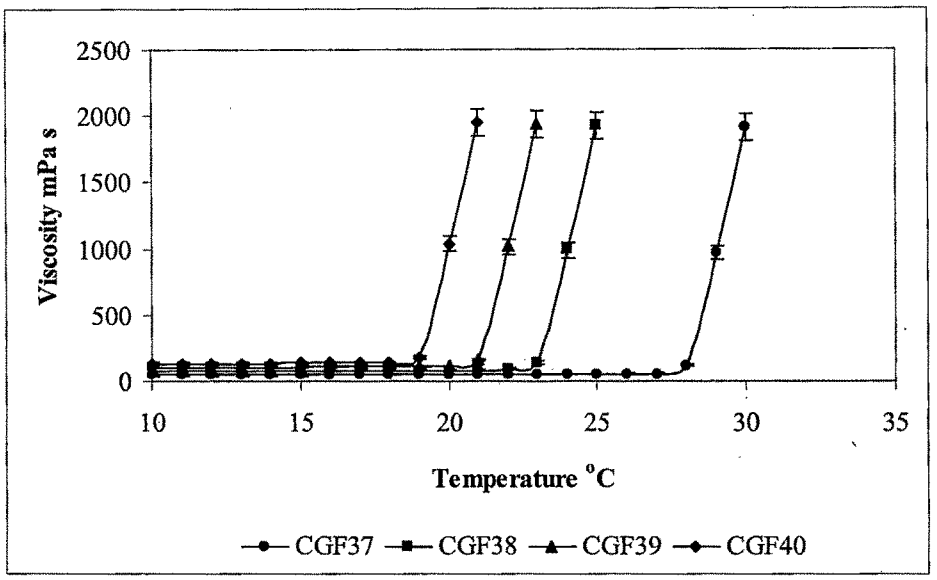
Values are expressed as mean ± SD (n =3).

Figure 4.49: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w HEC and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3).

Figure 4.50: Effect of temperature on the viscosity of various HEC-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.75 % w/w HEC and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

4.8.3 Gel strength

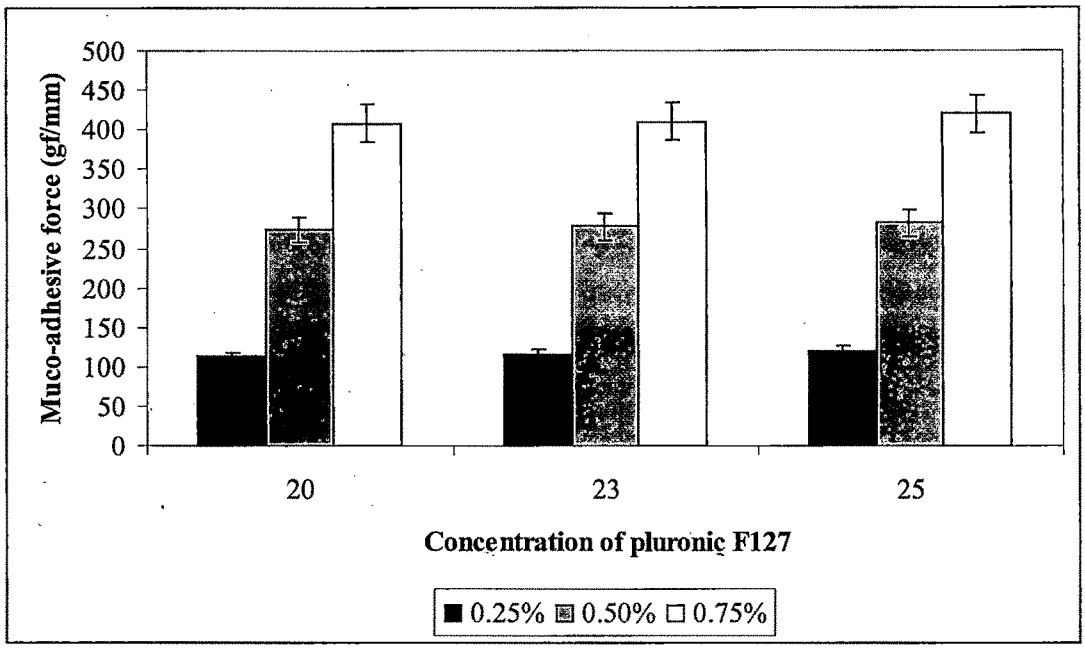
The gel strength of all the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.25 % w/w, 0.50 % w/w, and 0.75 % w/w HEC concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. There is no significant difference in the formulations containing minocycline hydrochloride and clindamycin phosphate was observed. Increase in the gel strength in presence of different concentration of HEC may be due to the bond formation between pluronic F127 and HEC resulting in high mucoadhesive property, increased residence time at the application site and thereby increased bioavailability of the drug.

4.8.4 Mucoadhesive strength

The assessment of the mucoadhesive strength for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.25% w/w, 0.50 % w/w, and 0.75% w/w HEC concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel

(Figure 4.51). However, their difference in the mucoadhesive strength is non significant (P>0.001). From the results it is evident that the availability of the hydroxyl groups determines the mucoadhesion. Thus HEC having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoprotein. There is evidence that the higher mucoadhesive strength delivery system possesses prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.51: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of HEC- pluronic F127



4.8.5 Syringeability

The assessment of the syringeability was made in terms of force required to syringe the formulation to the application site. Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.2% w/w, 0.3% w/w, 0.4% w/w and 0.5% w/w HEC concentration

increases significantly (P< 0.001) with respect to plain pluronic F127 gel. The results of the syringeability are shown in Table No4.74.

Table No 4.74: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of HEC- pluronic F127

Formulation Code	Syringeability
MGF29	189.41
MGF30	198.85
MGF31	203.19
MGF32	237.90
CGF29	193.50
CGF30	203.62
CGF31	210.85
CGF32	287.69

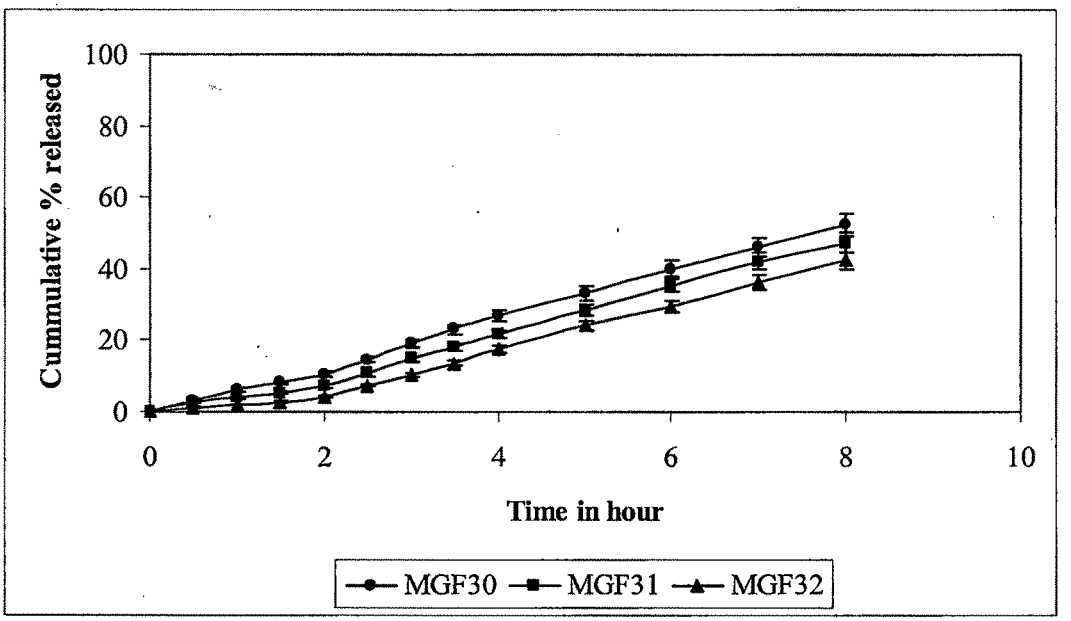
4.8.6 In vitro release study

The release profile of minocycline hydrochloride and clindamycin phosphate along with mucoadhesive polymer HEC is illustrated in Figure 4.52 and 4.53 respectively. The maximum release of minocycline hydrochloride from the thermoreversible gels was shown by the formulation MGF30 where as the least was shown by the formulation MGF32 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF30 where as the least was shown by the formulation CGF32 after 8 hours. The higher release of minocycline hydrochloride and clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study showed that the formulation MGF30 and CGF30 had low viscosity than MGF32 and CGF32. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF30 and CGF30. In addition, formulation MGF30 and CGF30 due to low viscosity have more availability of water to diffuse consequently showed more diffusion through the membrane. Similarly formulation MGF32 and CGF32 showed high viscosity, which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.75: In Vitro Release Profile of Minocycline hydrochloride from HEC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride released ± SD		
	MGF30	MGF31	MGF32
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	3.22 ± 0.006	2.65 ± 0.014	1.24 ± 0.053
1.00	6.20 ± 0.096	4.03 ± 0.050	2.03 ± 0.038
1.50	8.05 ± 0.034	5.42 ± 0.029	2.74 ± 0.055
2.00	10.60 ± 0.006	7.37 ± 0.005	4.22 ± 0.017
2.50	14.72 ± 0.007	10.63 ± 0.049	7.43 ± 0.010
3.00	19.05 ± 0.016	14.85 ± 0.069	10.39 ± 0.013
3.50	23.17 ± 0.057	18.31 ± 0.017	13.62 ± 0.028
4.00	26.78 ± 0.063	21.70 ± 0.053	17.56 ± 0.017
5.00	33.23 ± 0.038	28.68 ± 0.034	24.20 ± 0.030
6.00	39.96 ± 0.049	35.48 ± 0.020	29.39 ± 0.065
7.00	46.21 ± 0.041	42.06 ± 0.021	36.21 ± 0.035
8.00	52.52 ± 0.006	47.38 ± 0.030	42.35 ± 0.014

Figure 4.52: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from HEC - Pluronic F127 thermoreversible periodontal gel



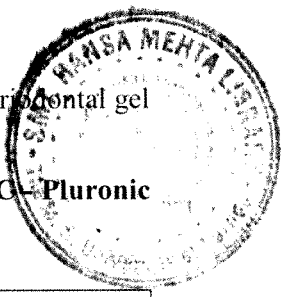


Table No 4.76: In Vitro Release Profile of Clindamycin phosphate from HEC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate released ± SD		
	CGF30	CGF31	CGF32
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.25 ± 0.022	1.06 ± 0.038	0.83 ± 0.018
1.00	5.24 ± 0.050	3.38 ± 0.025	2.02 ± 0.033
1.50	6.76 ± 0.008	4.79 ± 0.034	2.68 ± 0.011
2.00	9.81 ± 0.018	7.06 ± 0.058	4.86 ± 0.042
2.50	12.75 ± 0.020	10.05 ± 0.041	6.30 ± 0.019
3.00	16.11 ± 0.022	12.93 ± 0.031	8.65 ± 0.027
3.50	20.22 ± 0.051	16.55 ± 0.025	11.82 ± 0.028
4.00	22.92 ± 0.022	19.03 ± 0.009	14.34 ± 0.006
5.00	29.84 ± 0.027	25.44 ± 0.024	20.23 ± 0.035
6.00	36.73 ± 0.006	32.16 ± 0.048	26.95 ± 0.020
7.00	43.76 ± 0.035	39.24 ± 0.042	33.89 ± 0.052
8.00	50.29 ± 0.045	45.48 ± 0.045	40.44 ± 0.062

Figure 4.53: Cumulative percentage release profile of clindamycin phosphate in mcg/cm^2 from HEC - Pluronic F127 thermoreversible periodontal gel

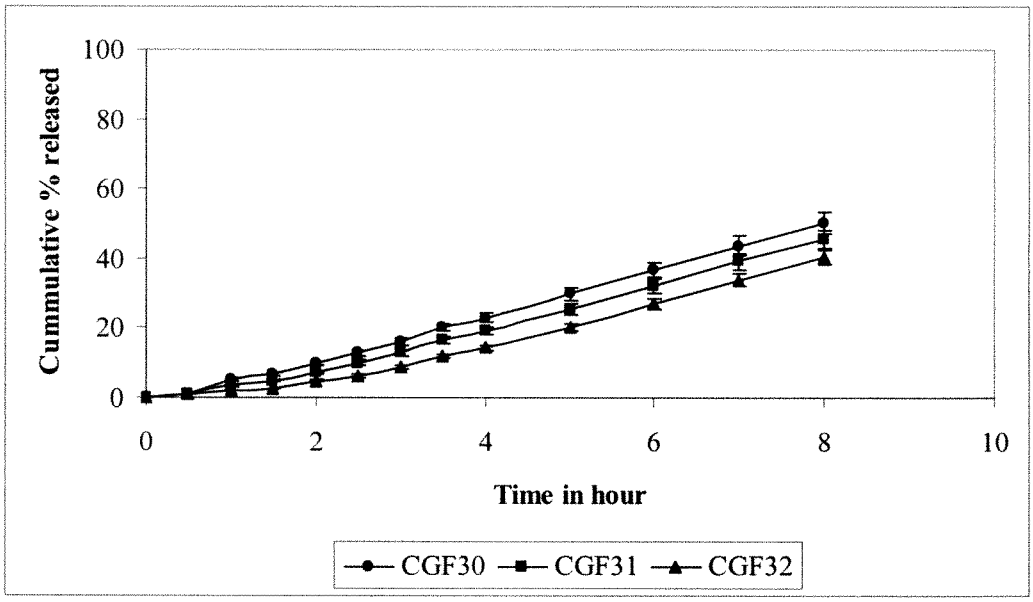


Table no. 4.77: Release kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded HEC - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				N (Release exponent)	K (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF30	**0.9972**	0.4195	0.9013	0.5847	6.737	5.457
MGF31	**0.9902**	0.3187	0.8642	0.1357	6.212	1.629
MGF32	**0.9527**	0.2126	0.8244	0.5686	9.069	1.172
CGF30	**0.9963**	0.3449	0.8828	0.6008	6.455	2.851
CGF31	**0.9898**	0.2507	0.8549	0.5975	5.874	7.482
CGF32	**0.9691**	0.1524	0.8033	0.574	5.187	1.538

4.8.7 In Vitro permeation study

4.8.7.1 Determination of saturated drug concentration

The saturated concentration of minocycline hydrochloride/ clindamycin phosphate was done in phosphate buffer pH 6.75 using the method as described in section 4.6.6.1 and was found to be 106.994 mg ml-1 and 103.900 mg ml^{-1} respectively.

4.8.7.2 Preparation of mucosal tissue

The mucosal tissue was prepared as described in the section 4.6.6.2.

4.8.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of sheep cheek mucous membrane was measured as described earlier in section 4.6.6.3. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ µm, which is the mean of 3 measurements.

The cationic polymer HEC is reported to demonstrate permeation enhancing property with high mucoadhesive property. The effective permeability coefficient determined for minocycline hydrochloride and clindamycin phosphate in each gel formulations are given in Table No4.80 and the cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various HEC- pluronic F127 gels formulations are given in the Figure 4.54 and 4.55. It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for HEC-pluronic F127 thermoreversible gels

than compared to the pure drug solution. Since the pluronic F127 gels are viscous, isotropic liquid crystals containing micelles, it may be hypothesized that the drug release may be by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing HEC ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. The presence of HEC results in very rapid dissolution and release of drug due to swelling and dissolution of HEC at pH 6.75. However presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micelle structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations which may be due to the formations of larger concentrations of the micelle. Addition of the HEC increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in wetability and swelling of the polymers. The swelling of the polymers was also due to ionic strength and pH (Park and Robinson, 1985). At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the HEC. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of ionized drug molecules help in the formation of hydrogen binding site and relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability, formulations containing 0.25 % w/w HEC along with 20 % w/w and 23 % w/w pluronic F127 (MGF30, CGF30, MGF31and CGF31) was found to be the best as the formulation with 0.25 % w/w HEC along with 25 % pluronic F127 shows low gelling temperature, low permeation profile with high syringeability which may make difficulty to administer the drug into the periodontal cavity, hence selected for the further study.

Table No 4.78: In Vitro Permeation Profile of Minocycline hydrochloride from HEC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF 30	MGF 31	MGF 32
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.24 ± 0.023	1.04 ± 0.110	0.84 ± 0.035
1.00	3.17 ± 0.034	2.05 ± 0.029	1.84 ± 0.005
1.50	6.04 ± 0.021	4.35 ± 0.038	3.32 ± 0.016
2.00	8.62 ± 0.034	6.36 ± 0.044	5.23 ± 0.014
2.50	11.26 ± 0.055	9.62 ± 0.029	7.41 ± 0.037
3.00	14.26 ± 0.036	12.49 ± 0.019	10.40 ± 0.027
3.50	17.59 ± 0.045	15.53 ± 0.054	13.10 ± 0.032
4.00	20.68 ± 0.059	18.26 ± 0.007	15.69 ± 0.042
5.00	26.32 ± 0.039	23.78 ± 0.032	20.55 ± 0.051
6.00	32.09 ± 0.028	29.42 ± 0.040	26.56 ± 0.025
7.00	38.39 ± 0.014	35.45 ± 0.002	32.34 ± 0.060
8.00	44.60 ± 0.067	41.35 ± 0.054	38.31 ± 0.036

Figure 4.54: Cumulative permeation profile of minocycline hydrochloride from HEC - pluronic F127 thermoreversible periodontal gel

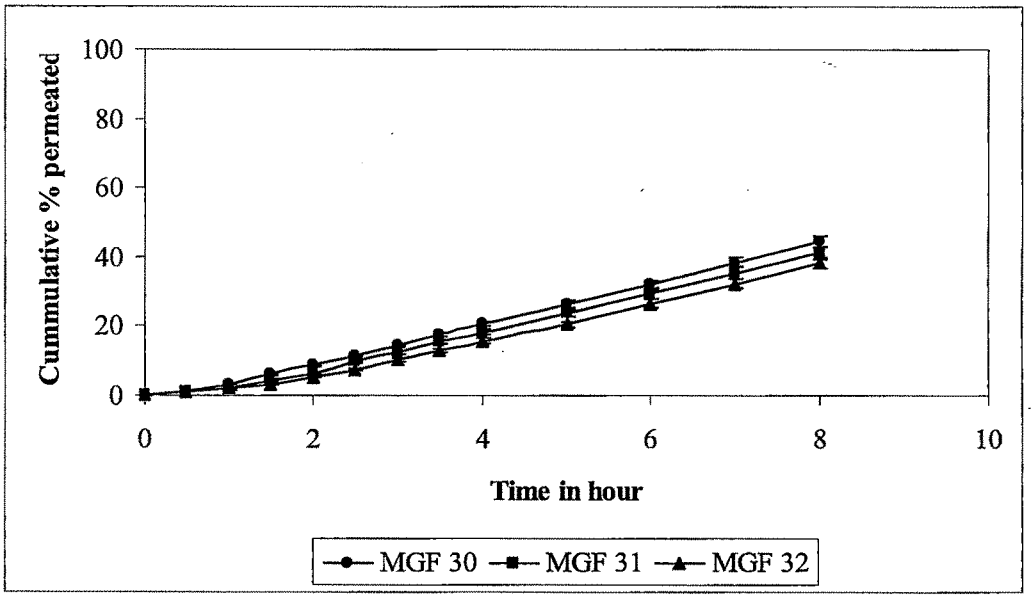


Table No 4.79: In Vitro Permeation Profile of Clindamycin phosphate from HEC - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF 30	CGF 31	CGF 32
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.03 ± 0.021	0.99 ± 0.111	0.61 ± 0.029
1.00	1.94 ± 0.034	1.83 ± 0.030	1.02 ± 0.009
1.50	4.16 ± 0.023	3.64 ± 0.038	2.47 ± 0.014
2.00	6.62 ± 0.031	5.70 ± 0.043	4.46 ± 0.013
2.50	9.31 ± 0.048	7.52 ± 0.030	6.49 ± 0.039
3.00	12.33 ± 0.033	10.53 ± 0.019	8.59 ± 0.026
3.50	15.54 ± 0.043	13.48 ± 0.051	10.71 ± 0.033
4.00	18.70 ± 0.061	16.34 ± 0.007	13.45 ± 0.041
5.00	24.36 ± 0.041	21.83 ± 0.031	18.70 ± 0.048
6.00	30.10 ± 0.028	27.44 ± 0.038	24.41 ± 0.020
7.00	36.37 ± 0.013	33.34 ± 0.012	30.25 ± 0.059
8.00	43.02 ± 0.065	39.25 ± 0.054	36.87 ± 0.040

Figure 4.55: Cumulative permeation profile of clindamycin phosphate from HEC - Pluronic F127 thermoreversible periodontal gel

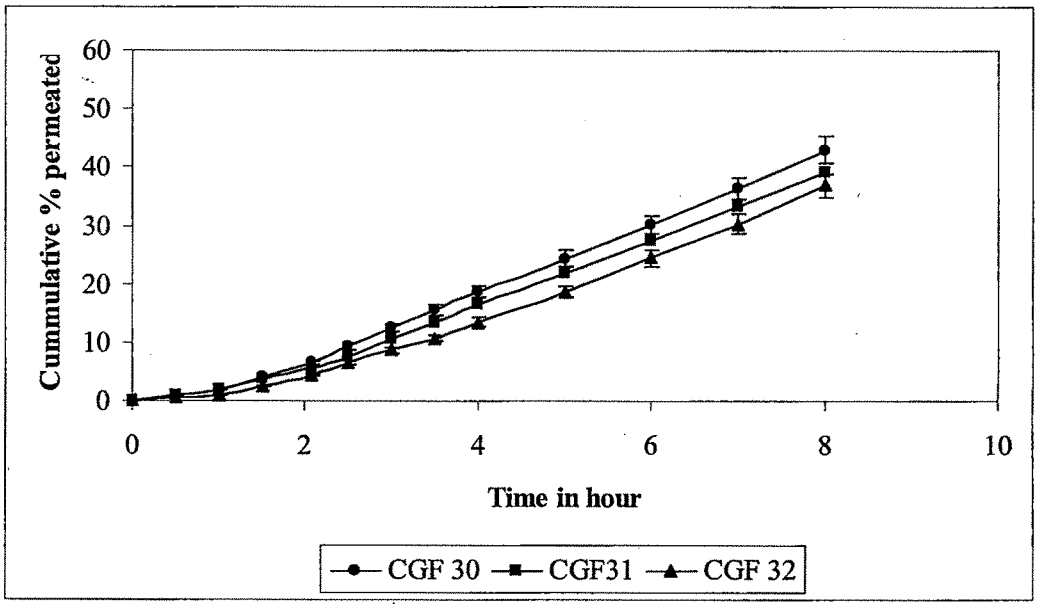


Table no. 4.80: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal gels

Formulations	Permeation flux $J(mcg.cm^{-2}.hr^{-1})$	Lag time ($t_L$hr)	Diffusion coefficient $(D\times10^{-8}cm^2.sec^{-1})$	Permeability coefficient $(P\times10^{-8}cm.sec^{-1})$
MGF30	5.92	1.00	1.070	1.536
MGF31	5.72	1.25	0.855	1.485
MGF32	5.42	1.50	0.713	1.407
CGF30	5.92	0.90	1.180	1.582
CGF31	5.58	1.35	0.792	1.491
CGF32	5.43	1.75	0.611	1.451

4.8.8 Stability Study

The formulations MGF30, CGF30, MGF31and CGF31 were studied for the stability of the formulation at Freeze condition (4°C) and at RT (25 °C). All the four formulations showed good physical and chemical stability with no discoloration, precipitation or any physical changes after storage. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of all the formulations was within the range of 5.90 to 5.95, which is the neutral pH.

Table no. 4.81: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF30	98.50 ± 0.92	5.90	32.3
MGF31	97.15 ± 0.72	5.95	29.8
CGF30	97.12 ± 0.79	5.91	31.7
CGF31	99.55 ± 0.75	5.93	29.1

Table no. 4.82: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF30	99.22 ± 0.95	5.92	32.2
MGF31	98.36 ± 0.87	5.96	29.5
CGF30	97.75 ± 0.76	5.96	31.5
CGF32	98.15 ± 0.88	5.95	29.0

4.8.9 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with mucoadhesive polymer HEC by incorporating antibiotics minocycline hydrochloride and clindamycin phosphate. Gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. At 4°C all the formulations were at liquid state with viscosity ranging from 17.89 mPas to 30.36 mPas for 19 % w/w to 25 % w/w pluronic F127 with 0.25-0.75 w/w % HEC. Rheological behavior of all the formulations measured was shown to exhibit Newtonian behavior at 4°C, where, all the formulations remained as liquid with no gel formation. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer HEC lowered the gelation temperature. It is also noted that addition of increasing concentration of HEC from 0.25-0.75% w/w further lowered the gelation temperature. The gelation temperature lowering effect of mucoadhesive polymer might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of HEC which may be due to bond formation between pluronic F127 and HEC. Increase in gel strength shows that the addition of HEC increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property thereby increasing the residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of HEC. From the study it was evidenced that the availability of the carboxyl groups determines the mucoadhesion. Presence of mucoadhesive polymer HEC having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.25% w/w HEC concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The higher release of minocycline hydrochloride/ clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation prepared with 20%w/w pluronic F127 along with 0.25% HEC (MGF30 and CGF30) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.25% HEC (MGF31 and CGF31). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF30 and CGF30. In addition, formulation MGF30 and CGF30 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF31 and CGF31 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for HEC- pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it may be hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and

clindamycin phosphate was significantly different in formulations containing the HEC ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of HEC results in very rapid dissolution of the drug due to swelling and dissolution of HEC. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations this may be due to the formations of larger concentrations of the micelles. Addition of the HEC increases the drug permeation compared to the plain pluronic F127 formulations , this may be due to increase in concentrations of ionized hydroxyl group to a level require to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized hydroxyl group results in de-coiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized HEC.

The investigation of in vitro release and permeation data showed that the mechanism of drug release followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stability during the storage period of six months, and their chemical and mechanical property does not change significantly.

It may be concluded that the mucoadhesive polymer HEC increases mucoadhesive, physico-chemical and mechanical properties than compared to the plain periodontal thermoreversible gels. Thermoreversible gel formulations maintained a satisfactory residence time in the periodontal cavity and ensured zero order of release of the drug over relatively longer period, which made them good candidate for drug delivery system through periodontal route for the treatment of infectious periodontal diseases.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.25% HEC along with 20% and 23 % w/w pluronic F127 were found to be best among HEC-pluronic F127 thermoreversible gel formulations.

4.9 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND POLY VINYL PYRROLIDONE

4.9.1 Preparation of mixed PVP - pluronic F127 periodontal gels

Formulations containing the minocycline hydrochloride (1%) and clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with PVP. The compositions of the formulations are cited in table no 4.83 to 4.88.

TABLE NO 4.83: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVP (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF41	MGF42	MGF43	MGF44
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	40	34	30	28
Visual gel Temp. (°C)	40.7	34.5	30.6	28.7
Drug content	98.63 ± 0.38	99.65 ± 0.36	99.48 ± 0.59	97.76 ± 0.31
Mucoadhesion (gf/mm)	17.82 ± 0.18	20.36 ± 0.93	23.47 ± 1.03	27.76 ± 1.21
Gel strength (N/m)	9847.72 ± 111.28	16203.74 ± 121.39	19328.58 ± 99.32	21529.49 ± 132.39
pH (Sol)	5.71	5.78	5.85	5.79
pH (Gel)	5.73	5.82	5.89	5.82
Sol Viscosity mPas	16.85	23.92	25.67	28.29
Gel Viscosity mPas	2048	2937	3219	3439

Table No 4.84: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVP (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF45	MGF46	MGF47	MGF48
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	1.00	1.00	1.00	1.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	35	30	26	24
Visual gel Temp. (°C)	35.8	30.7	26.5	24.5
Drug content	97.39 ± 0.97	97.81 ± 0.38	98.29 ± 0.36	98.37 ± 0.56
Mucoadhesion (gf/mm)	18.91 ± 1.17	23.49 ± 0.98	27.59 ± 1.34	30.27 ± 1.46
Gel strength (N/m)	10195.63 ± 134.76	15697.73 ± 127.82	18824.82 ± 104.57	21937.81 ± 98.72
pH (Sol)	5.71	5.78	5.85	5.79
pH (Gel)	5.73	5.82	5.89	5.82
Sol Viscosity mPas	17.64	22.79	26.48	29.03
Gel Viscosity mPas	2204	3029	3783	3429

Table No 4.85: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVP (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF49	MGF50	MGF51	MGF52
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	2.00	2.00	2.00	2.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	31	27	25	23
Visual gel Temp. (°C)	31.7	28.5	26.2	24.1
Drug content	99.21 ± 0.28	98.76 ± 0.19	99.16 ± 0.33	98.12 ± 0.44
Mucoadhesion (gf/mm)	19.29 ± 0.93	24.73 ± 1.13	27.75 ± 0.47	31.79 ± 1.17
Gel strength (N/m)	10933.44 ± 124.69	17403.57 ± 101.76	19473.83 ± 99.84	22497.87 ± 132.79
pH (Sol)	5.83	5.92	5.96	6.01
pH (Gel)	5.84	5.94	5.98	6.03
Sol Viscosity mPas	18.46	23.94	27.89	30.17
Gel Viscosity mPas	2349	3318	3419	3631

Table No 4.86: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PVP (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF41	CGF42	CGF43	CGF44
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	39	33	29	27
Visual gel Temp. (°C)	40.1	34.2	30.1	28.3
Drug content	97.6 ± 0.59	98.2 ± 0.75	98.38 ± 0.58	99.2 ± 0.63
Mucoadhesion (gf/mm)	18.04 ± 0.27	20.19 ± 0.30	23.48 ± 1.19	26.84 ± 1.04
Gel strength (N/m)	10137.49 ± 97.07	16738.59 ± 111.59	18974.62 ± 104.72	20934.84 ± 119.34
pH (Sol)	5.62	5.71	5.82	5.79
pH (Gel)	5.65	5.75	5.84	5.83
Sol Viscosity mPas	17.02	23.34	26.14	29.33
Gel Viscosity mPas	2092	3021	3364	3494

Table No 4.87: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PVP (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF45	CGF46	CGF47	CGF48
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	1.00	1.00	1.00	1.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	34	29	25	23
Visual gel Temp. (°C)	34.6	30.4	26.5	24.2
Drug content	99.19 ± 0.38	98.67 ± 0.58	99.21 ± 0.28	99.13 ± 0.49
Mucoadhesion (gf/mm)	19.23 ± 1.02	23.79 ± 1.11	27.86 ± 1.89	29.78 ± 1.43
Gel strength (N/m)	10234.69 ± 109.47	15923.79 ± 193.28	19382.48 ± 134.58	22389.79 ± 101.88
pH (Sol)	5.78	5.85	5.79	5.86
pH (Gel)	5.82	5.89	5.82	5.89
Sol Viscosity mPas	18.03	23.13	26.78	29.49
Gel Viscosity mPas	2279	3173	3824	3488

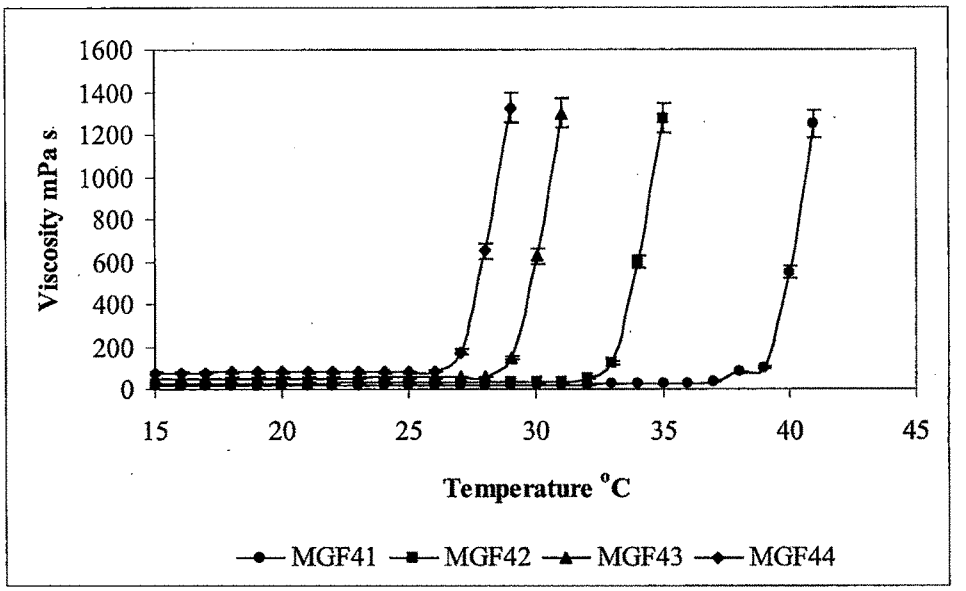
Table No 4.88: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PVP (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF49	CGF50	CGF51	CGF52
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVP (%w/w)	2.00	2.00	2.00	2.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	30	27	24	22
Visual gel Temp. (°C)	30.9	27.8	25.1	23.2
Drug content	99.61 ± 0.28	99.25 ± 0.56	98.67 ± 0.47	97.82 ± 0.32
Mucoadhesion (gf/mm)	19.63 ± 0.27	25.12 ± 0.93	28.32 ± 1.11	31.93 ± 0.86
Gel strength (N/m)	11244.38 ± 124.69	17892.39 ± 99.83	20278.47 ± 97.38	23147.79 ± 113.93
pH (Sol)	5.92	5.96	6.01	5.99
pH (Gel)	5.94	5.98	6.03	6.02
Sol Viscosity mPas	19.29	24.32	28.45	30.84
Gel Viscosity mPas	2431	3352	3478	3729

4.9.2 Viscosity and Gelling Temperature Determination

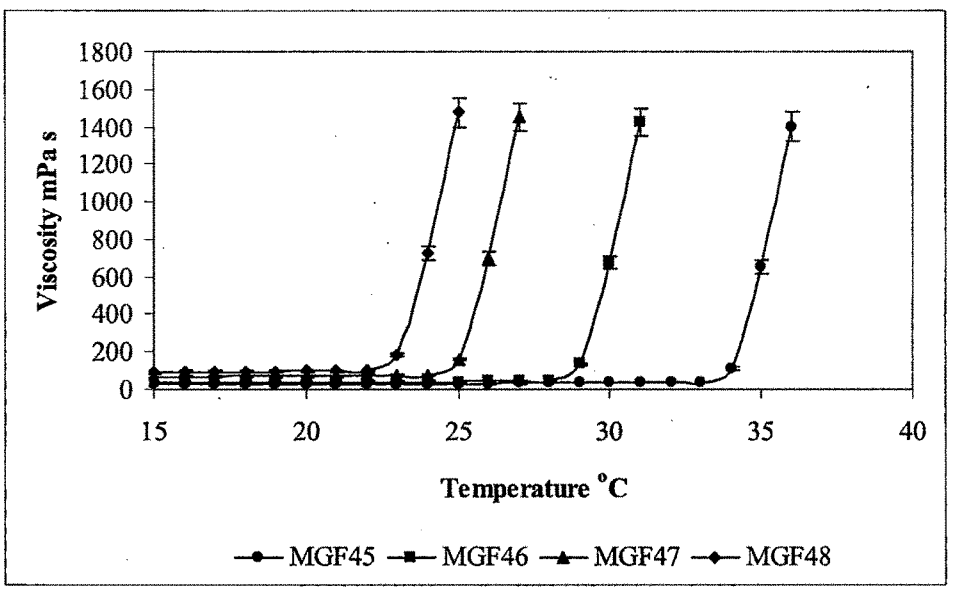
As evident from the results in table 4.83 to 4.88 the gelling temperature of pluronic F127 vehicle as determined by rheological method were lowered by the addition of increasing concentration of the mucoadhesive polymer PVP i.e. 0.50 % w/w, 1.00% w/w and 2.00 % w/w. Figure 4.56 to 4.61 shows the viscosity of various pluronic F127 gels with varying concentration of PVP measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method did not vary more than ± 1.5°C. The decrease in the gelation temperature with increase in PVP concentration may be due to enhanced viscosity of the gel formulation. The formulations showing the gelling temperature between 25°C to 37°C seemed to be proper for in situ gelling of the various vehicles at the periodontal cavity, minimizing the loss of administered drug due to clearance from the site of application.

Figure 4.56: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w PVP and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



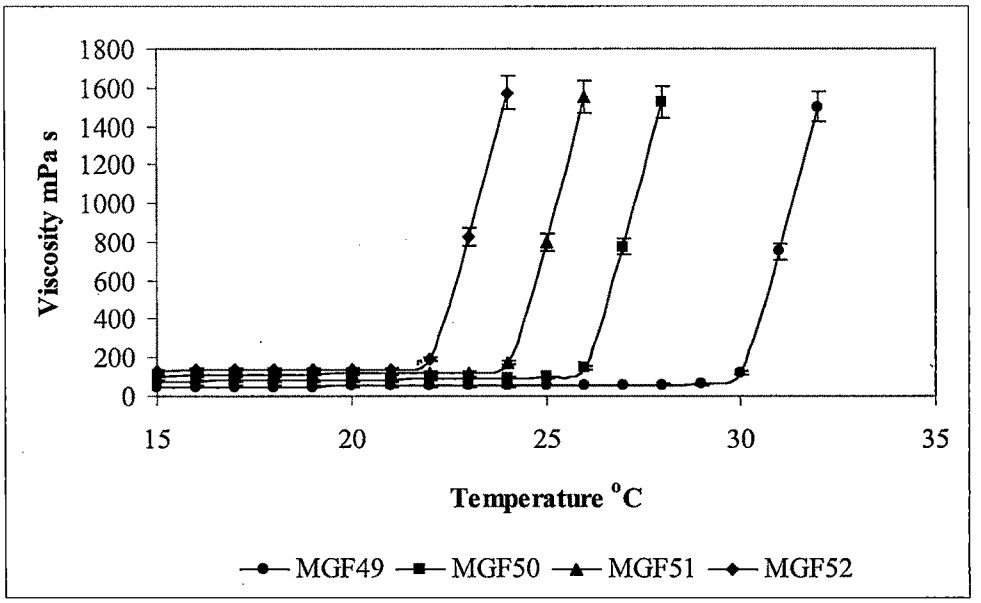
Values are expressed as mean ± SD (n =3)

Figure 4.57: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w PVP and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



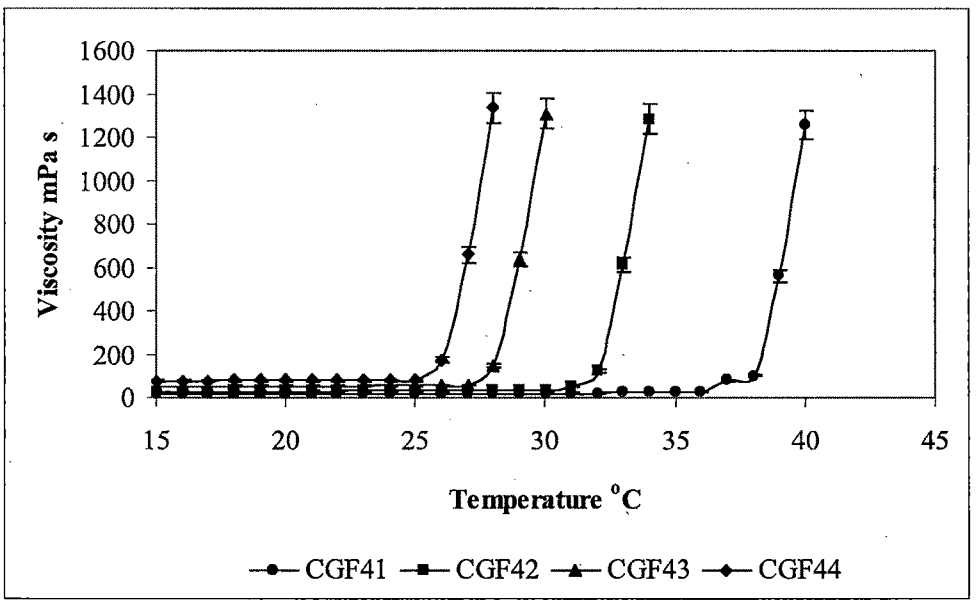
Values are expressed as mean ± SD (n =3).

Figure 4.58: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w PVP and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



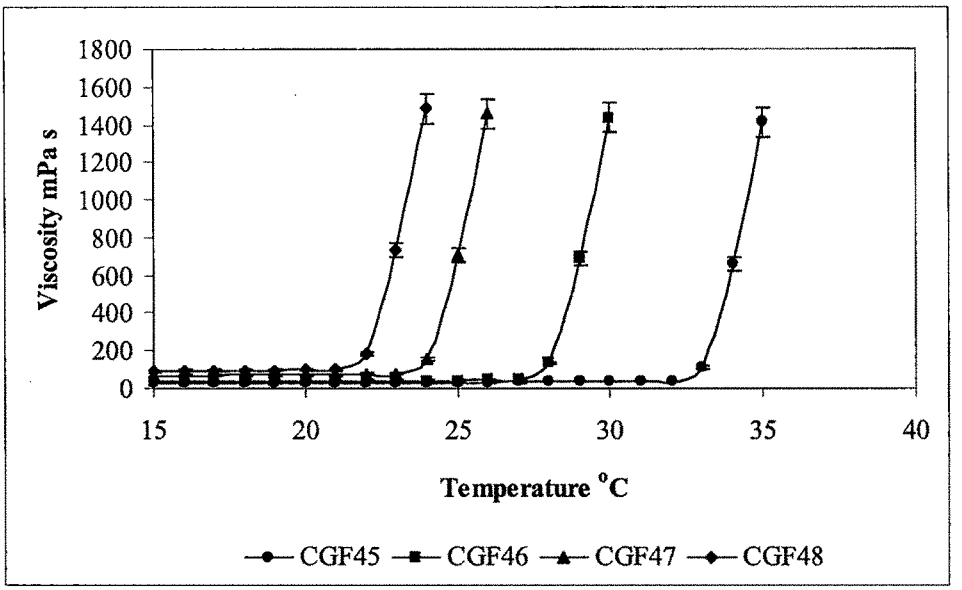
Values are expressed as mean ± SD (n =3)

Figure 4.59: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w PVP and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



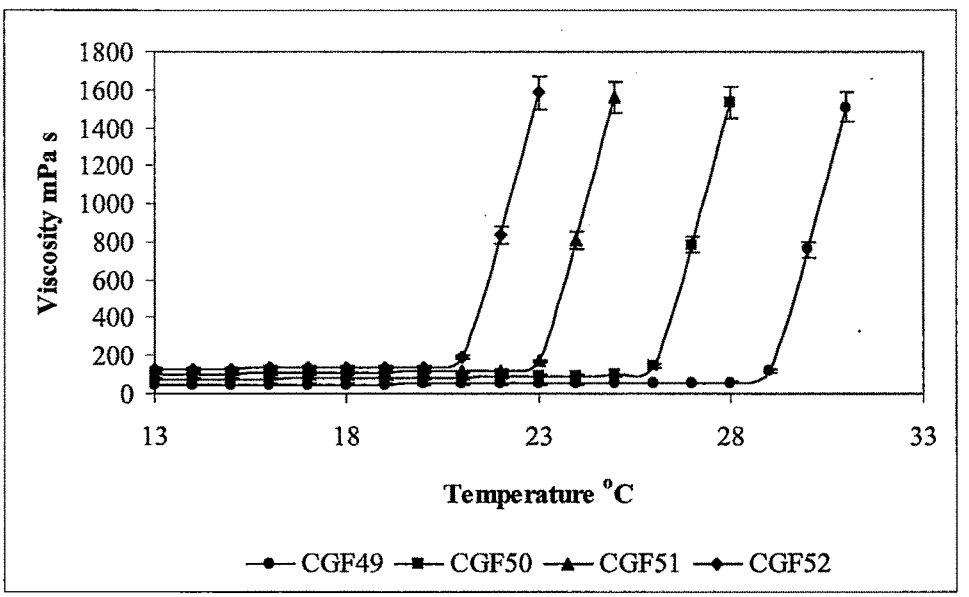
Values are expressed as mean ± SD (n =3).

Figure 4.60: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w PVP and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate.



Values are expressed as mean ± SD (n =3)

Figure 4.61: Effect of temperature on the viscosity of various PVP-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w PVP and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3).

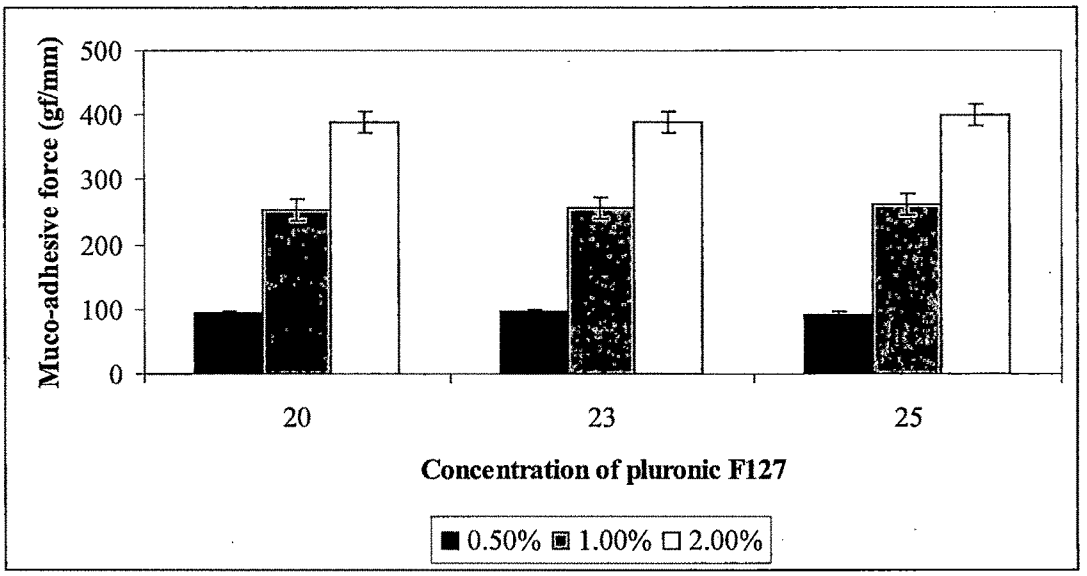
4.9.3 Gel Strength

The gel strength of the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.50 % w/w, 1.00 % w/w, and 2.00 % w/w PVP concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. There is no significant difference in the formulations containing minocycline hydrochloride and clindamycin phosphate. Increase in the gel strength, increase in stiffness, in presence of different concentration of PVP may be due to bond formation between pluronic F127 and PVP. Higher gel strength formulations possess the higher mucoadhesive property and increased residence time at the application site and thereby increasing the bioavailability of the drug.

4.9.4 Mucoadhesive Strength

The assessment of the mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of (Figure 4.62) increased concentration of PVP (0.50 %w/w, 1.00 %w/w, and 2.00 %w/w). Difference in the mucoadhesive strength of the formulations of PVP is non significant ($P>0.001$). From the results it is evident that the availability of number of the hydroxyl groups present may determine the mucoadhesion. Thus PVP having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoprotein. There is evidence that the drug delivery systems with high mucoadhesive strength possess prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.62: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of PVP- pluronic F127



4.9.5 Syringeability

The assessment of the syringeability was done in terms of force required to syringe the formulation to the application site. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations increases significantly ($P< 0.001$) with increase in PVP concentration with respect to plain pluronic F127 gel. The results of the syringeability are shown in table no 4.89.

Table No 4.89: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of PVP- pluronic F127

Formulation Code	Syringeability
MGF42	155.62
MGF43	201.55
MGF44	235.74
CGF42	178.38
CGF43	213.75
CGF44	250.49

4.9.6 In vitro release study

The in vitro release profile of minocycline hydrochloride/ clindamycin phosphate is illustrated in Figure 4.63 and 4.64 respectively. The maximum release of minocycline hydrochloride from the thermoreversible gels was shown by the formulation MGF42 where as the least was shown by the formulation MGF 44 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF42 where as the least was shown by the formulation CGF44 after 8 hours. The high release of minocycline hydrochloride and clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation MGF42 and CGF42 had low viscosity than MGF44 and CGF44. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF42 and CGF42. In addition, formulation MGF42 and CGF42 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF44 and CGF44 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.90: In Vitro Release Profile of Minocycline hydrochloride from PVP - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride released ± SD		
	MGF42	MGF43	MGF44
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	4.09 ± 0.025	2.38 ± 0.007	1.24 ± 0.049
1.00	6.30 ± 0.025	4.05 ± 0.041	2.41 ± 0.051
1.50	8.48 ± 0.037	6.49 ± 0.022	4.03 ± 0.014
2.00	11.83 ± 0.047	8.41 ± 0.011	6.06 ± 0.046
2.50	14.68 ± 0.006	11.61 ± 0.011	8.44 ± 0.047
3.00	18.78 ± 0.001	14.72 ± 0.078	10.93 ± 0.032
3.50	22.71 ± 0.008	18.62 ± 0.010	14.04 ± 0.019
4.00	26.64 ± 0.074	22.86 ± 0.004	17.57 ± 0.040
5.00	34.64 ± 0.005	30.81 ± 0.004	25.23 ± 0.047
6.00	42.32 ± 0.030	37.81 ± 0.050	32.16 ± 0.041
7.00	50.34 ± 0.046	45.62 ± 0.045	39.72 ± 0.038
8.00	57.72 ± 0.054	52.42 ± 0.049	47.13 ± 0.060

Figure 4.63: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from PVP - Pluronic F127 thermoreversible periodontal gel

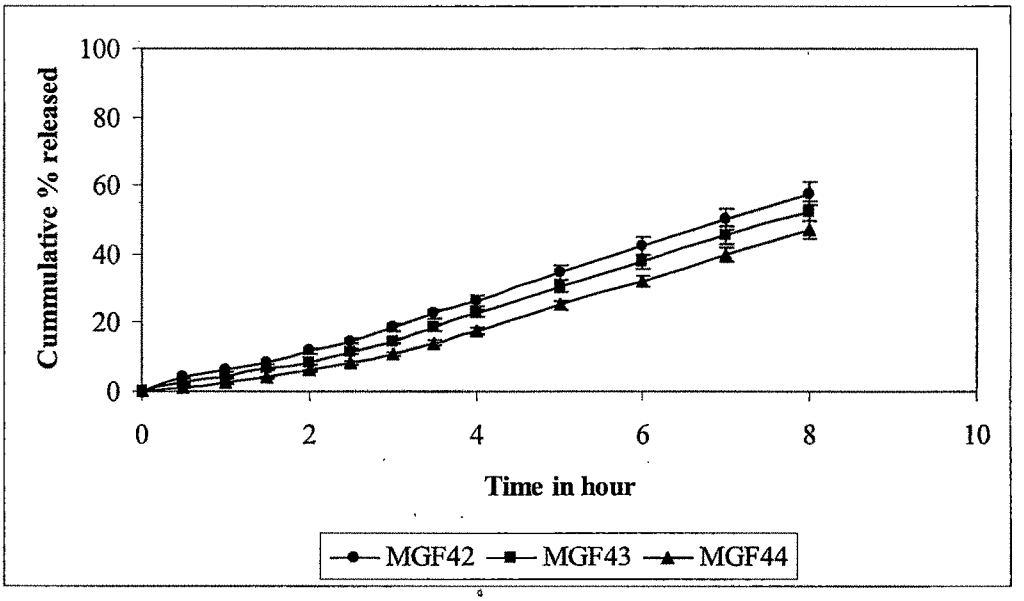


Table No 4.91: In Vitro Release Profile of clindamycin phosphate from PVP - Pluronic F127 thermoreversible periodontal gel

Time in Hour	% Clindamycin Released ± SD		
	CGF42	CGF43	CGF44
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.76 ± 0.032	1.51 ± 0.050	1.06 ± 0.039
1.00	5.13 ± 0.035	3.17 ± 0.004	1.97 ± 0.035
1.50	7.39 ± 0.041	5.51 ± 0.040	3.13 ± 0.077
2.00	11.13 ± 0.035	8.31 ± 0.057	5.37 ± 0.037
2.50	15.12 ± 0.033	11.77 ± 0.027	8.62 ± 0.038
3.00	19.05 ± 0.033	15.84 ± 0.021	12.05 ± 0.057
3.50	23.72 ± 0.035	19.05 ± 0.043	15.52 ± 0.029
4.00	27.84 ± 0.069	23.34 ± 0.031	19.22 ± 0.040
5.00	35.46 ± 0.068	30.97 ± 0.046	26.25 ± 0.045
6.00	42.24 ± 0.029	37.97 ± 0.046	32.38 ± 0.006
7.00	49.15 ± 0.020	44.14 ± 0.064	39.27 ± 0.023
8.00	56.72 ± 0.030	50.48 ± 0.034	45.63 ± 0.032

Figure 4.64: Cumulative percentage release profile of clindamycin phosphate in mcg/cm^2 from PVP - Pluronic F127 thermoreversible periodontal gel

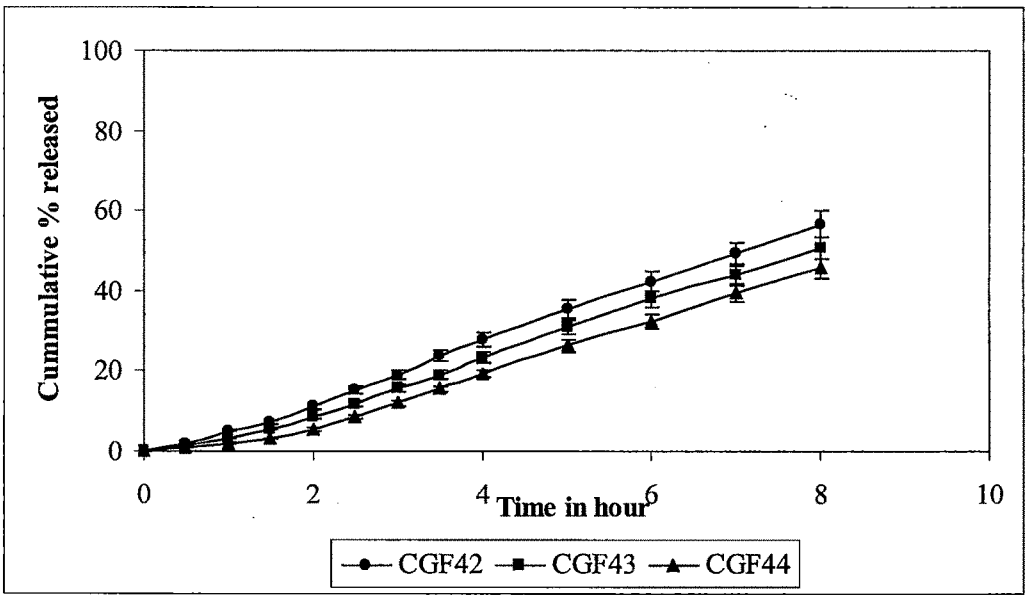


Table no. 4.92: Release Kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded PVP - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				n (Release exponent)	K (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF42	0.9953	0.4814	0.8818	0.5579	7.291	1.954
MGF43	0.9892	0.3891	0.8563	0.144	6.775	5.957
MGF44	0.9527	0.2755	0.8187	0.575	9.069	1.172
CGF42	0.9971	0.4647	0.8919	0.6082	7.358	2.280
CGF43	0.9927	0.3593	0.8693	0.6048	6.680	4.786
CGF44	0.9834	0.2592	0.8366	0.5922	6.046	1.112

4.9.7 In Vitro permeation study

4.9.7.1 Determination of saturated drug concentration

A saturated minocycline hydrochloride/ clindamycin phosphate was prepared as described in section 4.6.6.1. The saturated concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was 106.994 mg ml^{-}1 and 103.900 mg ml^{-1} respectively.

4.9.7.2 Preparation of mucosal tissue

The mucosal tissue was prepared as described in the section 4.6.6.2.

4.9.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane was measured as described in section 4.6.6.3. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ µm, which is the mean of 3 measurements.

PVP polymers were shown to express a high mucoadhesive property. The effective permeability coefficient determined for minocycline hydrochloride and clindamycin phosphate in each gel formulations are given in Table No4.95 and the cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various PVP-pluronic F127 gels formulations are given in the Figure 4.65 and 4.66 respectively. It is evident from the results that effective

permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for PVP-pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution. Since the pluronic F127 gels are viscous, isotropic liquid crystals containing micelles, it was hypothesized that the drug may release by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate significantly differs in formulations containing the PVP ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. The presence of PVP results in very rapid dissolution and release of drug due to swelling and dissolution of PVP at pH 6.75. However presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micelle structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations which may be due to the formation of larger concentrations of the micelle's. Addition of the PVP increases the drug permeation compared to the plain pluronic F127 formulations, which may be due to increase in wetability and swelling of the polymers. The swelling of the polymers was also due to ionic strength and pH (Park and Robinson, 1985). Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of ionized drug molecules helps in the formation of hydrogen binding site and relaxation of the polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability, formulations containing 0.25 % w/w PVP along with 20% and 23 % w/w pluronic F127 were found to be the best. However formulations containing 0.25 %w/w PVP along with 25 %w/w pluronic F127 showed lower gelling temperature, low permeation profile and high syringeability which may make difficult to administer the drug to the periodontal cavity. Formulations containing the higher concentrations of PVP (1.00 % w/w and 2.00 % w/w) showed a high syringeability and blockage of the syringe which may be due to high viscous solution. Hence MGF42, CGF42, MGF43 and CGF43 was selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No 4.93: In Vitro Permeation Profile of Minocycline hydrochloride from PVP - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF 42	MGF 43	MGF 44
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.24 ± 0.025	1.04 ± 0.109	0.77 ± 0.031
1.00	3.27 ± 0.031	2.86 ± 0.018	2.01 ± 0.015
1.50	5.11 ± 0.025	4.38 ± 0.027	3.93 ± 0.027
2.00	7.28 ± 0.036	6.96 ± 0.054	5.63 ± 0.034
2.50	9.57 ± 0.051	8.97 ± 0.039	7.05 ± 0.027
3.00	12.83 ± 0.032	11.85 ± 0.081	10.16 ± 0.057
3.50	15.71 ± 0.043	14.59 ± 0.034	12.35 ± 0.042
4.00	18.83 ± 0.056	17.52 ± 0.017	16.41 ± 0.012
5.00	26.48 ± 0.035	25.19 ± 0.028	23.55 ± 0.015
6.00	34.08 ± 0.023	32.82 ± 0.032	31.02 ± 0.045
7.00	42.00 ± 0.024	40.45 ± 0.017	39.27 ± 0.052
8.00	50.26 ± 0.058	48.86 ± 0.046	46.92 ± 0.019

Figure 4.65: Cumulative permeation profile of minocycline hydrochloride from PVP - pluronic F127 thermoreversible periodontal gel

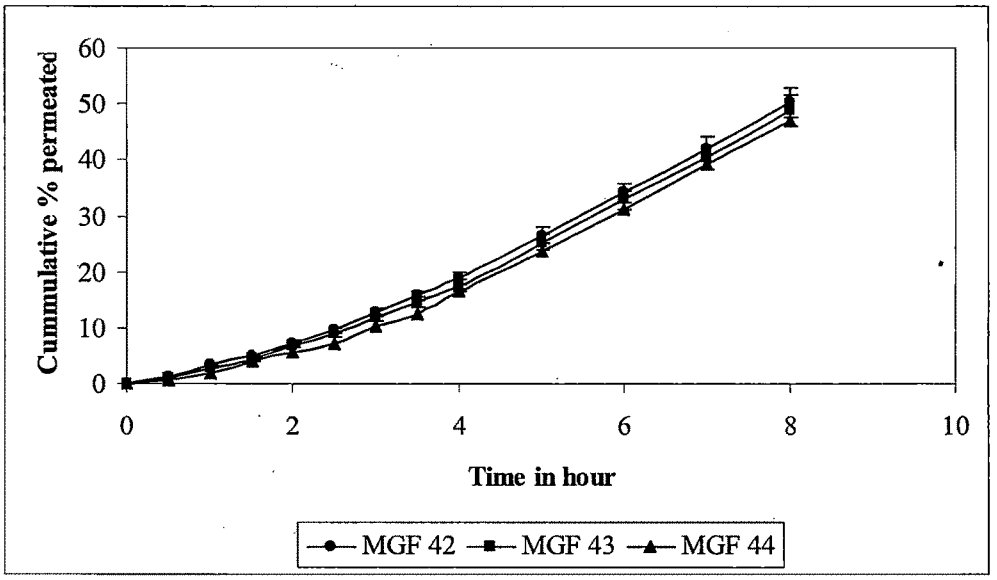


Table No 4.94: In Vitro Permeation Profile of Clindamycin phosphate from PVP - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF 42	CGF 43	CGF 44
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.20 ± 0.017	0.96 ± 0.105	0.54 ± 0.022
1.00	2.87 ± 0.029	1.96 ± 0.025	1.05 ± 0.016
1.50	4.42 ± 0.018	3.77 ± 0.033	2.47 ± 0.017
2.00	6.97 ± 0.027	5.55 ± 0.037	4.13 ± 0.009
2.50	8.93 ± 0.041	8.04 ± 0.026	5.98 ± 0.033
3.00	11.67 ± 0.028	10.39 ± 0.013	8.24 ± 0.021
3.50	14.34 ± 0.039	13.44 ± 0.046	11.11 ± 0.029
4.00	17.70 ± 0.057	16.20 ± 0.016	13.84 ± 0.038
5.00	25.11 ± 0.037	22.86 ± 0.028	20.61 ± 0.042
6.00	32.90 ± 0.023	30.37 ± 0.031	28.16 ± 0.017
7.00	40.53 ± 0.009	38.37 ± 0.009	35.93 ± 0.051
8.00	48.42 ± 0.059	45.80 ± 0.046	43.74 ± 0.039

Figure 4.66: Cumulative permeation profile of clindamycin phosphate from PVP - Pluronic F127 thermoreversible periodontal gel

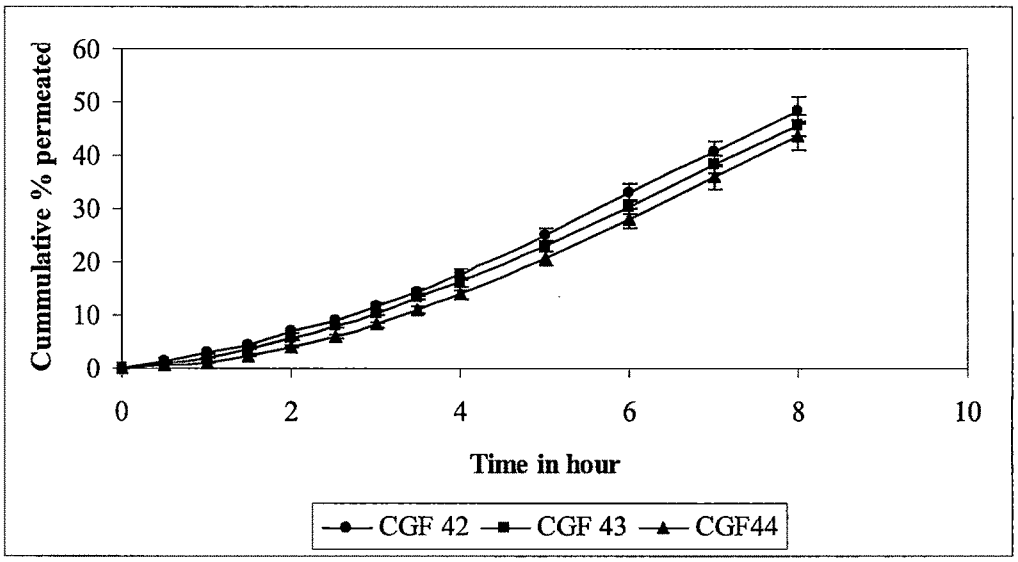


Table no. 4.95: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal gels

Formulations	Permeation flux J(mcg.cm^{-2}.hr^{-1})	Lag time ($t_L$hr)	Diffusion coefficient (D×10^{-8}cm^2.sec^{-1})	Permeability coefficient (P×10^{-8}cm.sec^{-1})
MGF42	6.82	1.35	0.792	1.778
MGF43	6.66	1.65	0.648	1.729
MGF44	6.56	2.00	0.534	1.703
CGF42	6.87	1.65	0.648	1.836
CGF43	6.58	2.00	0.534	1.761
CGF44	6.47	2.25	0.475	1.729

4.9.8 Stability Study

The Minocycline hydrochloride/Clindamycin phosphate loaded periodontal thermoreversible gels prepared using 20 % w/w and 23 % w/w pluronic F127 along with 0.50 % w/w PVP were studied for the stability of the formulation at Freeze condition (4°C) and at room temperature. All the four formulations showed good physical stability, as there was no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of all the formulations was within the range of 5.67 to 5.82, which is the neutral pH.

Table no. 4.96: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF42	97.45 ± 0.95	5.75	34.4
MGF43	98.35 ± 0.78	5.82	30.2
CGF42	99.11 ± 0.89	5.68	33.9
CGF43	98.85 ± 0.85	5.76	29.7

Table no. 4.97: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature.

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF42	98.12 ± 0.85	5.74	34.2
MGF43	97.46 ± 0.91	5.80	30.0
CGF42	98.35 ± 0.77	5.67	33.7
CGF43	97.65 ± 0.78	5.72	29.4

4.9.9 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with mucoadhesive polymer PVP by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate. Gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. At 4°C all the formulations were at liquid state with viscosity ranging from 16.85 mPas to 30.84 mPas for 19 % w/w to 25 %w/w pluronic F127 with 0.50-2.00 %w/w PVP. Rheological behavior of all the formulations was measured and shown to exhibit Newtonian behavior at 4°C; all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant.

It is evident from the data that the presence of mucoadhesive polymer PVP lowered the gelation temperature. It is also noted that addition of increasing concentration of PVP from 0.50-2.00 % w/w further lowered the gelation temperature. The gelation temperature lowering effect of mucoadhesive polymer might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the PVP is exposed to water the polymer begins to uncoil and generating an increase in viscosity and gel formation. The uncoiling and expansion of the molecule result in polymer swelling and elastic gel formation.

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of PVP, which may be due to bond formation between pluronic F127 and PVP. Increase in gel strength shows that the addition of PVP increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property and increases the residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that presence of mucoadhesive polymer PVP having high density of available ionized groups would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues. Hence the pluronic F127 preparations possess adhesive properties that increase with addition of PVP.

Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; which may be due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.50% w/w PVP concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation study showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The higher release of minocycline hydrochloride/ clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation prepared with 20%w/w pluronic F127 along with 0.50% PVP (MGF42 and CGF42) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.50 % PVP (MGF43 and CGF43). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF42 and CGF42. In addition, formulation MGF42 and CGF42 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane. Similarly formulation MGF43 and CGF43 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for PVP-pluronic F127 thermoreversible gels than plain pluronic F127 gels. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in

formulations containing the PVP (P>0.001) compared to the plain pluronic F127 thermoreversible gels. Presence of PVP results in very rapid dissolution of the drug due to swelling and dissolution of PVP. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations, which may be due to the formations of larger concentrations of the micelles. Addition of the PVP increases the drug permeation compared to the plain pluronic F127 formulations , this may be due to increase in concentrations of ionized group to a level require to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized group results in de-coiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized PVP.

The investigation of in vitro release and permeation data showed that the diffusion is the mechanism of drug release and followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stability during the storage period of six months, and their chemical and mechanical property does not change significantly.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.50 % PVP along with 20% and 23 % w/w pluronic F127 were found to be best for periodontal among PVP-pluronic F127 formulations.

4.10 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND CARBOPOL 934P

4.10.1 Preparation of mixed carbopol 934P - pluronic F127 periodontal gels

Formulations containing the minocycline hydrochloride (1%) and clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with carbopol 934P. The compositions of the formulations are cited in Table No4.98 to 4.105.

Table No 4.98: Composition and Characteristics of MnHCl loaded mixed periodontal gels of carbopol 934P (0.20%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF53	MGF54	MGF55	MGF56
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.20	0.20	0.20	0.20
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	39	30	28	26
Visual gel Temp. (°C)	40.2	31.2	29.2	27.1
Drug content	98.6 ± 0.29	98.95 ± 0.11	99.38 ± 0.41	97.45 ± 0.54
Mucoadhesion (gf/mm)	13.78 ± 0.38	14.28 ± 0.47	16.93 ± 0.72	19.21 ± 0.57
Gel strength (N/m)	8764.93 ± 19.23	9567.73 ± 23.68	13022.74 ± 11.97	15364.78 ± 62.42
pH (Sol)	6.02	6.07	6.17	6.19
pH (Gel)	6.05	6.10	6.19	6.21
Sol Viscosity mPas	11.24	17.62	19.86	23.67
Gel Viscosity mPas	1923	2919	3172	3764

Table No 4.99: Composition and Characteristics of MnHCl loaded mixed periodontal gels of carbopol 934P (0.30%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF57	MGF58	MGF59	MGF60
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.30	0.30	0.30	0.30
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	36	28	25	22
Visual gel Temp. (°C)	37.3	29.1	25.8	23.1
Drug content	98.61 ± 0.18	98.73 ± 1.12	99.26 ± 0.27	98.79 ± 0.91
Mucoadhesion (gf/mm)	13.98 ± 0.17	15.92 ± 1.03	18.58 ± 0.48	19.89 ± 0.28
Gel strength (N/m)	9782.37 ± 73.22	10278.37 ± 49.21	14102.38 ± 29.76	15978.28 ± 57.29
pH (Sol)	6.12	6.18	6.21	6.27
pH (Gel)	6.14	6.21	6.23	6.29
Sol Viscosity mPas	14.81	18.92	20.31	26.73
Gel Viscosity mPas	2289	3476	3672	4012

Table No 4.100: Composition and Characteristics of MnHCl loaded mixed periodontal gels of carbopol 934P (0.40%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF61	MGF62	MGF63	MGF64
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.40	0.40	0.40	0.40
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	34	25	23	20
Visual gel Temp. (°C)	35.2	26.3	24.2	21.1
Drug content	98.7 ± 0.71	99.28 ± 0.37	97.68 ± 0.38	98.38 ± 0.41
Mucoadhesion (gf/mm)	15.21 ± 0.37	16.48 ± 0.29	19.27 ± 0.72	21.02 ± 0.41
Gel strength (N/m)	9986.28 ± 38.02	10567.39 ± 38.03	14527.58 ± 52.14	16372.28 ± 41.33
pH (Sol)	6.25	6.47	6.34	6.51
pH (Gel)	6.27	6.51	6.38	6.53
Sol Viscosity mPas	16.24	19.23	21.27	27.17
Gel Viscosity mPas	2447	3628	3984	4438

Table No 4.101: Composition and Characteristics of MnHCl loaded mixed periodontal gels of carbopol 934P (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF65	MGF66	MGF67	MGF68
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	32	23	21	17
Visual gel Temp. (°C)	33.1	24.5	22.3	18.2
Drug content	98.35 ± 0.38	99.21 ± 0.11	97.94 ± 0.19	99.38 ± 0.33
Mucoadhesion (gf/mm)	15.31 ± 0.25	17.71 ± 0.39	20.38 ± 0.33	22.09 ± 0.52
Gel strength (N/m)	10137.38 ± 27.38	10637.83 ± 38.29	13873.49 ± 29.54	15687.56 ± 52.38
pH (Sol)	6.29	6.33	6.48	6.49
pH (Gel)	6.32	6.37	6.51	6.52
Sol Viscosity mPas	17.04	19.32	24.75	29.69
Gel Viscosity mPas	2702	3892	4012	4315

Table No 4.102: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of carbopol 934P (0.20%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF53	CGF54	CGF55	CGF56
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.20	0.20	0.20	0.20
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Rheological gel Temp. (°C)	38	29	28	25
Visual gel Temp. (°C)	38.7	30.2	29.2	25.6
Drug content	97.63 ± 0.54	98.15 ± 0.28	99.29 ± 0.38	99.04 ± 0.57
Mucoadhesion (gf/mm)	14.12 ± 0.29	14.42 ± 0.27	17.21 ± 0.38	19.76 ± 0.41
Gel strength (N/m)	8803.38 ± 22.73	9704.28 ± 27.46	13219.77 ± 19.34	15472.43 ± 38.91
pH (Sol)	6.07	6.17	6.19	6.23
pH (Gel)	6.10	6.19	6.21	6.28
Sol Viscosity mPas	11.36	17.89	20.01	23.82
Gel Viscosity mPas	1955	3029	3357	3804

Table No 4.103: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of carbopol 934P (0.30%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF57	CGF58	CGF59	CGF60
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.30	0.30	0.30	0.30
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	35	27	24	21
Visual gel Temp. (°C)	35.7	28.2	25.1	21.8
Drug content	99.25 ± 0.38	99.38 ± 0.28	98.57 ± 0.35	99.19 ± 0.54
Mucoadhesion (gf/mm)	14.12 ± 0.21	16.25 ± 0.75	18.93 ± 0.57	20.38 ± 0.42
Gel strength (N/m)	9324.37 ± 44.27	10346.72 ± 39.67	14379.72 ± 22.38	20213.27 ± 47.93
pH (Sol)	6.18	6.21	6.27	6.47
pH (Gel)	6.21	6.23	6.29	6.50
Sol Viscosity mPas	14.95	19.45	20.48	26.84
Gel Viscosity mPas	2317	3495	3798	4048

Table No 4.104: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of carbopol 934P (0.40%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF61	CGF62	CGF63	CGF64
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.40	0.40	0.40	0.40
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	33	24	22	19
Visual gel Temp. (°C)	34.2	25.1	23.2	20.5
Drug content	99.15 ± 0.17	98.38 ± 0.27	98.91 ± 0.36	97.89 ± 0.29
Mucoadhesion (gf/mm)	15.34 ± 0.21	16.52 ± 0.31	19.46 ± 0.38	21.45 ± 0.37
Gel strength (N/m)	9994.27 ± 48.27	10672.37 ± 24.38	14616.78 ± 38.23	16487.29 ± 33.51
pH (Sol)	6.47	6.34	6.51	6.32
pH (Gel)	6.51	6.38	6.53	6.35
Sol Viscosity mPas	16.38	19.43	21.48	27.52
Gel Viscosity mPas	2495	3673	4011	4483

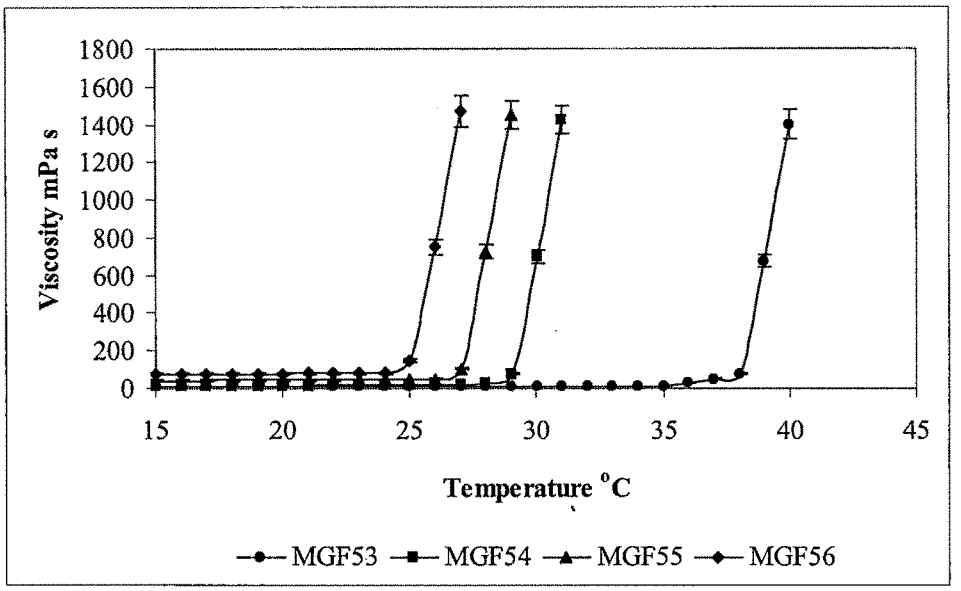
Table No 4.105: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of carbopol 934P (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF65	CGF66	CGF67	CGF68
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Carbopol934P (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH(%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	31	22	20	16
Visual gel Temp. (°C)	31.5	22.7	20.8	17.1
Drug content	99.23 ± 0.29	98.69 ± 0.33	99.25 ± 0.57	98.26 ± 0.37
Mucoadhesion (gf/mm)	15.42 ± 0.32	17.81 ± 0.36	20.43 ± 0.22	22.35 ± 0.47
Gel strength (N/m)	10348.72 ± 36.72	10822.38 ± 29.76	13927.63 ± 22.67	15789.76 ± 37.24
pH (Sol)	6.33	6.48	6.49	6.51
pH (Gel)	6.37	6.51	6.52	6.53
Sol Viscosity mPas	18.61	19.89	25.05	30.04
Gel Viscosity mPas	2730	3922	4031	4476

4.10.2 Viscosity and Gelling Temperature Determination

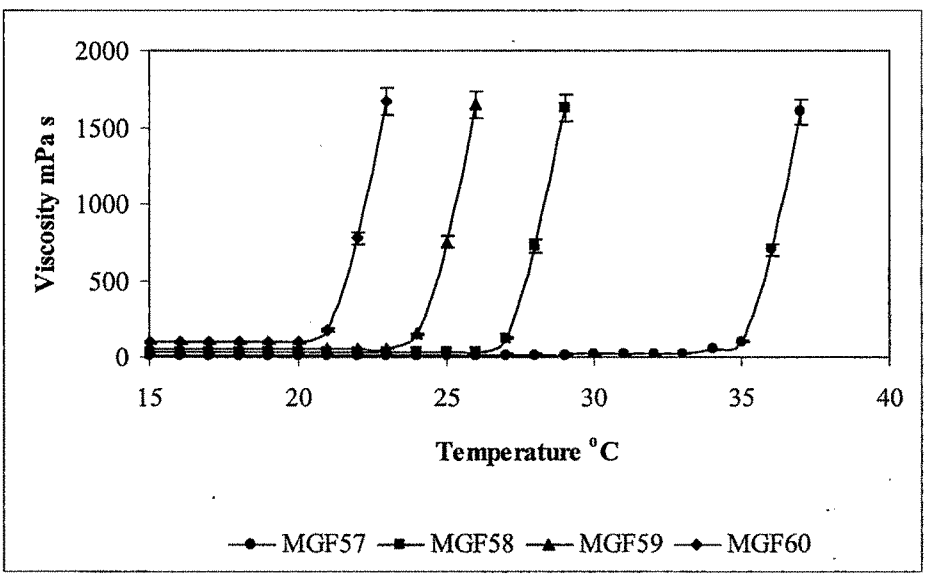
As evident from the results in Table No4.98 to 4.105, the gelling temperature of pluronic vehicle as determined by rheological method were lowered by the addition of increasing concentration of the mucoadhesive polymer carbopol 934P i.e. 0.20 % w/w to 0.50 % w/w. Figure 4.67 to 4.74 shows the viscosity of various pluronic gels with varying concentration of carbopol 934P measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method did not vary more than ± 1.5°C. The decrease in the gelation temperature with increase in carbopol 934P concentration may be due to enhanced viscosity of the gel formulation. The formulations showing the gelling temperature between 25°C to 37°C seems to be proper for in situ gelling of the various vehicles at the periodontal cavity, minimizing the loss of administered drug due to clearance from the site of application.

Figure 4.67: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.20 % w/w carbopol 934P and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



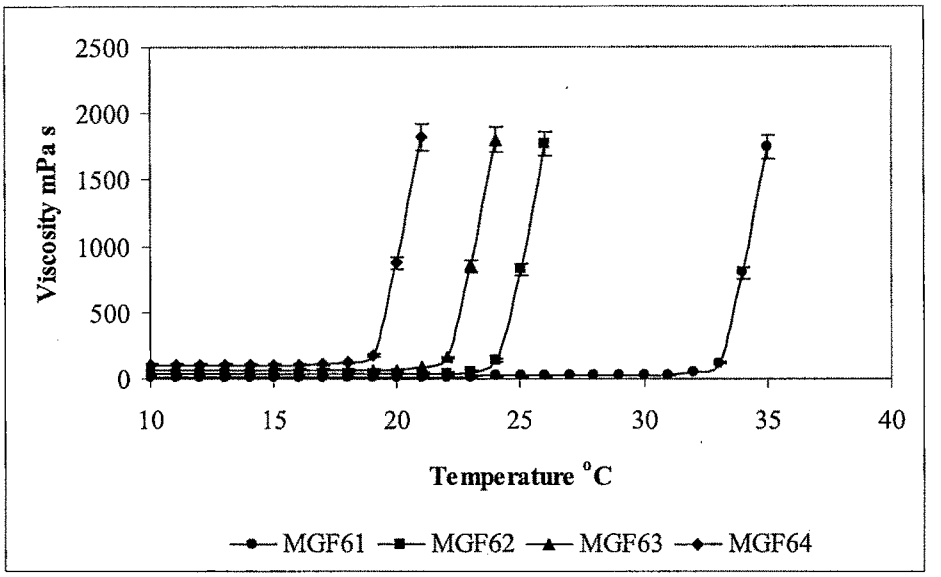
Values are expressed as mean ± SD (n =3)

Figure 4.68: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.30 % w/w carbopol 934P and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



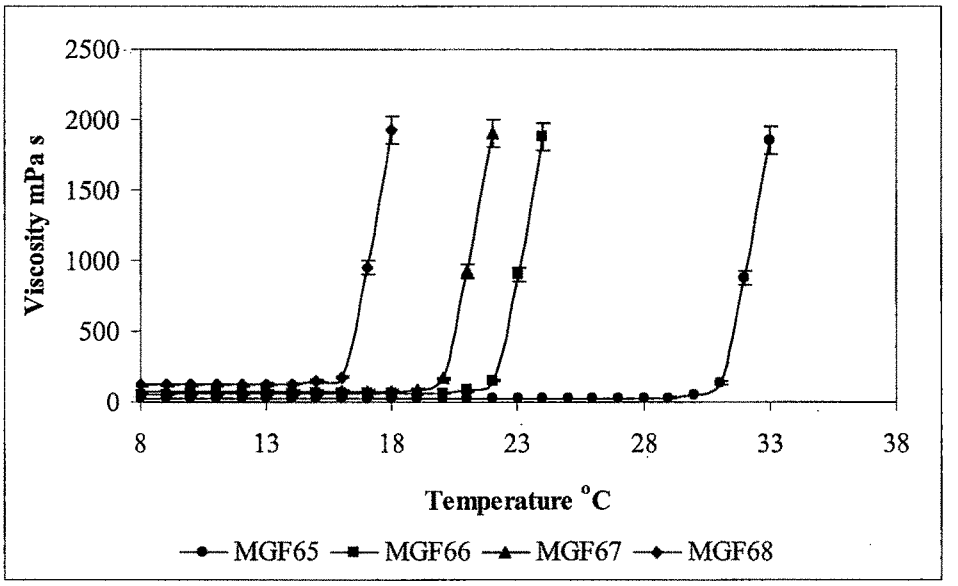
Values are expressed as mean ± SD (n =3).

Figure 4.69: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.40 % w/w carbopol 934P and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



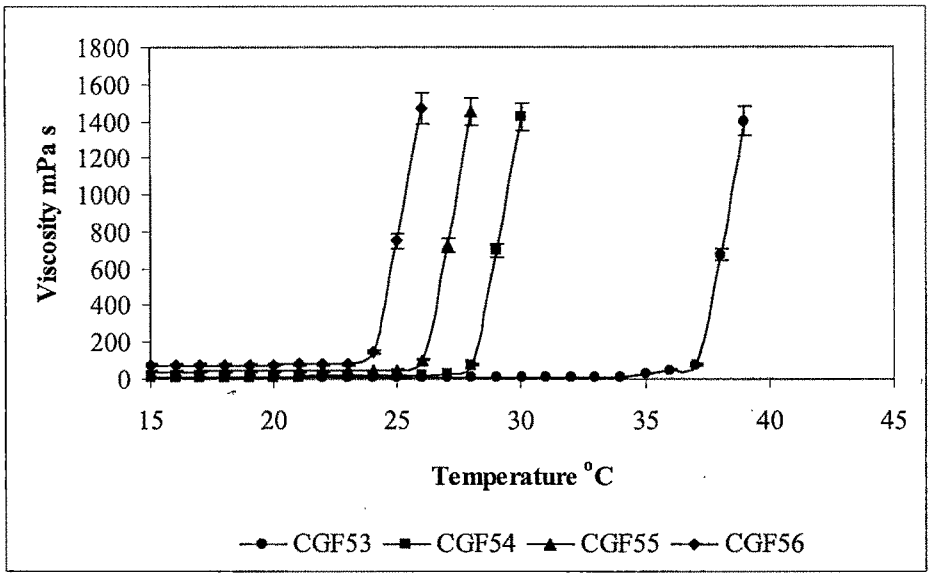
Values are expressed as mean ± SD (n =3)

Figure 4.70: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w carbopol 934P and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



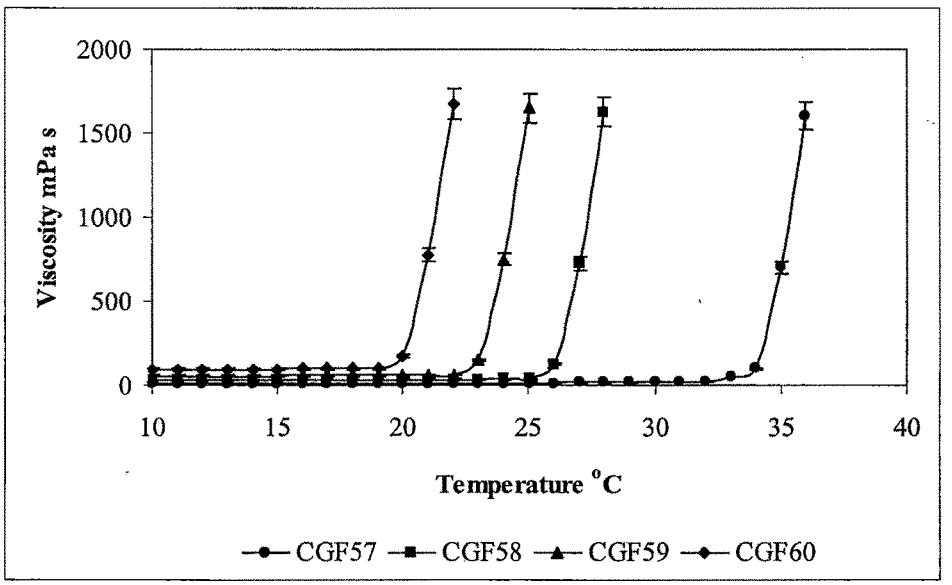
Values are expressed as mean ± SD (n =3).

Figure 4.71: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.20 % w/w carbopol 934P and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



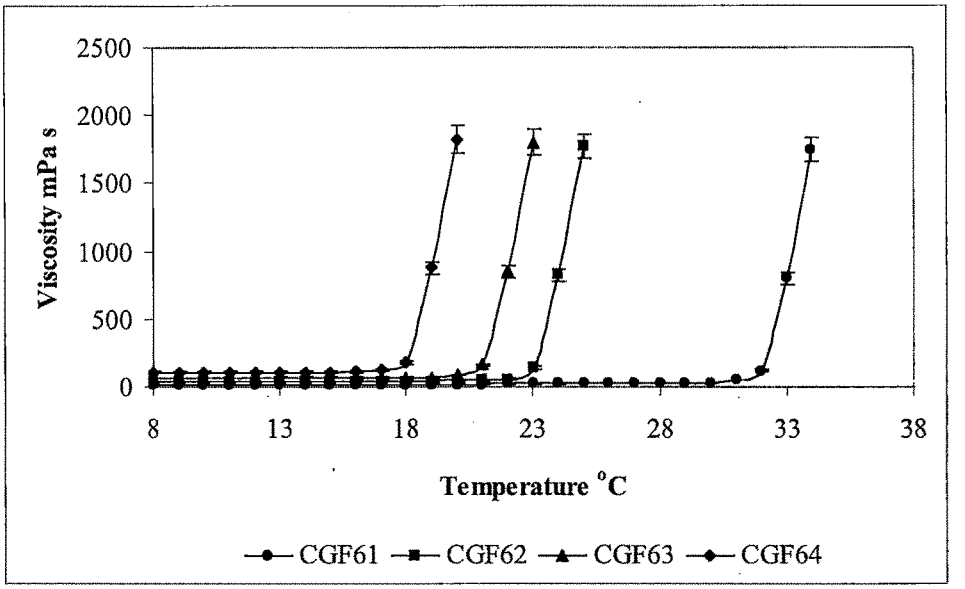
Values are expressed as mean ± SD (n =3)

Figure 4.72: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.30 % w/w carbopol 934P and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



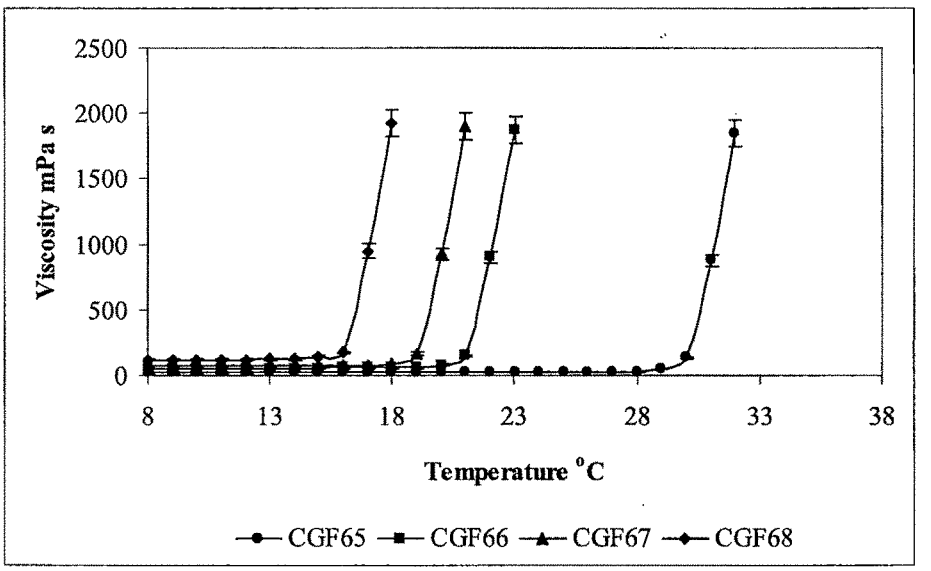
Values are expressed as mean ± SD (n =3)

Figure 4.73: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.40 % w/w carbopol 934P and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

Figure 4.74: Effect of temperature on the viscosity of various carbopol 934P-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w carbopol 934P and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

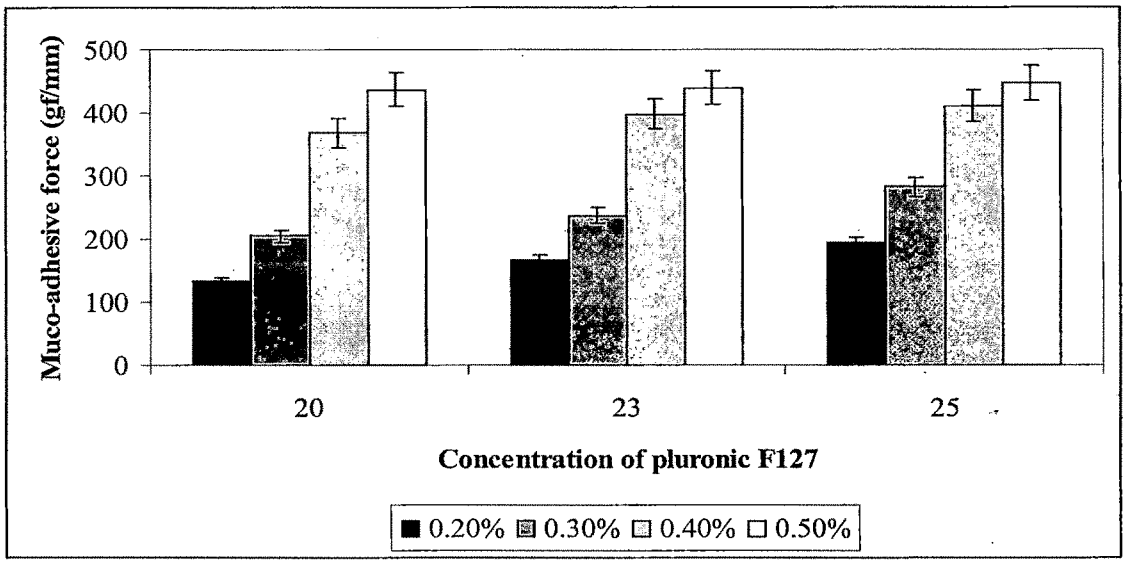
4.10.3 Gel strength

All the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.20 % w/w, 0.30 % w/w, 0.40 % w/w and 0.50 % w/w carbopol 934P concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel thereby increasing the stiffness of the gel, which may be due to bond formation between pluronic F127 and carbopol 934P. Higher gel strength formulations possess the high mucoadhesive property and increased residence time at the application site and thereby increase the bioavailability of the drug.

4.10.4 Mucoadhesive strength

The assessment of the mucoadhesive strength for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.20 % w/w, 0.30 % w/w, 0.40 % w/w and 0.50 % w/w carbopol 934P concentration increases significantly ($P<0.001$) with respect to plain pluronic F127 gel (Figure no. 4.75). Difference in the mucoadhesive strength of formulations prepared with 23% w/w and 25% w/w pluronic F127 along with 0.30 % w/w, 0.40 % w/w, and 0.50 % concentrations of carbopol 934P is non significant ($P>0.001$). Earlier work with carbopol polymers has clearly indicated that the number of carboxyl groups available determines the mucoadhesion; (Vlachou et al., 1996) carbopol 934P has very high percentage (58 % - 68 %) of oligosaccharide chains in the mucus membrane resulting in formation of strengthened network between polymer and mucus membrane. Thus carbopol having high density of available hydrogen bonding groups would be able to interact favorably the macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of delivery system may lead to the prolonged retention and increased absorption across mucosal tissue (Kunisawa et al., 2000).

Figure 4.75: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of carbopol 934P- pluronic F127



4.10.5 Syringeability

Syringeability for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.2% w/w, 0.3% w/w, 0.4% w/w and 0.5% w/w carbopol 934P concentration was done in similar manner as described in section 4.6.4 increases significantly ($P < 0.001$) with respect to plain pluronic F127 gel and was found to be viscosity dependent. The results of the syringeability are shown in Table No4.106.

Table No 4.106: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of carbopol 934P- pluronic F127

Formulation Code	Syringeability
MGF54	163.42
MGF55	201.5
MGF56	238.9
CGF54	189.5
CGF55	222.3
CGF56	295.6

4.10.6 In vitro release study

The release profile of minocycline hydrochloride and clindamycin phosphate is illustrated in Figure 4.76 and 4.77 respectively. The maximum release of minocycline hydrochloride from the thermoreversible gels was shown by the formulation MGF54 where as the least was shown by the formulation MGF56 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF54 where as the least was shown by the formulation CGF56 after 8 hours. A preliminary study showed that the formulation MGF54 and CGF54 had low viscosity than MGF56 and CGF56. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF54 and CGF54. In addition, formulation MGF56 and CGF56 due to low viscosity have more available waters to diffuse, hence consequently showed more diffusion through the membrane, similarly formulation MGF56 and CGF56 showed high viscosity which in turn have less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.107: In Vitro Release Profile of Minocycline hydrochloride from Carbopol 934P - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride released ± SD		
	MGF54	MGF55	MGF56
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	4.12 ± 0.034	3.52 ± 0.004	1.98 ± 0.035
1.00	7.57 ± 0.039	5.87 ± 0.024	4.30 ± 0.037
1.50	12.73 ± 0.024	9.45 ± 0.036	7.55 ± 0.037
2.00	16.91 ± 0.021	13.50 ± 0.025	10.76 ± 0.035
2.50	22.24 ± 0.039	17.20 ± 0.051	14.56 ± 0.045
3.00	26.75 ± 0.029	22.53 ± 0.027	18.45 ± 0.005
3.50	31.58 ± 0.102	27.07 ± 0.044	23.74 ± 0.007
4.00	37.11 ± 0.034	32.01 ± 0.022	28.30 ± 0.056
5.00	46.49 ± 0.002	41.57 ± 0.020	38.24 ± 0.040
6.00	56.56 ± 0.012	51.54 ± 0.001	48.44 ± 0.045
7.00	66.98 ± 0.027	60.57 ± 0.001	57.19 ± 0.030
8.00	75.77 ± 0.167	70.20 ± 0.042	66.11 ± 0.031

Figure 4.76: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from carbopol 934P - Pluronic F127 thermoreversible periodontal gel

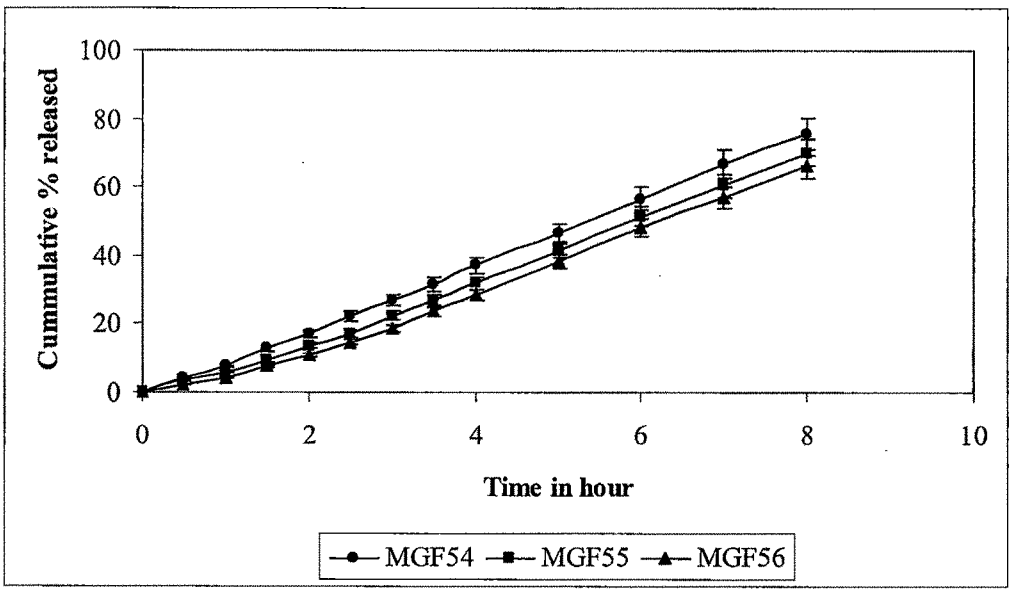


Table No 4.108: In vitro release profile of clindamycin phosphate from carbopol 934p - pluronic F127 thermoreversible periodontal gel

Time in Hour	% Clindamycin Phosphate Released ± SD		
	CGF54	CGF55	CGF56
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	3.11 ± 0.067	2.15 ± 0.037	1.06 ± 0.034
1.00	6.05 ± 0.019	4.09 ± 0.035	2.51 ± 0.043
1.50	12.06 ± 0.040	8.10 ± 0.042	6.13 ± 0.045
2.00	18.14 ± 0.007	13.22 ± 0.012	10.16 ± 0.052
2.50	24.15 ± 0.017	19.08 ± 0.045	15.19 ± 0.009
3.00	29.35 ± 0.054	24.06 ± 0.012	20.10 ± 0.010
3.50	34.08 ± 0.039	29.12 ± 0.046	24.21 ± 0.002
4.00	40.18 ± 0.046	34.10 ± 0.081	29.16 ± 0.020
5.00	50.02 ± 0.012	43.17 ± 0.058	37.06 ± 0.052
6.00	59.33 ± 0.036	52.09 ± 0.009	45.04 ± 0.030
7.00	68.10 ± 0.040	60.16 ± 0.027	53.14 ± 0.024
8.00	75.19 ± 0.058	67.10 ± 0.024	60.06 ± 0.036

Figure 4.77: Cumulative percentage release profile of clindamycin phosphate from carbopol 934P - pluronic F127 thermoreversible periodontal gel

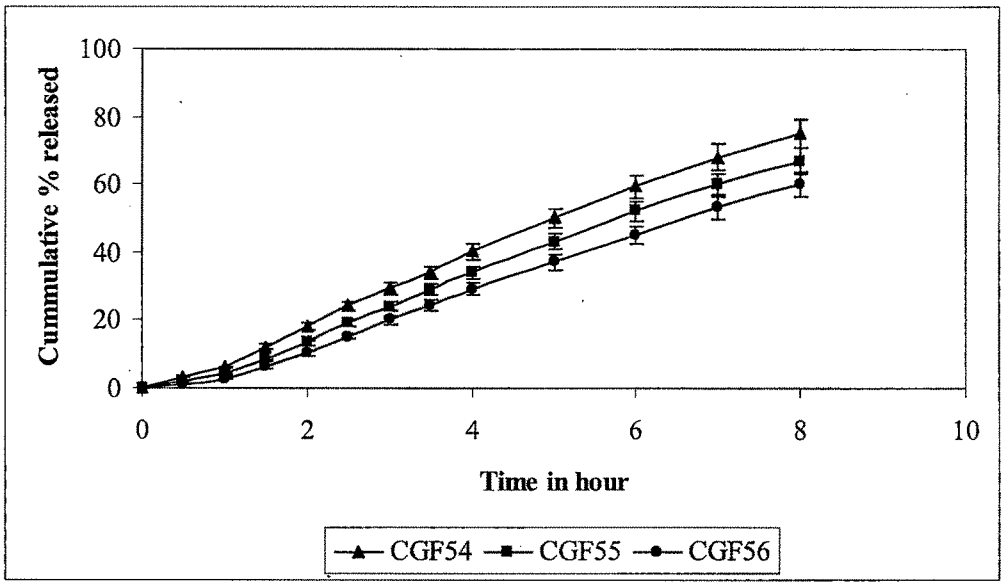


Table no. 4.109: Release Kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded Carbopol-934P - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				n (Release exponent)	k (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF54	0.9991	0.7059	0.9012	0.5915	9.649	4.456
MGF55	0.9953	0.6356	0.8775	0.1376	8.981	9.572
MGF56	0.9527	0.5823	0.8564	0.5945	9.069	1.172
CGF54	0.9985	0.695	0.9145	0.6078	9.439	2.748
CGF55	0.9978	0.5658	0.896	0.6029	8.214	1.637
CGF56	0.9920	0.4546	0.8614	0.6064	7.501	3.169

4.10.7 In Vitro permeation study

4.10.7.1 Determination of saturated drug concentration

A saturated minocycline hydrochloride/ clindamycin phosphate was done as described in section 4.6.6.1. The saturated concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was found to be 106.994 mg ml^{-1} and 103.900 mg ml^{-1} **respectively.**

4.10.7.2 Preparation of mucosal tissue

The mucosal tissue was prepared as described in the section 4.6.6.2.

4.10.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane was measured as described in section 4.6.6.3. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ μm, which is the mean of 3 measurements.

The anionic polymer carbopol 934P were shown to express a high Ca^{++} binding ability. It is also reported to possess permeation enhancing property. Therefore, it was important to found out the extent of increase in in-vitro permeation across the oral mucosal membrane that could be attained by the thermoreversible gels of pluronic F127 along with carbopol 934P. Effective permeability coefficient determined for minocycline hydrochloride and clindamycin phosphate containing thermoreversible periodontal gel formulations are given in

Table No4.110 and 4.111 and the cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various carbopol 934P-pluronic F127 gels formulations are given in the Figure 4.78 and 4.79. It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for carbopol 934P pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution. Since the pluronic F127 gels are viscous, isotropic liquid crystals containing micelles, it was hypothesized that the drug release may be by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the carbopol 934P ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. The presence of carbopol 934P results in very rapid dissolution and release of drug due to swelling and dissolution of carbopol 934P at pH 6.75. However presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to 20% w/w and 23% w/w pluronic F127 containing formulations, which may be due to the formations of larger concentrations of the micelle. Addition of the carbopol 934P increases the drug permeation compared to the plain pluronic F127 formulations, this may be due to increase in concentrations of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network (Chen et al, 1997). At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized carbopol 934P (Chen et al, 1997). Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of carbopol 934P not only increase in the Ca^{++} binding site but also increase in interaccessibility of Ca^{++} binding sites due to relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.2% carbopol 934P along with 20% and 23 % w/w pluronic F127 found to be the best. However formulations containing 0.2% w/w carbopol 934P along with 25 % pluronic F127 shows lower gelling

temperature, low permeation profile and high syringeability which may make difficult to administer the drug to the periodontal cavity. Formulations containing the higher concentrations of carbopol 934P (0.3, 0.4 and 0.5% w/w) showed a high syringeability and blockage of the syringe which may be due to high viscous solution. Hence MGF54, CGF54, MGF55 and CGF55 was selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No 4.110: Ex Vivo Permeation Profile of Minocycline hydrochloride from Carbopol 934P - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF54	MGF55	MGF56
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	2.31 ± 0.061	1.98 ± 0.490	1.04 ± 0.556
1.00	5.59 ± 0.002	4.44 ± 0.424	2.92 ± 0.454
1.50	8.27 ± 0.016	7.33 ± 0.416	5.39 ± 0.564
2.00	11.11 ± 0.074	10.22 ± 0.425	8.28 ± 0.557
2.50	14.59 ± 0.030	13.12 ± 0.051	11.27 ± 0.456
3.00	18.48 ± 0.062	16.26 ± 0.194	14.04 ± 0.067
3.50	22.15 ± 0.022	19.56 ± 0.404	17.54 ± 0.559
4.00	26.25 ± 0.027	23.77 ± 0.620	21.07 ± 0.479
5.00	35.08 ± 0.006	31.72 ± 0.334	28.66 ± 0.390
6.00	43.64 ± 0.005	41.02 ± 0.391	36.76 ± 0.271
7.00	52.72 ± 0.007	49.06 ± 0.285	46.05 ± 0.323
8.00	62.32 ± 0.006	57.78 ± 0.014	54.24 ± 0.298

Figure 4.78: Cumulative permeation profile of minocycline hydrochloride from carbopol 934P - pluronic F127 thermoreversible periodontal gel

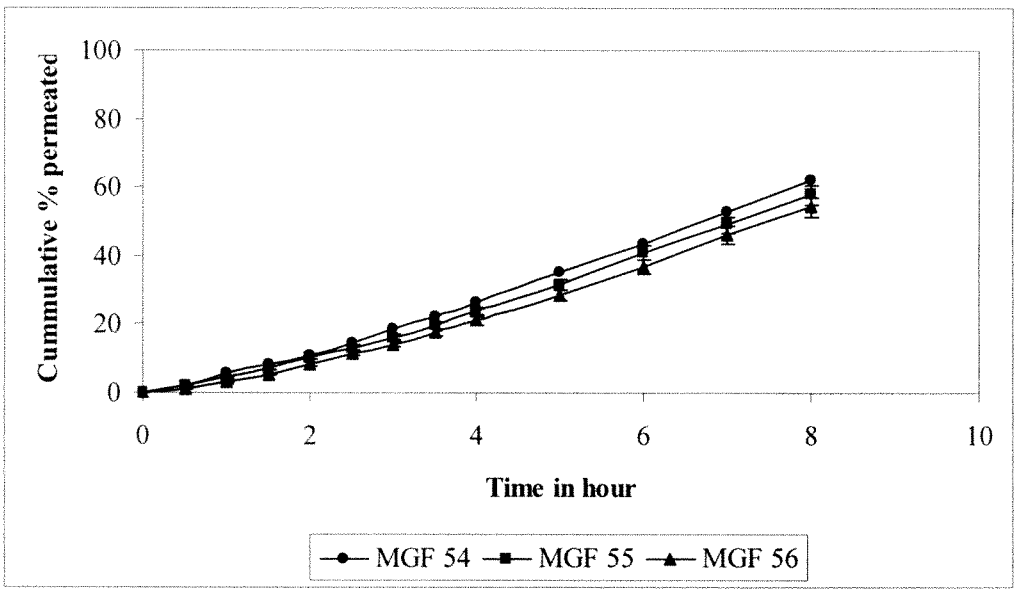


Table No 4.111: Ex Vivo Permeation Profile of Clindamycin phosphate from Carbopol 934P - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hours	% Clindamycin phosphate Permeated ± SD		
	CGF54	CGF55	CGF56
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	2.08 ± 0.033	1.83 ± 0.020	1.28 ± 0.057
1.00	6.81 ± 0.039	4.72 ± 0.007	2.52 ± 0.015
1.50	9.65 ± 0.071	7.79 ± 0.042	4.64 ± 0.033
2.00	13.54 ± 0.059	10.82 ± 0.044	7.06 ± 0.086
2.50	18.03 ± 0.048	14.75 ± 0.046	10.44 ± 0.050
3.00	22.42 ± 0.068	18.51 ± 0.043	14.13 ± 0.083
3.50	27.09 ± 0.071	22.94 ± 0.035	17.54 ± 0.088
4.00	30.86 ± 0.068	25.91 ± 0.049	20.70 ± 0.056
5.00	38.90 ± 0.070	33.34 ± 0.061	28.07 ± 0.040
6.00	46.15 ± 0.034	41.11 ± 0.020	35.06 ± 0.050
7.00	53.68 ± 0.063	48.25 ± 0.099	42.72 ± 0.040
8.00	60.66 ± 0.061	55.50 ± 0.080	50.58 ± 0.084

Figure 4.79: Cumulative permeation profile of clindamycin phosphate from carbopol 934P - Pluronic F127 thermoreversible periodontal gel

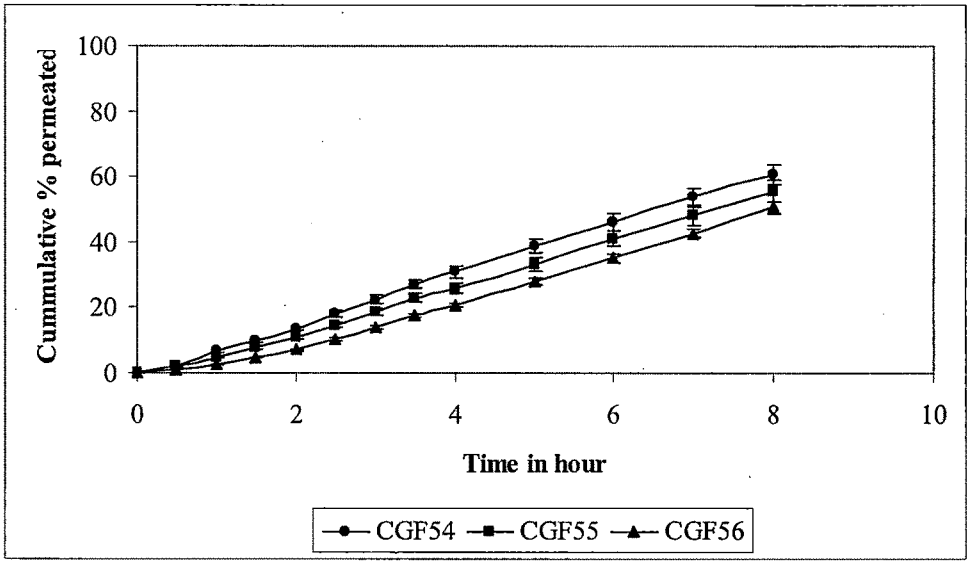


Table no. 4.112: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal gels

Formulations	Permeation flux $J(mcg.cm^{-2}.hr^{-1})$	Lag time ($t_L$hr)	Diffusion coefficient $(D\times10^{-8}cm^2.sec^{-1})$	Permeability coefficient $(P\times10^{-8}cm.sec^{-1})$
MGF54	8.19	0.90	1.180	2.126
MGF55	7.71	1.25	0.855	2.000
MGF56	7.39	1.50	0.713	1.918
CGF54	7.49	1.25	0.855	2.000
CGF55	7.31	1.75	0.611	1.954
CGF56	6.86	2.25	0.475	1.834

4.10.8 Stability Study

The Minocycline hydrochloride/ clindamycin phosphate loaded periodontal thermoreversible gels prepared using 20 % w/w and 23 % w/w pluronic F127 along with 0.20 % w/w carbopol 934P were studied for the stability of the formulation at Freeze condition (4°C) and at RT (25°C). All the four formulations showed good physical stability, as there was no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of all the formulations was within the range of 6.04 to 6.17, which is the neutral pH.

Table no. 4.113: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF54	99.35 ± 0.75	6.05	31.0
MGF55	97.85 ± 0.88	6.10	29.1
CGF54	98.41 ± 0.99	6.12	30.1
CGF55	97.75 ± 0.65	6.15	28.7

Table no. 4.114: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature

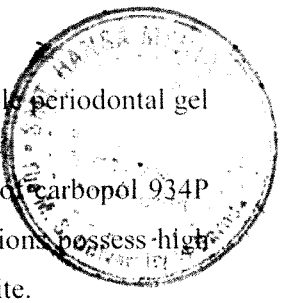
Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF54	97.72 ± 0.81	6.04	31.1
MGF55	97.96 ± 0.71	6.11	28.7
CGF54	97.65 ± 0.47	6.13	29.7
CGF55	98.75 ± 0.68	6.17	28.5

4.10.9 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with mucoadhesive polymer carbopol 934P by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate. Carbopol 934P gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. At 4°C all the formulations were at liquid state with viscosity ranging from 11.24 mPas to 30.04 mPas for 19 % w/w to 25 % w/w pluronic F127 with 0.20-0.50 w/w.% carbopol 934P. Rheological behavior of all the formulations was measured. All the formulations exhibit Newtonian behavior at 4°C, all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer carbopol 934P lowered the gelation temperature. The addition of increasing concentration of carbopol 934P from 0.20-0.50 % w/w further lowered the gelation temperature, which might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the carbopol 934P is exposed to water the polymer begins to uncoil generating an increase in viscosity and gel formation.

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of increased concentrations of carbopol 934P. Increase in the gel strength in presence of different concentration of carbopol 934P may be due to bond formation between pluronic



F127 and carbopol 934P. Increase in gel strength shows that the addition of carbopol 934P increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property and increases the residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of carbopol 934P. From the study it was evidenced that the availability of the carboxyl groups determines the mucoadhesion. Presence of mucoadhesive polymer carbopol 934P having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Syringeability of the formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.20% w/w carbopol 934P concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The preliminary study showed that the formulation prepared with 20%w/w pluronic F127 along with 0.20% carbopol 934P (MGF54 and CGF54) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.20 % carbopol 934P (MGF55 and CGF55). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF54 and CGF54. In addition, formulation MGF54 and CGF54 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF55 and CGF55 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for carbopol 934P-pluronic F127 thermoreversible gels than plain pluronic F127 thermoreversible gels compared to the pure drug solution.

As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations this may be due to the formations of larger concentrations of the micelles. Addition of the carbopol 934P increases the drug permeation compared to the plain pluronic F127 formulations , this may be due to increase in concentrations of ionized carboxyl group to a level require to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized carbopol 934P. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of carbopol 934P not only increase in the Ca^{++} binding site but also increase the inter accessibility of Ca^{++} binding sites due to relaxation of polymer network.

The investigation of in vitro release and permeation data showed that the diffusion is the mechanism of drug release and followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stable during the storage period of 6 months, and their chemical and mechanical property does not change significantly.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.20 % carbopol 934P along with 20% and 23 % w/w pluronic F127 were found to be best for periodontal thermoreversible gel delivery of minocycline hydrochloride and clindamycin phosphate.

4.11 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND POLY VINYL ALCOHOL

4.11.1 Preparation of mixed PVA - pluronic F127 periodontal gels

Formulations containing minocycline hydrochloride (1%) and clindamycin phosphate (1%) were prepared by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with PVA. The compositions of the formulations are cited in Table No4.115 and 4.120.

Table No 4.115: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVA (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF69	MGF70	MGF71	MGF72
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	38	32	28	26
Visual gel Temp. (°C)	38.7	33.1	29.5	27.4
Drug content	99.25 ± 0.32	98.67 ± 0.26	99.17 ± 0.35	99.1 ± 0.47
Mucoadhesion (gf/mm)	19.34 ± 0.25	20.82 ± 0.38	23.87 ± 0.76	28.33 ± 0.82
Gel strength (N/m)	9921.28 ± 38.69	16321.25 ± 35.23	19476.63 ± 86.35	22017.83 ± 57.84
pH (Sol)	5.55	5.67	5.72	5.64
pH (Gel)	5.57	5.69	5.75	5.67
Sol Viscosity mPas	17.21	24.11	25.89	28.73
Gel Viscosity mPas	2328	3057	3392	3478

Table No 4.116: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVA (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF73	MGF74	MGF75	MGF76
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	1.00	1.00	1.00	1.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	33	28	24	22
Visual gel Temp. (°C)	34.5	29.4	25.1	23.3
Drug content	97.6 ± 0.35	98.4 ± 0.47	99.81 ± 0.51	99.19 ± 0.22
Mucoadhesion (gf/mm)	19.17 ± 11.47	24.03 ± 34.22	28.11 ± 10.84	31.13 ± 21.37
Gel strength (N/m)	10347.68 ± 19.28	15982.35 ± 24.45	19326.48 ± 17.86	22435.67 ± 67.83
pH (Sol)	5.65	5.69	5.73	5.78
pH (Gel)	5.66	5.71	5.76	5.81
Sol Viscosity mPas	17.94	22.87	26.67	29.65
Gel Viscosity mPas	2276	3046	3849	3563

Table No 4.117: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PVA (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF77	MGF78	MGF79	MGF80
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	2.00	2.00	2.00	2.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	29	26	23	21
Visual gel Temp. (°C)	30.2	26.8	24.1	22.4
Drug content	98.38 ± 0.51	99.14 ± 0.49	99.19 ± 0.21	97.28 ± 0.35
Mucoadhesion (gf/mm)	20.06 ± 10.19	25.32 ± 12.27	28.47 ± 11.13	32.19 ± 12.38
Gel strength (N/m)	11389.37 ± 68.92	17893 ± 85.97	19673.87 ± 93.64	22837.60 ± 79.63
pH (Sol)	5.68	5.72	5.76	5.81
pH (Gel)	5.71	5.74	5.78	5.83
Sol Viscosity mPas	18.69	24.59	28.31	30.85
Gel Viscosity mPas	2387	3376	3457	3689

Table No 4.118: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PVA (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF69	CGF70	CGF71	CGF72
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	0.50	0.50	0.50	0.50
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temperature (°C)	37	31	27	25
Visual gel Temperature (°C)	37.8	32.1	28.5	26.4
Drug content	99.23 ± 0.31	99.19 ± 0.27	98.17 ± 0.11	98.76 ± 0.33
Mucoadhesion (gf/mm)	18.37 ± 0.35	20.26 ± 0.26	23.63 ± 0.93	27.32 ± 0.67
Gel strength (N/m)	10345.27 ± 86.73	16932.67 ± 92.37	19237.83 ± 86.79	21347.98 ± 91.34
pH (Sol)	5.67	5.72	5.64	5.62
pH (Gel)	5.69	5.75	5.67	5.64
Sol Viscosity mPas	17.46	23.78	26.61	29.79
Gel Viscosity mPas	2139	3126	3378	3524

'able No 4.119: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal els of PVA (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF73	CGF74	CGF75	CGF76
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	1.00	1.00	1.00	1.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temperature (°C)	32	27	23	21
Visual gel Temperature (°C)	33.1	28.5	24.1	21.9
Drug content	97.67 ± 0.61	97.21 ± 0.83	98.5 ± 0.41	97.86 ± 0.32
Mucoadhesion (gf/mm)	19.35 ± 0.47	24.36 ± 0.93	27.29 ± 0.45	30.18 ± 0.67
Gel strength (N/m)	10427.38 ± 93.02	14237.58 ± 85.76	19487.37 ± 93.28	23011.63 ± 79.33
pH (Sol)	5.69	5.73	5.78	5.81
pH (Gel)	5.71	5.76	5.81	5.83
Sol Viscosity mPas	18.25	23.38	27.01	29.87
Gel Viscosity mPas	2286	3203	3941	3789

'able No 4.120: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal els of PVA (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF77	CGF78	CGF79	CGF80
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
PVA (%w/w)	2.00	2.00	2.00	2.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
Purified water	qs	qs	qs	qs
Rheological gel Temperature (°C)	28	24	22	20
Visual gel Temperature (°C)	28.7	25.2	23.2	21.1
Drug content	99.21 ± 0.81	97.67 ± 0.57	98.69 ± 0.21	99.21 ± 0.11
Mucoadhesion (gf/mm)	19.97 ± 0.31	25.47 ± 1.04	28.76 ± 0.89	32.31 ± 0.68
Gel strength (N/m)	11244.38 ± 124.69	17892.39 ± 99.83	20278.47 ± 97.38	23147.79 ± 113.93
pH (Sol)	5.72	5.76	5.81	5.86
pH (Gel)	5.74	5.78	5.83	5.89
Sol Viscosity mPas	19.37	24.52	28.67	31.02
Gel Viscosity mPas	2478	3382	3491	3763

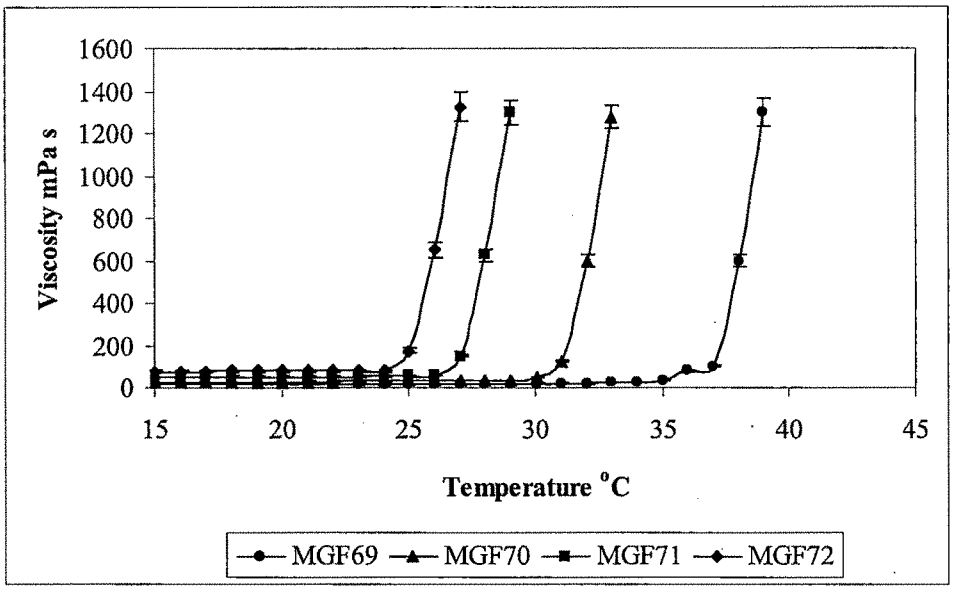
4.11.2 Viscosity and Gelling Temperature Determination

As evident from the results in table no. 4.115 to 4.120, the gelling temperature of pluronic F127 vehicle as determined by rheological method were lowered by the addition of increasing concentration of the mucoadhesive polymer PVA i.e. 0.50 % w/w to 2.00 % w/w. Figure 4.80 to 4.85 showed the viscosity of various pluronic gels with varying concentration of PVA measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method did not vary more than ± 1.5°C. The decrease in the gelation temperature with increase in PVA concentration may be due to enhanced viscosity of the gel formulation. The formulations showing the gelling temperature between 25°C to 37°C seemed to be proper for in situ gelling of the various vehicles at the periodontal cavity thereby minimizing the loss of administered drug due to clearance from the site of application.

4.11.3 Gel strength

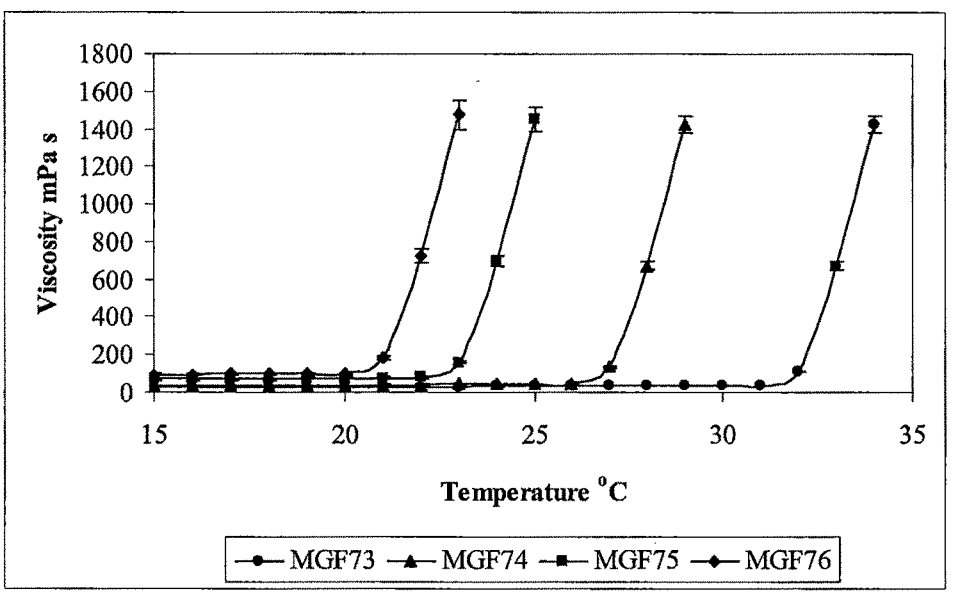
All the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.50 % w/w, 1.00 % w/w, and 2.00 % w/w PVA concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. Increase in the gel strength in presence of different concentration of PVA may be due to bond formation between pluronic F127 and PVA. Higher gel strength formulations possess the high mucoadhesive property and increases the residence time at the application site resulting in increased bioavailability of the drug.

Figure 4.80: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w PVA and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



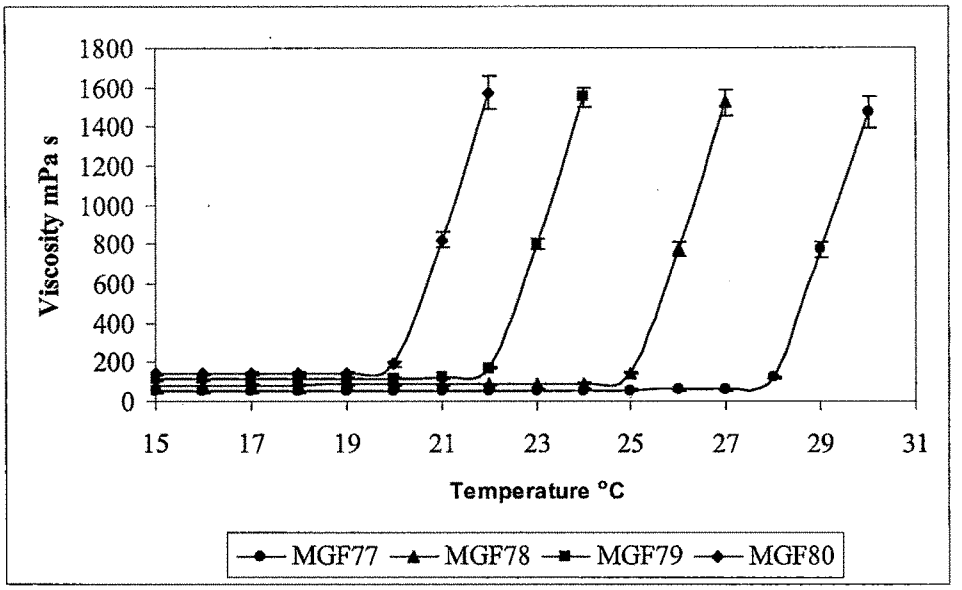
Values are expressed as mean ± SD (n =3)

Figure 4.81: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w PVA and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



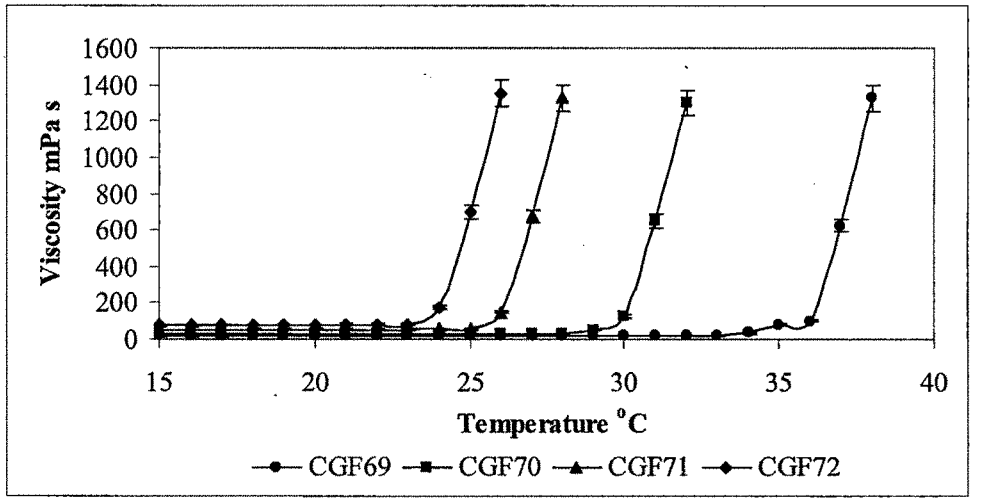
Values are expressed as mean ± SD (n =3)

Figure 4.82: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w PVA and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



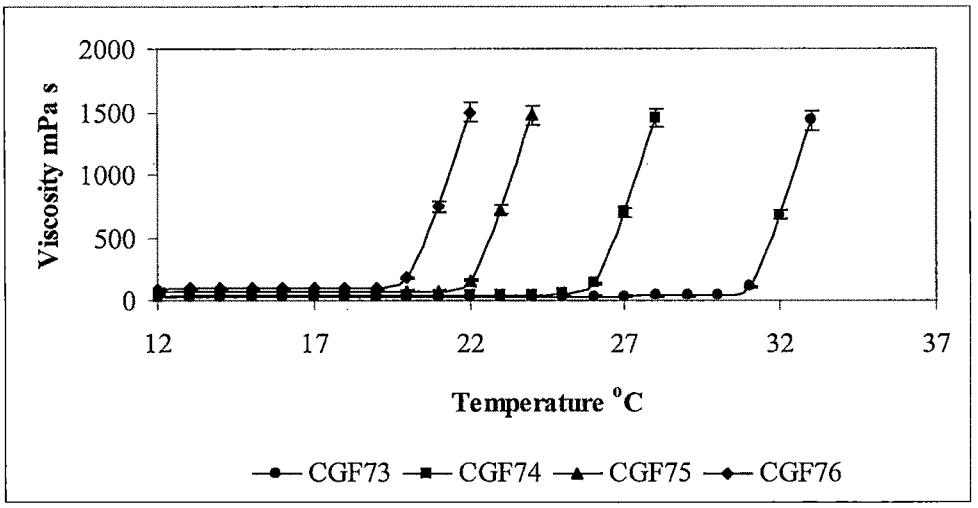
Values are expressed as mean ± SD (n =3)

Figure 4.83: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w PVA and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



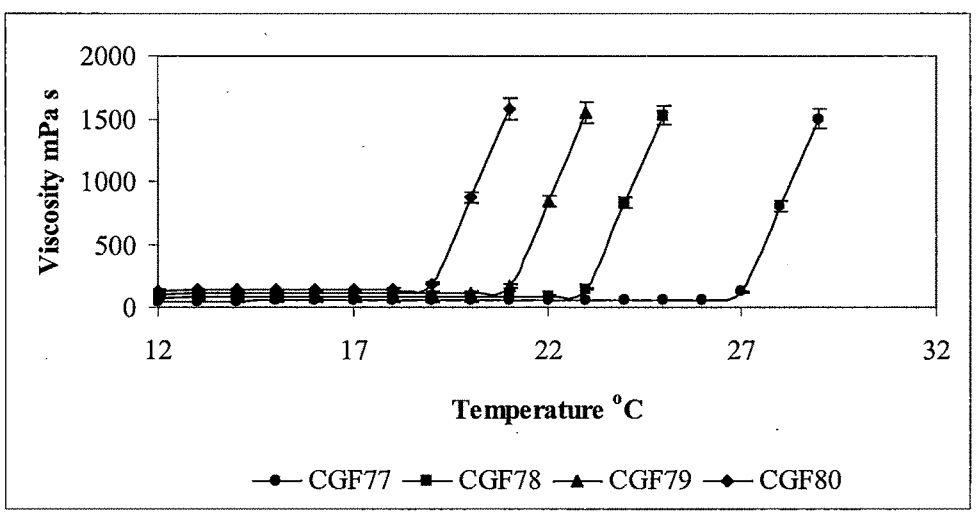
Values are expressed as mean ± SD (n =3)

Figure 4.84: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w PVA and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

Figure 4.85: Effect of temperature on the viscosity of various PVA-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w PVA and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate

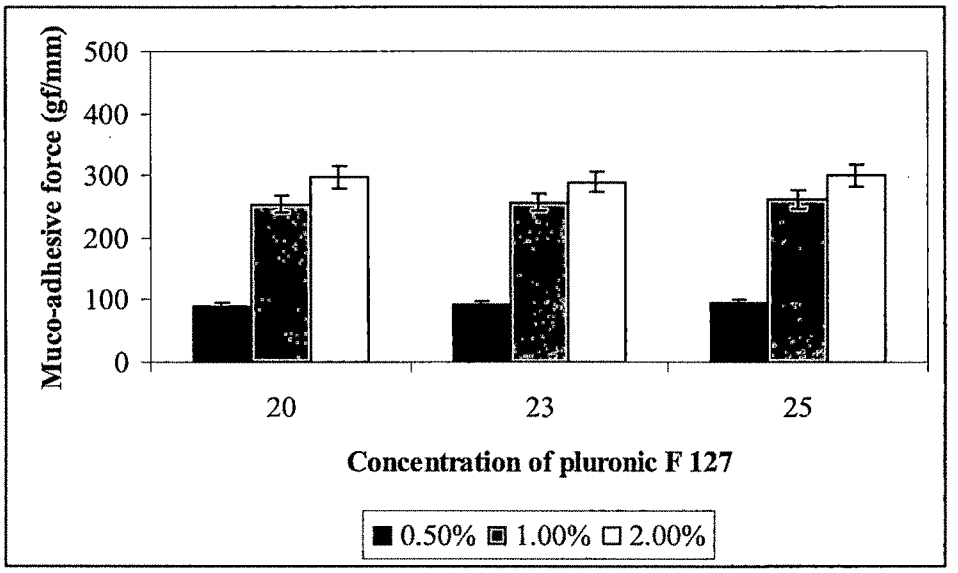


Values are expressed as mean ± SD (n =3)

4.11.4 Mucoadhesive Strength

The assessment of the mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase significantly ($P< 0.001$) with addition of increasing concentration of PVA (Figure 4.86). Difference in the mucoadhesive strength of formulations was found to be non significant ($P>0.001$). From the results it shows that the availability of the hydroxyl groups determines the mucoadhesion. Thus PVA having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoprotein. It is evidenced that the higher mucoadhesive strength delivery system possesses prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.86: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of PVA- pluronic F127



4.11.5 Syringeability

The assessments of the syringeability may be done in terms of force required to syringe the formulation to the application site. Syringeability of various formulations depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive

polymer. Syringeability for formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.5% w/w, 1.00% w/w, and 2.00% w/w PVA concentration increases significantly (P< 0.001) with respect to plain pluronic F127 gel. The results of the syringeability are shown in Table No4.121.

Table No 4.121: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of PVA- pluronic F127

Formulation Code	Syringeability
MGF70	201.35
MGF71	239.47
MGF72	278.90
CGF70	215.76
CGF71	265.89
CGF72	315.81

4.11.6 In vitro release study

The in vitro release profile of minocycline hydrochloride and clindamycin phosphate is illustrated in Figure 4.87 and 4.88. The maximum release of minocycline hydrochloride from the thermoreversible periodontal gels were shown by the formulation MGF70 where as the least was shown by the formulation MGF72 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible periodontal gels were shown by the formulation CGF70 where as the least was shown by the formulation CGF72 after 8 hours. The higher release of minocycline hydrochloride and clindamycin phosphate from gels can be explained reversibly by the viscosity of the polymer solution. A preliminary study shows that the formulation MGF70 and CGF70 had low viscosity than MGF72 and CGF72. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF70 and CGF70. In addition, formulation MGF70 and CGF70 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF72 and CGF72 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.122: In Vitro Release Profile of Minocycline hydrochloride from PVA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline Hydrochloride Released ± SD		
	MGF70	MGF71	MGF72
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	4.06 ± 0.025	2.38 ± 0.007	1.21 ± 0.049
1.00	6.40 ± 0.025	4.05 ± 0.041	2.43 ± 0.051
1.50	8.22 ± 0.037	6.49 ± 0.022	4.03 ± 0.014
2.00	11.78 ± 0.047	8.41 ± 0.011	6.06 ± 0.046
2.50	14.77 ± 0.006	11.61 ± 0.011	8.44 ± 0.047
3.00	18.67 ± 0.001	14.72 ± 0.078	10.93 ± 0.032
3.50	22.54 ± 0.008	18.62 ± 0.010	14.04 ± 0.019
4.00	26.79 ± 0.074	22.86 ± 0.004	17.57 ± 0.040
5.00	34.47 ± 0.005	30.64 ± 0.004	25.16 ± 0.047
6.00	42.31 ± 0.030	37.74 ± 0.050	32.36 ± 0.041
7.00	50.33 ± 0.046	45.61 ± 0.045	39.89 ± 0.038
8.00	57.15 ± 0.054	52.42 ± 0.049	47.30 ± 0.060

Figure 4.87: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from PVA - Pluronic F127 thermoreversible periodontal gel

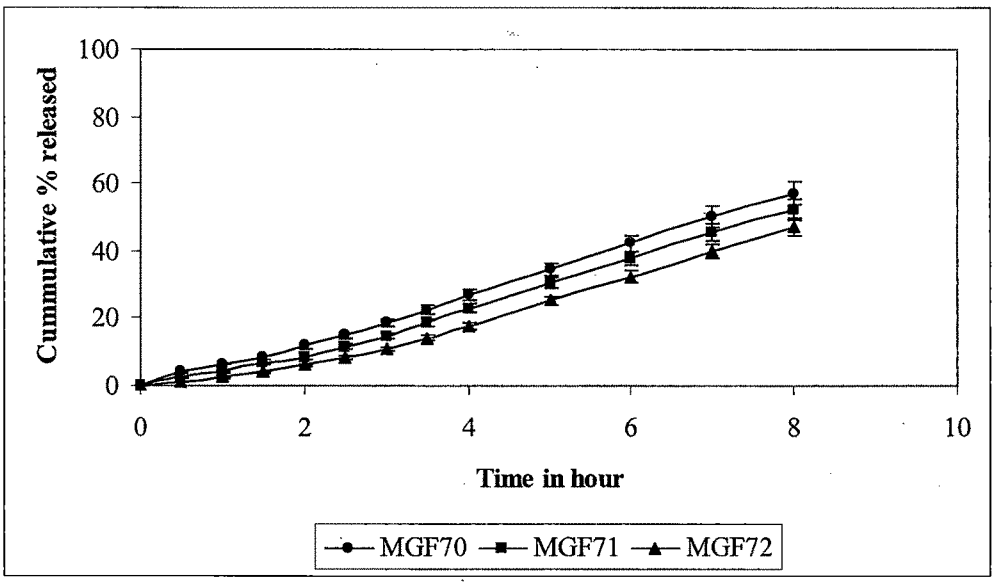


Table No 4.123: In Vitro Release Profile of Clindamycin phosphate from PVA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin Phosphate Released ± SD		
	CGF70	CGF71	CGF72
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.76 ± 0.032	1.51 ± 0.050	1.09 ± 0.039
1.00	5.20 ± 0.035	3.17 ± 0.004	1.97 ± 0.035
1.50	7.33 ± 0.041	5.31 ± 0.040	3.07 ± 0.077
2.00	11.13 ± 0.035	8.40 ± 0.057	5.37 ± 0.037
2.50	15.24 ± 0.033	11.77 ± 0.027	8.58 ± 0.038
3.00	19.18 ± 0.033	15.90 ± 0.021	12.05 ± 0.057
3.50	23.57 ± 0.035	19.37 ± 0.043	15.48 ± 0.029
4.00	27.88 ± 0.069	23.22 ± 0.031	19.22 ± 0.040
5.00	35.56 ± 0.068	30.98 ± 0.046	26.22 ± 0.045
6.00	42.15 ± 0.029	37.66 ± 0.046	32.37 ± 0.006
7.00	49.15 ± 0.020	44.46 ± 0.064	39.26 ± 0.023
8.00	55.76 ± 0.030	50.40 ± 0.034	45.79 ± 0.032

Figure 4.88: Cumulative percentage release profile of clindamycin phosphate in mcg/cm^2 from PVA - Pluronic F127 thermoreversible periodontal gel

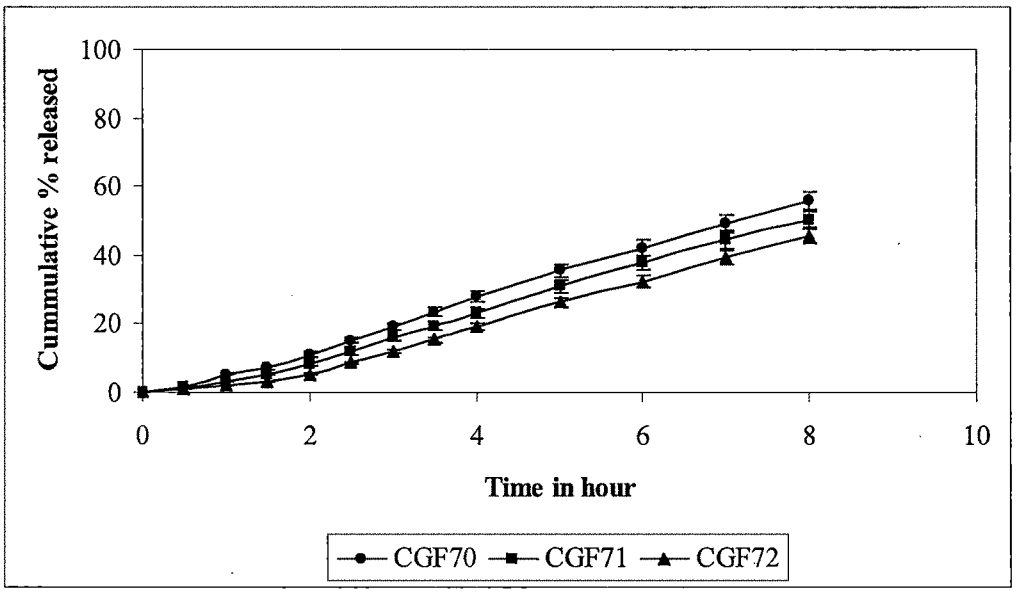


Table No 4.124: Release Kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded PVA - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				n (Release exponent)	k (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF70	0.9953	0.4779	0.8827	0.5597	7.258	1.811
MGF71	0.9892	0.3882	0.8562	0.1438	6.768	5.861
MGF72	0.9527	0.2783	0.8177	0.576	9.069	1.172
CGF70	0.9970	0.459	0.8945	0.6112	7.296	1.977
CGF71	0.9929	0.3595	0.8697	0.6052	6.682	4.808
CGF72	0.9830	0.2601	0.8356	0.5897	6.055	1.135

4.11.7 In Vitro permeation study

4.11.7.1 Determination of saturated drug concentration

A saturated minocycline hydrochloride/ clindamycin phosphate was done as described in section 4.6.6.1. The saturated concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was found to be 106.994 mg ml^{-}1 and 103.900 mg ml^{-1} respectively.

4.11.7.2 Preparation of mucosal tissue

The mucosal tissue was prepared as described in the section 4.6.6.2.

4.11.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane was measured as described in section 4.6.6.3. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ μm, which is the mean of 3 measurements.

The anionic polymer PVA is reported to possess permeation enhancing property with high mucoadhesive property. Therefore, it was important to estimate the extent of increase in in-vitro permeation across the oral mucosal membrane that could be attained by the thermoreversible periodontal gels of pluronic F127 and PVA. Effective permeability coefficient determined for minocycline hydrochloride and clindamycin phosphate in each gel

formulations are given in Table No4.127 and the cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various PVA-pluronic F127 gels formulations are given in the Figure 4.89 and 4.90 respectively. It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for PVA-pluronic F127 thermoreversible periodontal gels compared to the pure drug solution. Since the pluronic F127 gels are viscous, isotropic liquid crystals containing micelles, it was hypothesized that the drug release may be due to diffusion through the extra micellar water channels of the gel matrix. The permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing PVA ($P>0.001$) compared to the pure drug solutions. The presence of PVA resulted in very rapid dissolution and release of drug due to swelling and dissolution of PVA at pH 6.75. However, presence of pluronic F127 in the periodontal gel retard the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micelle structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23 w/w pluronic F127 containing formulations which may be due to the formations of larger concentrations of the micelle's. Addition of the PVA increases the drug permeation compared to the plain pluronic F127 formulations, this may be due to increase in wetability and swelling of the polymers. The swelling of the polymers was also due to ionic strength and pH (Park and Robinson, 1985). At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the PVA. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of ionized drugs molecules helps in the formation of hydrogen binding site and relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability of various formulations MGF70, CGF70, MGF71 and CGF71 were selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No 4.125: Ex vivo Permeation Profile of Minocycline hydrochloride from PVA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF70	MGF71	MGF72
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	3.05 ± 0.025	2.15 ± 0.007	1.02 ± 0.049
1.00	5.85 ± 0.025	4.41 ± 0.041	2.99 ± 0.051
1.50	8.26 ± 0.037	6.73 ± 0.022	5.45 ± 0.014
2.00	10.59 ± 0.047	9.10 ± 0.011	6.44 ± 0.046
2.50	13.83 ± 0.006	11.79 ± 0.011	10.10 ± 0.047
3.00	16.86 ± 0.001	14.57 ± 0.078	12.49 ± 0.032
3.50	19.53 ± 0.008	17.36 ± 0.010	15.12 ± 0.019
4.00	22.51 ± 0.074	20.16 ± 0.004	17.46 ± 0.040
5.00	28.76 ± 0.005	26.32 ± 0.004	22.97 ± 0.047
6.00	35.69 ± 0.030	32.62 ± 0.050	29.52 ± 0.041
7.00	42.49 ± 0.046	39.39 ± 0.045	35.57 ± 0.038
8.00	50.11 ± 0.054	45.30 ± 0.049	42.09 ± 0.060

Figure 4.89: Cumulative permeation profile of minocycline hydrochloride from PVA - pluronic F127 thermoreversible periodontal gel

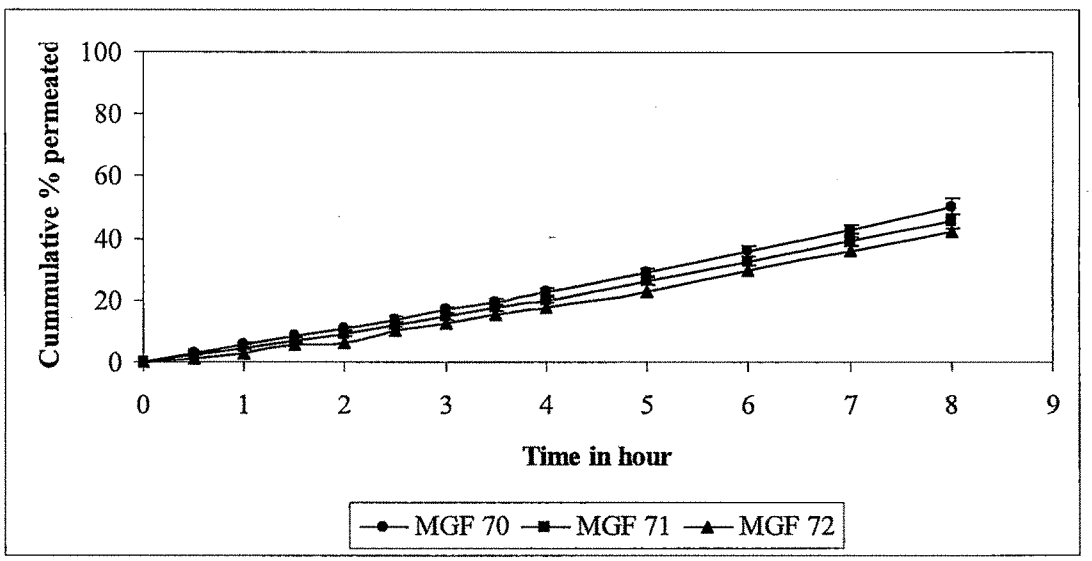


Table 4.126: Ex vivo Permeation Profile of Clindamycin phosphate from PVA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF70	CGF71	CGF72
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	1.35 ± 0.032	0.64 ± 0.050	0.58 ± 0.039
1.00	3.74 ± 0.035	2.37 ± 0.004	1.95 ± 0.035
1.50	6.36 ± 0.041	4.77 ± 0.040	3.47 ± 0.077
2.00	8.46 ± 0.035	7.04 ± 0.057	5.68 ± 0.037
2.50	11.83 ± 0.033	9.87 ± 0.027	8.14 ± 0.038
3.00	14.83 ± 0.033	12.48 ± 0.021	10.38 ± 0.057
3.50	17.46 ± 0.035	15.19 ± 0.043	13.00 ± 0.029
4.00	20.60 ± 0.069	17.96 ± 0.031	15.70 ± 0.040
5.00	26.95 ± 0.068	24.28 ± 0.046	21.15 ± 0.045
6.00	33.76 ± 0.029	30.41 ± 0.046	27.39 ± 0.006
7.00	40.41 ± 0.020	37.00 ± 0.064	33.61 ± 0.023
8.00	48.13 ± 0.030	43.72 ± 0.034	40.28 ± 0.032

Figure 4.90: Cumulative permeation profile of clindamycin phosphate from PVA - Pluronic F127 thermoreversible periodontal gel

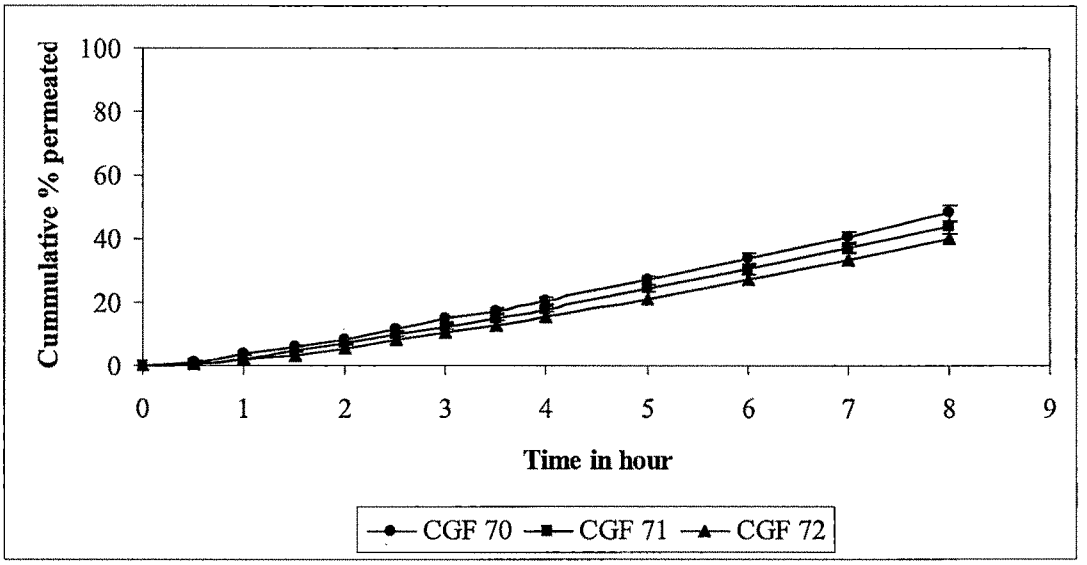


Table No 4.127: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal gels

Formulations	Permeation flux J(mcg.cm^{-2}.hr^{-1})	Lag time ($t_L$hr)	Diffusion coefficient (D×10^{-8}cm^2.sec^{-1})	Permeability coefficient (P×10^{-8}cm.sec^{-1})
MGF70	6.31	1.00	1.070	2.212
MGF71	5.91	1.25	0.855	1.534
MGF72	5.60	1.35	0.792	1.453
CGF70	6.32	1.00	1.070	1.689
CGF71	5.92	1.35	0.792	1.582
CGF72	5.52	1.75	0.611	1.475

4.11.8 Stability Study

The optimized Minocycline hydrochloride/ clindamycin phosphate loaded PVA containing periodontal thermoreversible gels were studied for the stability of the formulation at Freeze condition (4°C) and at RT (25 °C). All the four formulations showed good physical stability, as there was no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of all the formulations was within the range of 5.60 to 5.71, which is the neutral pH.

Table No 4.128: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF70	98.25 ± 0.55	5.65	33.0
MGF71	97.25 ± 0.98	5.71	29.4
CGF70	97.61 ± 0.79	5.62	31.8
CGF71	98.45 ± 0.85	5.66	28.2

Table No 4.129: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF70	97.82 ± 0.71	5.64	32.8
MGF71	97.56 ± 0.91	5.70	29.3
CGF70	97.95 ± 0.77	5.60	31.7
CGF71	98.25 ± 0.98	5.63	28.1

4.11.9 Conclusion

Pluronic F127 thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with mucoadhesive polymer PVA by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate. Gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. Rheological behavior of all the formulations was measured and shown to exhibit Newtonian behavior at 4°C; all the formulations were remained as liquid and no gel formation were observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxyethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxyethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer PVA lowered the gelation temperature. It is also noted that addition of increasing concentration of PVA from 0.50-2.00 % w/w further lowered the gelation temperature. The gelation temperature lowering effect of mucoadhesive polymer might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the PVA is exposed to water the polymer begins to uncoil and generating an increase in viscosity and gel formation. The uncoiling and expansion of the molecule result in polymer swelling and elastic gel formation.

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of PVA, which may be due to PVA- pluronic F127 bond formation. Increase in gel strength shows that the addition of PVA increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property and increases the residence time at the application site.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of PVA. Presence of mucoadhesive polymer PVA interacts more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Syringeability of the formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; this is due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.20% w/w PVA concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The higher release of minocycline hydrochloride/ clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation prepared with 20%w/w pluronic F127 along with 0.50% PVA (MGF70 and CGF70) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.50 % PVA (MGF71 and CGF71). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF70 and CGF70. In addition, formulation MGF70 and CGF70 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF71 and CGF71 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for PVA-pluronic F127 thermoreversible gels than pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the PVA ($P>0.001$) compared to the plain pluronic F127

thermoreversible gels. Presence of PVA results in very rapid dissolution of the drug due to swelling and dissolution of PVA. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results addition of the PVA increases the drug permeation compared to the plain pluronic F127 formulations , which may again be due to formation of ionized group to a level require to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized PVA. The investigation of in vitro release and permeation data showed that the diffusion is the mechanism of drug release which followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stable during the storage period of 6 months, and their chemical and mechanical property does not change significantly.

It may be concluded that the mucoadhesive polymer PVA increases mucoadhesive, physico-chemical and mechanical properties than compared to the plain periodontal thermoreversible gels. Thermoreversible gel formulations maintained a satisfactory residence time in the periodontal cavity and ensured zero order of release of the drug over relatively longer period, which made them good candidate for drug delivery system through periodontal route for the treatment of infectious periodontal diseases.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.50 % PVA along with 20% and 23 % w/w pluronic F127 were found to be best for periodontal thermoreversible gel delivery of minocycline hydrochloride and clindamycin phosphate.

4.12 MIXED MUCOADHESIVE PERIODONTAL GEL OF PLURONIC F127 AND POLY ACRYLIC ACID

4.12.1 Preparation of mixed PAA - pluronic F127 periodontal gels

Formulations containing the minocycline hydrochloride (1%) and clindamycin phosphate (1%) were prepared along with PAA by adopting the cold method (Schmolka et al 1972, Choi et al. 1998) as described earlier in section 4.5.1 by replacing polycarbophil with PAA. The compositions of the formulations are cited in Table No4.130 to 4.135.

Table No 4.130: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PAA (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF81	MGF82	MGF83	MGF84
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	0.50	0.50	0.50	0.50
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	41	35	33	31
Visual gel Temp. (°C)	42.2	36.1	34.2	32.5
Drug content	99.15 ± 0.12	98.38 ± 0.25	98.27 ± 0.72	98.59 ± 0.54
Mucoadhesion (gf/mm)	18.04 ± 0.35	20.46 ± 0.57	23.51 ± 0.68	27.91 ± 0.23
Gel strength (N/m)	9901.23 ± 47.84	16754.31 ± 57.72	19879.36 ± 76.54	21736.36 ± 84.34
pH (Sol)	6.45	6.38	6.28	6.21
pH (Gel)	6.47	6.40	6.31	6.22
Sol Viscosity mPas	17.11	24.02	25.78	28.79
Gel Viscosity mPas	2083	2968	3276	3491

Table No 4.131: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PAA (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF85	MGF86	MGF87	MGF88
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	1.00	1.00	1.00	1.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	38	31	29	25
Visual gel Temp. (°C)	39.1	32.4	30.2	26.1
Drug content	97.28 ± 0.29	98.21 ± 0.52	98.67 ± 0.42	98.76 ± 0.37
Mucoadhesion (gf/mm)	19.23 ± 0.93	23.52 ± 0.47	27.81 ± 0.69	30.52 ± 1.14
Gel strength (N/m)	10195.63 ± 134.76	15697.73 ± 127.82	18824.82 ± 104.57	21937.81 ± 98.72
pH (Sol)	6.31	6.27	6.20	6.11
pH (Gel)	6.32	6.31	6.21	6.13
Sol Viscosity mPas	17.78	22.92	26.78	29.49
Gel Viscosity mPas	2217	3167	3849	3537

Table No 4.132: Composition and Characteristics of MnHCl loaded mixed periodontal gels of PAA (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	MGF89	MGF90	MGF91	MGF92
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	2.00	2.00	2.00	2.00
Sodium metabisulphite (%w/w)	0.50	0.50	0.50	0.50
PEG 1000 (%w/w)	15.00	15.00	15.00	15.00
MnHCl (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	32	25	23	20
Visual gel Temp. (°C)	33.1	26.2	24.1	20.9
Drug content	98.36 ± 0.26	99.45 ± 0.57	99.28 ± 0.37	99.01 ± 0.49
Mucoadhesion (gf/mm)	19.78 ± 0.58	24.85 ± 0.95	27.89 ± 0.48	31.94 ± 0.67
Gel strength (N/m)	11253.57 ± 69.78	17483.36 ± 47.38	19627.48 ± 89.37	23123.48 ± 76.78
pH (Sol)	6.20	6.15	6.13	6.03
pH (Gel)	6.21	6.17	6.14	6.05
Sol Viscosity mPas	18.91	24.14	27.21	30.83
Gel Viscosity mPas	2401	3367	3472	3673

Table No 4.133: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PAA (0.50%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF81	CGF82	CGF83	CGF84
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	0.50	0.50	0.50	0.50
PEG1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5% NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp.(°C)	40	35	33	30
Visual gel Temp. (°C)	41.5	36.2	34.2	31.2
Drug content	97.63 ± 0.25	99.16 ± 0.23	98.63 ± 0.47	98.47 ± 0.35
Mucoadhesion (gf/mm)	18.12 ± 0.31	20.36 ± 0.72	23.63 ± 0.67	26.95 ± 0.59
Gel strength (N/m)	10248.93 ± 68.94	16894.64 ± 76.83	19102.37 ± 68.84	21287.37 ± 57.89
pH (Sol)	6.38	6.28	6.21	6.11
pH (Gel)	6.40	6.31	6.22	6.13
Sol Viscosity mPas	17.38	23.76	26.67	29.48
Gel Viscosity mPas	2117	3047	3382	3520

Table No 4.134: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PAA (1.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF85	CGF86	CGF87	CGF88
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	1.00	1.00	1.00	1.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp. (°C)	37	30	28	24
Visual gel Temp. (°C)	38.1	31.2	28.7	25.1
Drug content	98.36 ± 0.81	97.82 ± 0.49	99.18 ± 0.67	98.29 ± 0.34
Mucoadhesion (gf/mm)	19.57 ± 0.86	23.95 ± 0.76	28.01 ± 0.94	29.83 ± 0.58
Gel strength (N/m)	10374.46 ± 87.58	16203.73 ± 76.27	19478.39 ± 44.37	22836.38 ± 37.82
pH (Sol)	6.27	6.20	6.11	6.05
pH (Gel)	6.31	6.21	6.13	6.07
Sol Viscosity mPas	18.47	23.57	26.91	29.76
Gel Viscosity mPas	2328	3189	3867	3500

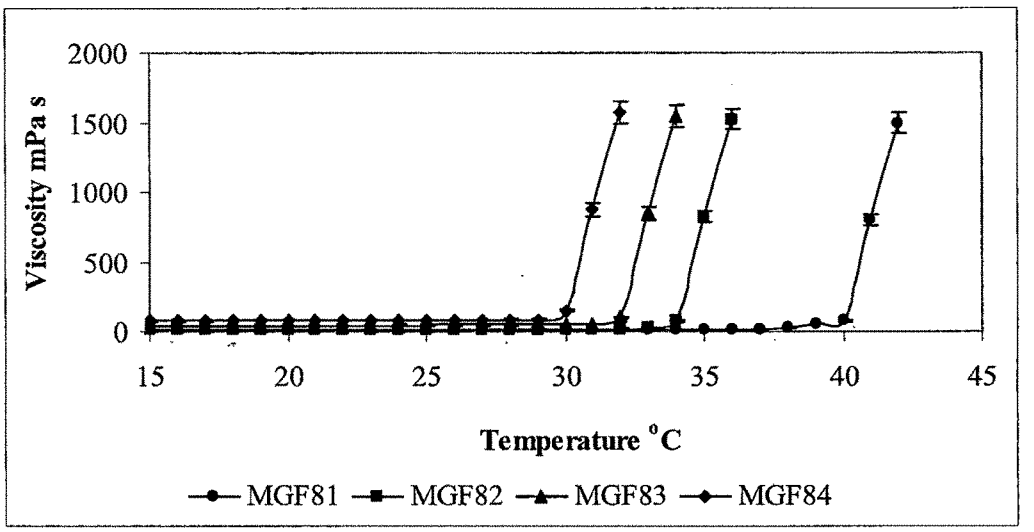
Table No 4.135: Composition and Characteristics of $ClPO_4$ loaded mixed periodontal gels of PAA (2.00%) - pluronic F127

Composition/ Characteristics	Formulation Code			
	CGF89	CGF90	CGF91	CGF92
PluronicF127 (%w/w)	19.00	20.00	23.00	25.00
Poly acrylic acid (%w/w)	2.00	2.00	2.00	2.00
PEG 1000(%w/w)	15.00	15.00	15.00	15.00
$ClPO_4$ (%w/w)	1.00	1.00	1.00	1.00
0.5%NaOH (%w/w)	2ml	2ml	2ml	2ml
Purified water	qs	qs	qs	qs
Rheological gel Temp (°C)	31	24	22	18
Visual gel Temp (°C)	31.7	25.4	23.1	18.5
Drug content	98.63 ± 0.29	99.19 ± 0.41	99.63 ± 0.21	98.11 ± 0.76
Mucoadhesion (gf/mm)	20.14 ± 0.31	25.75 ± 0.67	28.74 ± 0.89	32.21 ± 0.46
Gel strength (N/m)	11387.93 ± 101.27	18209.39 ± 90.26	20758.36 ± 87.57	23745.36 ± 97.35
pH (Sol)	6.15	6.13	6.03	5.95
pH (Gel)	6.17	6.14	6.05	5.97
Sol Viscosity mPas	19.37	24.86	28.79	30.89
Gel Viscosity mPas	2478	3369	3501	3787

4.12.2 Viscosity and Gelling Temperature Determination

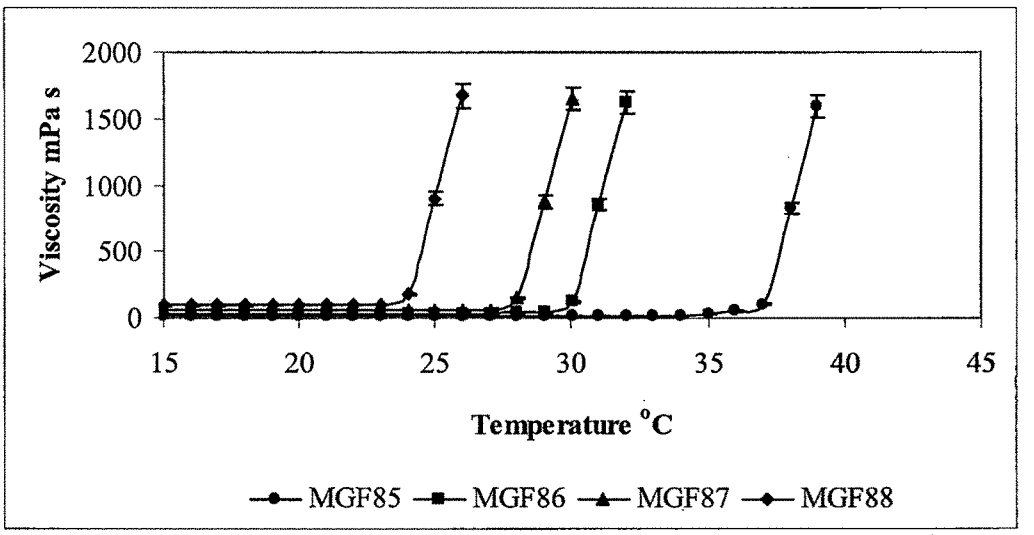
As evident from the results in table no.4.130 to 4.135 the gelling temperature of pluronic F127 vehicle as determined by rheological method were lowered by the addition of increasing concentration of the mucoadhesive polymer poly acrylic acid i.e. 0.50 % w/w, 1.00% and 2.00% w/w. Figure 4.91 to 4.96 shows the viscosity of various pluronic F127 gels with varying concentration of poly acrylic acid measured at 10 s^{-1} shear rate as a function of temperature. Gelation temperature determined by rheological method and visual method remained within ± 1.5°C. The decrease in the gelation temperature with increase in poly acrylic acid concentration may be due to enhanced viscosity of the gel formulation.

Figure 4.91: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w poly acrylic acid and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



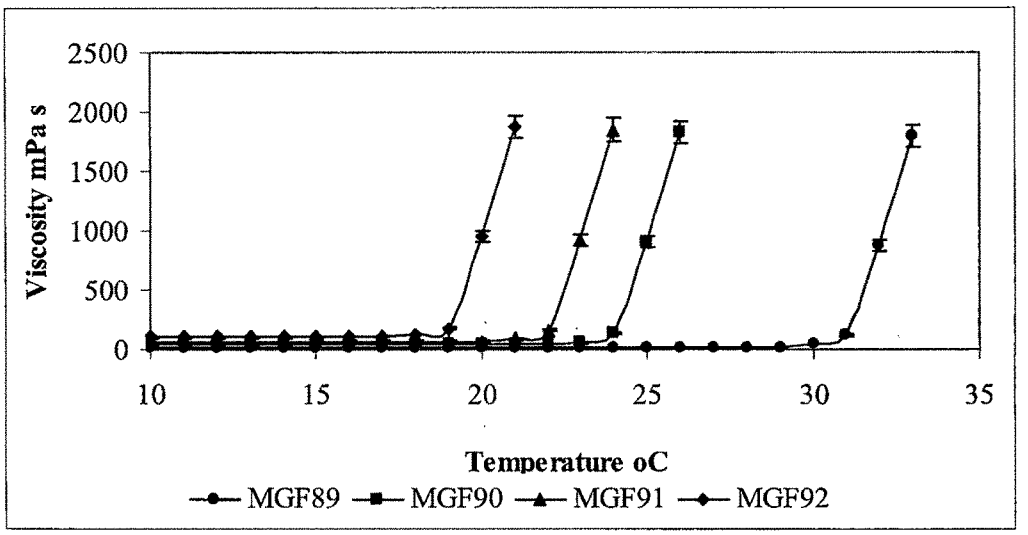
Values are expressed as mean ± SD (n =3)

Figure 4.92: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w poly acrylic acid and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



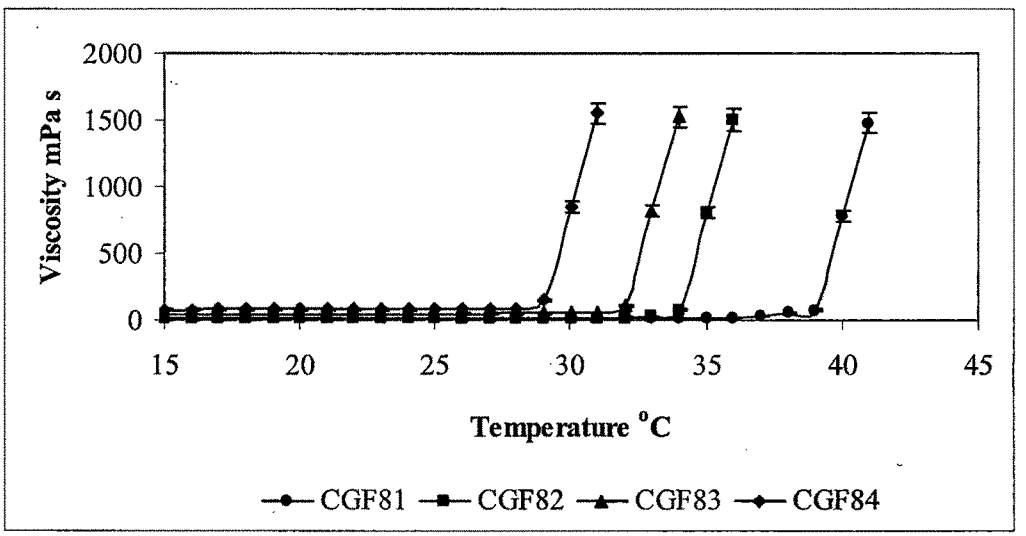
Values are expressed as mean ± SD (n =3)

Figure 4.93: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w poly acrylic acid and 1% w/w minocycline hydrochloride measured at 10 s^{-1} shear rate



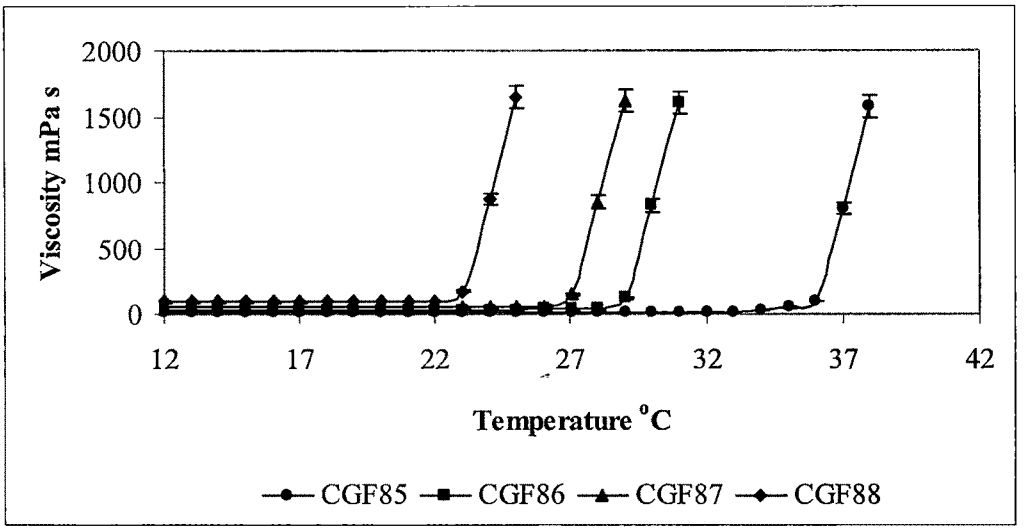
Values are expressed as mean ± SD (n =3)

Figure 4.94: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 0.50 % w/w poly acrylic acid and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



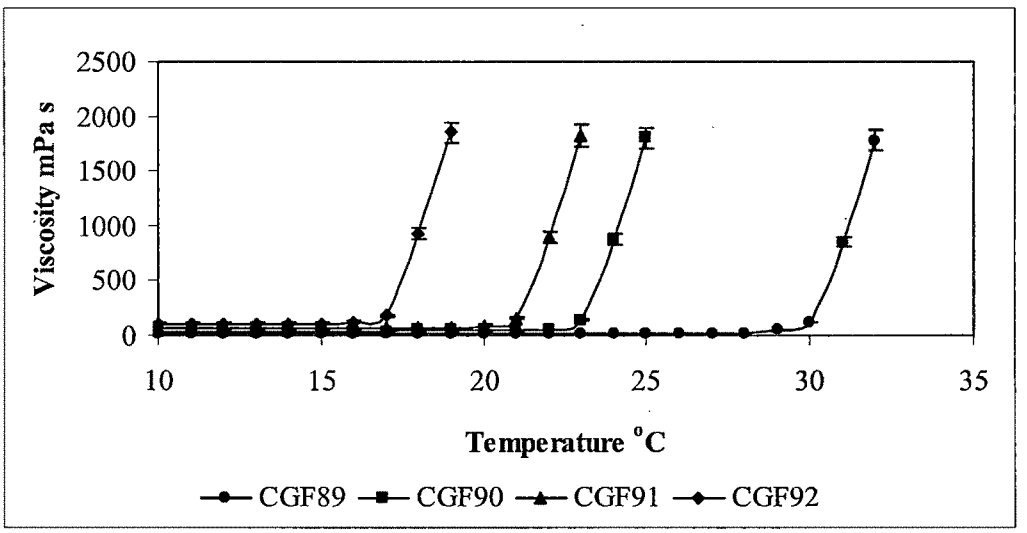
Values are expressed as mean ± SD (n =3)

Figure 4.95: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 1.00 % w/w poly acrylic acid and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

Figure 4.96: Effect of temperature on the viscosity of various poly acrylic acid-pluronic F127 periodontal gels with varying concentration of pluronic F127 along with 2.00 % w/w poly acrylic acid and 1% w/w clindamycin phosphate measured at 10 s^{-1} shear rate



Values are expressed as mean ± SD (n =3)

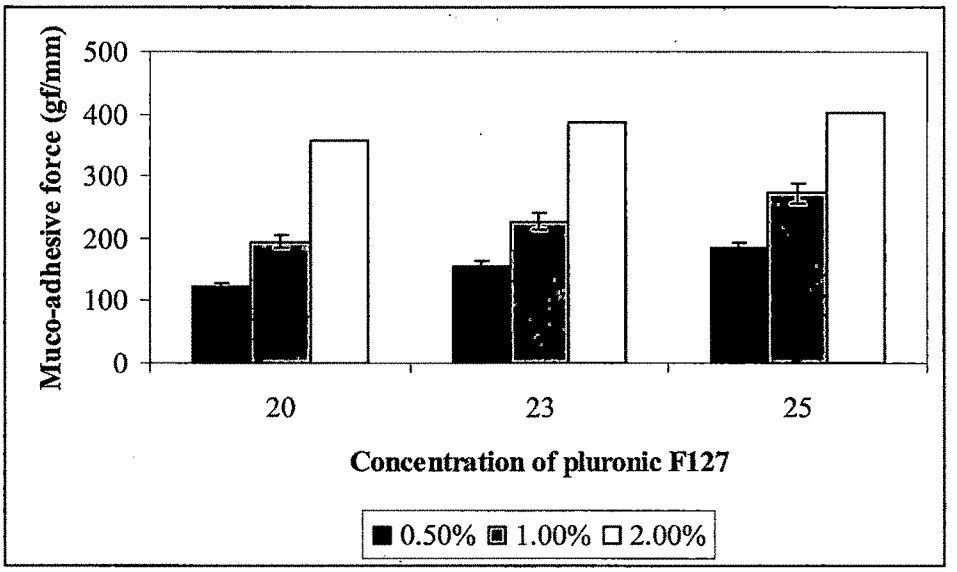
4.12.3 Gel Strength

All the formulations prepared using 20% w/w, 23% w/w and 25%w/w pluronic F127 along with 0.50 % w/w, 1.00 % w/w, and 2.00 % w/w poly acrylic acid concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel. Increase in the gel strength in presence of different concentration of poly acrylic acid may be due to bond formation between pluronic F127 and poly acrylic acid.

4.12.4 Mucoadhesive Strength

Mucoadhesive strength of formulations prepared using 20%w/w, 23%w/w and 25%w/w pluronic F127 along with 0.50%w/w, 1.00%w/w, and 2.00%w/w poly acrylic acid concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel (Figure 4.97). Their difference in the mucoadhesive strength is non significant ($P>0.001$). The result may be due to the presence of high density of available hydrogen bonding groups in poly acrylic acid; resulting in more strong interaction with mucin glycoprotein. The availability of the hydroxyl groups determines the mucoadhesion. There is evidence that the higher mucoadhesive strength delivery system possesses prolonged retention and increased absorption across mucosal tissues (Kunisawa et al., 2000).

Figure 4.97: The diagrammatic representation of mucoadhesive strength of mixed mucoadhesive periodontal gels of PAA- pluronic F127



4.12.5 Syringeability

Syringeability may be estimated in terms of force required to syringe the formulation at the application site, which depends on the viscosity of the formulations. Formulations containing the mucoadhesive polymers possess the higher syringeability force compared to that of plain pluronic F127 gel formulations; which may be due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w, 23% w/w and 25% w/w pluronic F127 along with 0.50 % w/w, 1.00 % w/w, and 2.00 % concentrations of poly acrylic acid concentration increases significantly ($P<0.001$) with respect to plain pluronic F127 gel. The results of the syringeability are shown in Table No4.111

Table No 4.136: Determination of syringeability of drug loaded mixed mucoadhesive periodontal gels of PAA- pluronic F127

Formulation Code	Syringeability
MGF82	225.67
MGF83	289.39
MGF84	320.05
CGF82	276.30
CGF83	305.25
CGF84	335.76

4.12.6 In vitro release study

The in vitro release profile of minocycline hydrochloride and clindamycin phosphate is illustrated in Figure 4.98 and 4.99 respectively. The maximum release of minocycline hydrochloride from the thermoreversible periodontal gel was shown by the formulation MGF82 where as the least was shown by the formulation MGF84 after 8 hours. Similarly the maximum release of clindamycin phosphate from the thermoreversible gels was shown by the formulation CGF82 where as the least was shown by the formulation CGF84 after 8 hours. The higher release of minocycline hydrochloride and clindamycin phosphate from gels can be explained by the viscosity of the polymer solution. A preliminary study shows that the formulation MGF82 and CGF82 had low viscosity than MGF84 and CGF84. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF82 and CGF82. In addition, formulation MGF82 and CGF82 due to low viscosity have more available waters to diffuse consequently shows more diffusion

through the membrane, similarly formulation MGF84 and CGF84 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

Table No 4.137: In Vitro Release Profile of Minocycline hydrochloride from PAA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride Released ± SD								
	MGF82			MGF83			MGF84		
0.00	0.00	±	0.000	0.00	±	0.000	0.00	±	0.000
0.50	6.90	±	0.010	4.78	±	0.028	2.88	±	0.015
1.00	11.54	±	0.020	8.34	±	0.027	4.84	±	0.047
1.50	16.01	±	0.022	12.78	±	0.046	8.05	±	0.022
2.00	21.65	±	0.008	16.63	±	0.047	11.67	±	0.068
2.50	27.56	±	0.021	21.27	±	0.019	14.50	±	0.065
3.00	33.36	±	0.041	25.75	±	0.011	19.22	±	0.020
3.50	39.59	±	0.037	31.18	±	0.037	23.17	±	0.059
4.00	45.49	±	0.062	37.00	±	0.045	27.50	±	0.052
5.00	55.71	±	0.056	48.22	±	0.043	37.73	±	0.028
6.00	66.44	±	0.055	58.39	±	0.016	47.54	±	0.038
7.00	76.95	±	0.115	67.74	±	0.039	57.44	±	0.069
8.00	87.41	±	0.070	77.61	±	0.057	67.52	±	0.045

Figure 4.98: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from PAA - Pluronic F127 thermoreversible periodontal gel

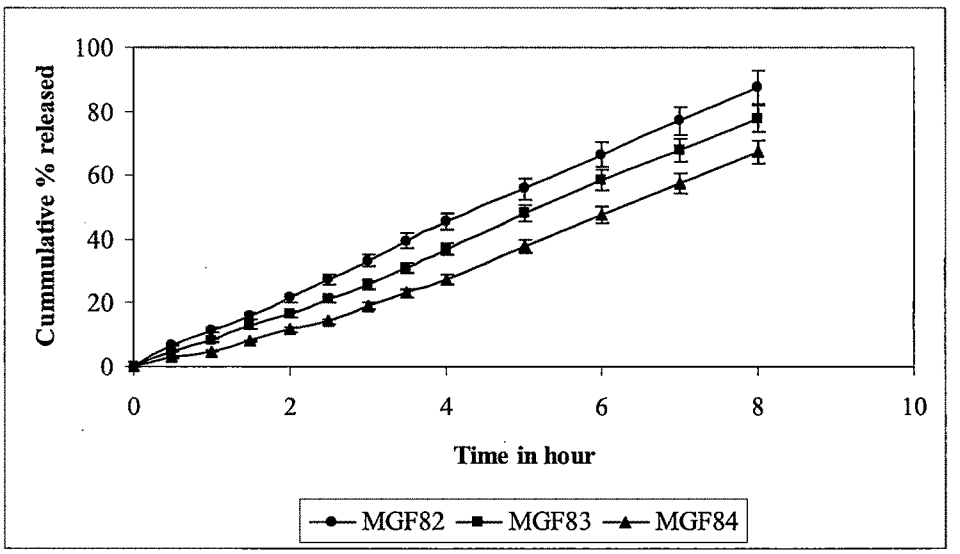


Table No 4.138: In Vitro Release Profile of Clindamycin phosphate from PAA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate released ± SD		
	CGF82	CGF83	CGF84
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	3.53 ± 0.046	2.56 ± 0.043	1.28 ± 0.041
1.00	7.35 ± 0.002	5.23 ± 0.034	3.26 ± 0.046
1.50	12.10 ± 0.019	8.54 ± 0.030	6.11 ± 0.041
2.00	17.08 ± 0.053	13.07 ± 0.052	9.20 ± 0.013
2.50	23.09 ± 0.068	17.61 ± 0.017	13.11 ± 0.044
3.00	28.05 ± 0.064	22.31 ± 0.074	17.29 ± 0.050
3.50	33.08 ± 0.046	27.13 ± 0.064	21.13 ± 0.005
4.00	38.13 ± 0.016	31.06 ± 0.035	25.06 ± 0.050
5.00	47.04 ± 0.039	39.65 ± 0.052	32.56 ± 0.045
6.00	56.09 ± 0.083	47.14 ± 0.016	41.82 ± 0.025
7.00	65.11 ± 0.071	55.20 ± 0.017	49.38 ± 0.047
8.00	73.26 ± 0.048	63.69 ± 0.026	57.69 ± 0.109

Figure 4.99: Cumulative percentage release profile of clindamycin phosphate from PAA - pluronic F127 thermoreversible periodontal gel

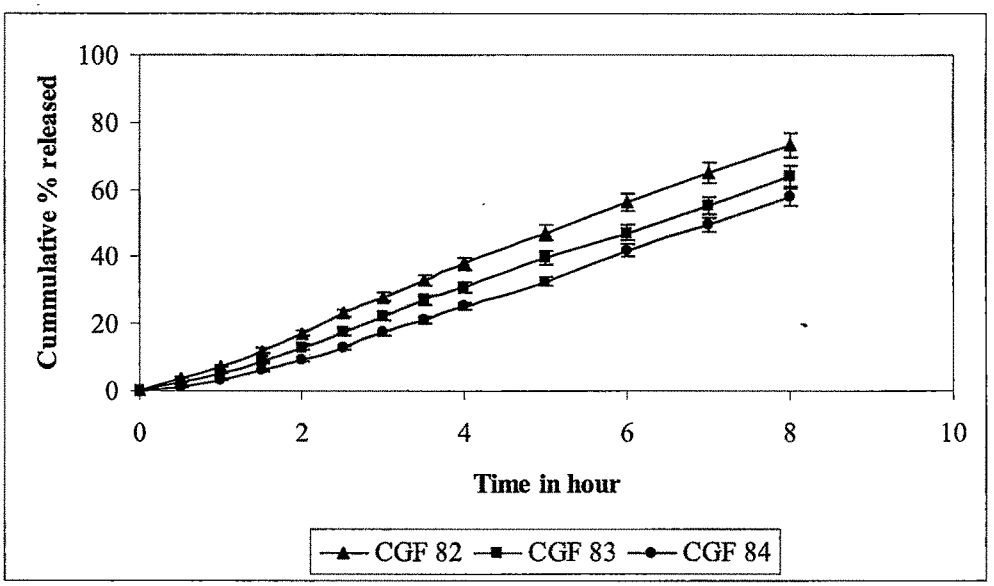


Table No 4.139: Release Kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded PAA - pluronic F127 mucoadhesive periodontal thermoreversible gel

Batch Code	Correlation coefficient				n (Release exponent)	k (Release rate constant)
	Zero order	First order	Higuchi	Peppas		
MGF82	0.9994	0.773	0.9186	0.58	10.952	8.953
MGF83	0.9974	0.7123	0.8924	0.1174	9.866	7.345
MGF84	0.9527	0.5794	0.8519	0.5699	9.069	1.172
CGF82	0.9962	0.7259	0.9145	0.6183	9.911	8.147
CGF83	0.9951	0.6297	0.8943	0.6173	8.962	9.162
CGF84	0.9938	0.5212	0.8784	0.6241	8.043	1.104

4.12.7 In Vitro permeation study

4.12.7.1 Determination of saturated drug concentration

A saturated minocycline hydrochloride/ clindamycin phosphate was done as described in section 4.6.6.1. The saturated concentration of minocycline hydrochloride/ clindamycin phosphate in phosphate buffer pH 6.75 was 106.994 mg ml-1 and 103.900 mg ml^{-1} respectively.

4.12.7.2 Preparation of mucosal tissue

The mucosal tissue was prepared as described in the section 4.6.6.2.

4.12.7.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of cheek mucous membrane was measured as described in section 4.6.6.3. The average thickness was found to be $1.52 \pm 0.325 \times 10^{-2}$ µm, which is the mean of 3 measurements.

The anionic polymer, poly acrylic acid is evidenced to show high Ca^{++} binding ability, thereby showing the permeation enhancing property. However, increase in in-vitro permeation across the oral mucosal membrane could be attained by the thermoreversible periodontal gels of pluronic F127 and poly acrylic acid. The cumulative amount of minocycline hydrochloride and clindamycin phosphate permeated as a function of time across the sheep mucous membrane for various poly acrylic acid pluronic F127 gels

formulations are given in the Figure 4.100 and 4.101 respectively. Since the pluronic F127 gels are viscous, isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra-micellar water channels of the gel matrix. The permeation of the minocycline hydrochloride/ clindamycin phosphate significantly differ in formulations containing the poly acrylic acid ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. The presence of poly acrylic acid results in very rapid dissolution and release of drug due to swelling and dissolutions of poly acrylic acid at pH 6.75. However presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micelle's structures. As seen from the results in presence of 25% w/w pluronic F127 drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations which may be due to the formations of larger concentrations of the micelle's. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of poly acrylic acid not only increase in the Ca^{++} binding site but also increase in inter-accessibility of Ca^{++} binding sites due to relaxation of polymer network.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.5 % w/w poly acrylic acid along with 21% and 23 %w/w pluronic F127 were found to be best. However, formulations containing 0.2%w/w poly acrylic acid along with 25 %w/w pluronic F127 shows lower gelling temperature, low permeation profile and high syringeability which may makes difficult to administer the drug to the periodontal cavity. Formulations containing the higher concentrations of poly acrylic acid (1.00 % w/w and 2.00 % w/w) showed a high syringeability and blockage of the syringe which may be due to high viscous solution. Hence MGF82, CGF82, MGF83 and CGF83 was selected as the optimized formulations exhibiting ideal characteristics with respect to gelation, mucoadhesion, gel strength, syringeability and permeability of drug through oral mucosal membrane and therefore selected for the further study.

Table No 4.140: Ex Vivo Permeation Profile of Minocycline hydrochloride from PAA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Minocycline hydrochloride permeated ± SD		
	MGF82	MGF83	MGF 84
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	5.09 ± 0.025	3.55 ± 0.005	1.78 ± 0.004
1.00	7.64 ± 0.030	5.47 ± 0.037	3.29 ± 0.016
1.50	12.94 ± 0.029	8.49 ± 0.052	5.88 ± 0.029
2.00	18.09 ± 0.039	12.95 ± 0.038	8.60 ± 0.032
2.50	23.17 ± 0.050	17.80 ± 0.020	11.51 ± 0.019
3.00	26.74 ± 0.048	21.14 ± 0.034	15.13 ± 0.009
3.50	32.33 ± 0.067	25.46 ± 0.041	19.13 ± 0.069
4.00	38.22 ± 0.053	31.19 ± 0.040	23.22 ± 0.010
5.00	47.13 ± 0.575	41.37 ± 0.048	32.38 ± 0.206
6.00	57.18 ± 0.023	50.76 ± 0.051	42.55 ± 0.044
7.00	67.01 ± 0.007	60.30 ± 0.049	51.28 ± 0.014
8.00	75.21 ± 0.089	68.06 ± 0.020	60.96 ± 0.043

Figure 4.100: Cumulative permeation profile of minocycline hydrochloride from PAA - pluronic F127 thermoreversible periodontal gel

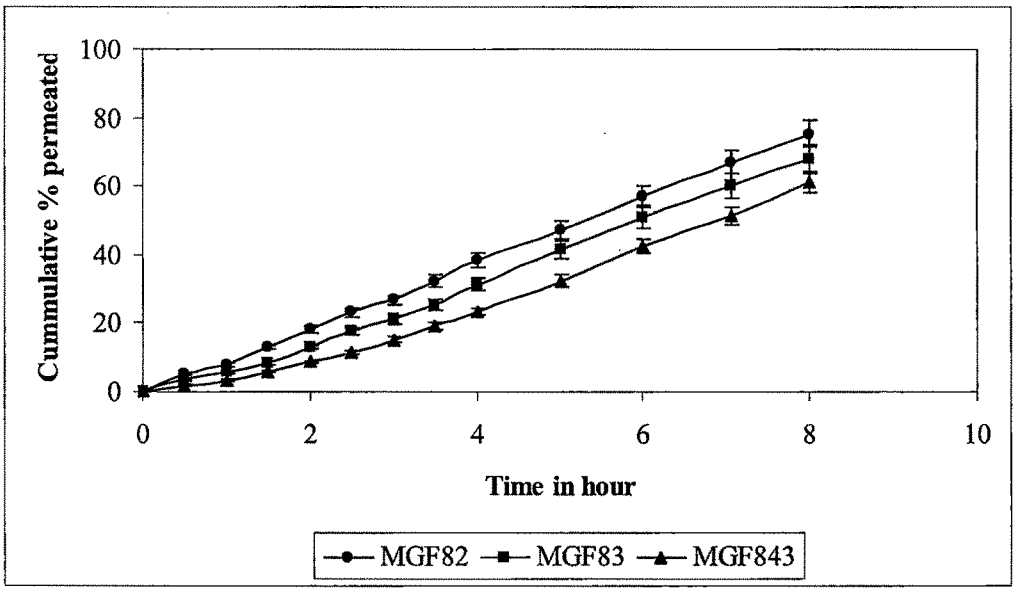


Table No 4.141:Ex Vivo Permeation Profile of Clindamycin phosphate from PAA - Pluronic F127 Thermoreversible Periodontal Gel

Time in Hour	% Clindamycin phosphate permeated ± SD		
	CGF82	CGF83	CGF84
0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
0.50	2.37 ± 0.034	1.38 ± 0.004	0.96 ± 0.035
1.00	4.36 ± 0.039	3.13 ± 0.024	1.96 ± 0.037
1.50	6.36 ± 0.024	4.86 ± 0.036	3.19 ± 0.037
2.00	8.84 ± 0.021	6.68 ± 0.025	5.37 ± 0.035
2.50	11.10 ± 0.039	9.27 ± 0.051	7.56 ± 0.045
3.00	14.07 ± 0.029	11.54 ± 0.027	9.70 ± 0.005
3.50	17.47 ± 0.102	14.85 ± 0.044	12.79 ± 0.007
4.00	21.21 ± 0.034	18.22 ± 0.022	15.33 ± 0.056
5.00	28.53 ± 0.002	25.80 ± 0.020	22.34 ± 0.040
6.00	36.36 ± 0.012	33.28 ± 0.001	29.93 ± 0.045
7.00	45.03 ± 0.027	42.28 ± 0.001	38.34 ± 0.030
8.00	55.21 ± 0.167	52.40 ± 0.042	48.26 ± 0.031

Figure 4.101: Cumulative permeation profile of clindamycin phosphate from PAA - Pluronic F127 thermoreversible periodontal gel

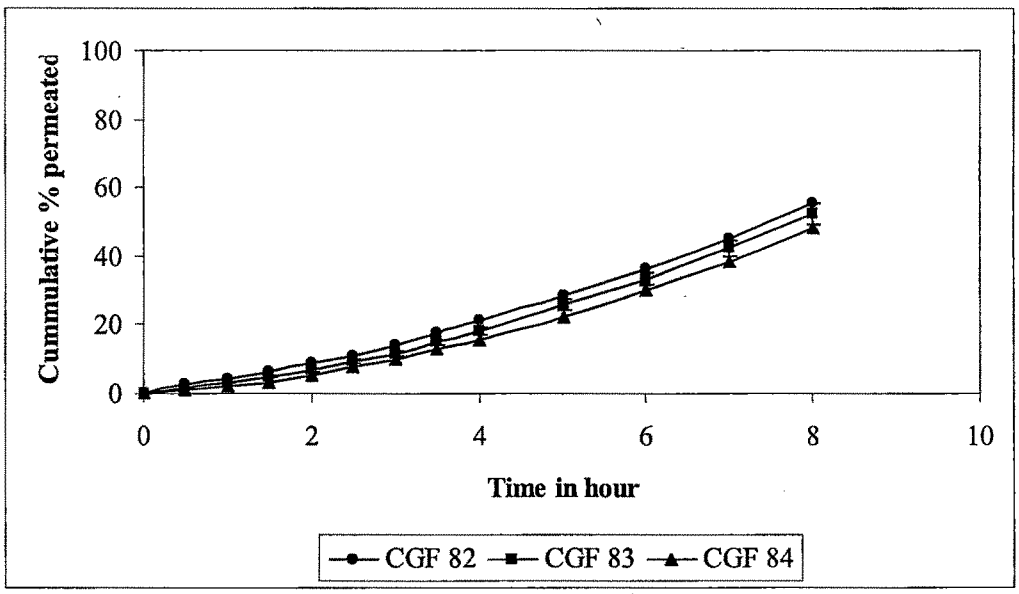


Table No 4.142: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal gels

Formulations	Permeation flux $J(mcg.cm^{-2}.hr^{-1})$	Lag time ($t_L$hr)	Diffusion coefficient $(D\times10^{-8}cm^2.sec^{-1})$	Permeability coefficient $(P\times10^{-8}cm.sec^{-1})$
MGF82	9.60	0.50	2.130	2.492
MGF83	9.22	0.75	1.430	2.393
MGF84	8.39	1.00	1.070	2.178
CGF82	9.43	0.25	4.280	2.521
CGF83	8.32	0.60	1.780	2.224
CGF84	7.51	1.00	1.060	2.000

4.12.8 Stability Study

The Minocycline hydrochloride/ clindamycin phosphate loaded periodontal thermoreversible gels prepared using 20 %w/w and 23 %w/w pluronic F127 along with 0.50 %w/w PAA were studied for the stability of the formulation at Freeze condition (4°C) and at RT (25 °C). All the four selected formulations showed good physical stability with no discoloration, precipitation or any physical changes after storage. Both minocycline hydrochloride and clindamycin phosphate showed good chemical stability in the gel formulation. The gel stability results were found to be similar to the published data (Katakam et al, 1995). The pH of all the formulations was within the range of 6.15 to 6.26, which is the neutral pH.

Table No 4.143: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at 4° C

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF82	97.15 ± 0.75	6.26	35.9
MGF83	98.29 ± 0.88	6.22	34.1
CGF82	98.71 ± 0.99	6.20	36.1
CGF83	99.45 ± 0.75	6.17	33.9

Table No 4.144: Drug Content and pH of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal thermoreversible gel after 180 days storage at room temperature

Formulation Code	Drug content (%)	pH	Gelling Temperature
MGF82	98.12 ± 0.91	6.25	35.8
MGF83	98.66 ± 0.51	6.20	34.0
CGF82	98.95 ± 0.67	6.18	36.0
CGF83	99.35 ± 0.68	6.15	33.8

4.12.9 Conclusion

Thermoreversible gel formulations for periodontal administration prepared using different concentrations of pluronic F127 along with mucoadhesive polymer PAA by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate were shown to exist as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. Rheological behavior of all the formulations was measured. All the formulations exhibit Newtonian behavior at 4°C and remained as liquid and no gel formation was observed. However at 37°C, the behavior of formulations changed, depending on the polymer concentration. At higher concentration a poly molecular micelle forms and micelles come together to minimize their interaction with water whereas at lower concentration monomolecular micelle is formed. At lower temperature water molecules around the polymer chain are ordered and hydrophilic interaction between poly (oxy ethylene) units of pluronic molecules and water molecules is dominant. With increasing temperature, hydrophobic interaction between poly (oxy ethylene) units of pluronic F127 molecules dominates polymer chains approach closer and squeeze ordered water molecule.

It is evident from the data that the presence of mucoadhesive polymer PAA lowered the gelation temperature. It is also noted that addition of increasing concentration of PAA from 0.50-2.00 % w/w further lowered the gelation temperature. The gelation temperature lowering effect of mucoadhesive polymer might be partly due to the increased viscosity after dissolution of mucoadhesive polymer. When the PAA is exposed to water the polymer begins to uncoil and generating an increase in viscosity and gel formation. The uncoiling and expansion of the molecule result in polymer swelling and elastic gel formation.

Increase in the gel strength in presence of different concentration of PAA may be due to bond formation between pluronic F127 and PAA. Increase in gel strength shows that the addition of PAA increases the strength or stiffness of the gel. Higher gel strength formulations possess high mucoadhesive property and increases the residence time at the application site.
Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of PAA. Presence of mucoadhesive polymer PAA having high density of available hydrogen bonding groups

would be able to interact more strongly with mucin glycoproteins and prolonged retention and increased absorption across mucosal tissues.

Gel formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; which may be due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20% w/w and 23% w/w pluronic F127 along with 0.20% w/w PAA concentration increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation showed a sustain release of the drug for a period of 8 hours compared to plain drugs. A preliminary study shows that the formulation prepared with 20%w/w pluronic F127 along with 0.50% PAA (MGF82 and CGF82) had low viscosity than formulation prepared with 23% w/w pluronic F127 along with 0.50 % PAA (MGF83 and CGF83). As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF82 and CGF82. In addition, formulation MGF82 and CGF82 due to low viscosity have more available waters to diffuse consequently shows more diffusion through the membrane, similarly formulation MGF83 and CGF83 shows high viscosity which in turn has less available water to diffuse which may be the cause of the slower drug release from the gel formulations.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for PAA pluronic F127 thermoreversible gels than the pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it was hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Permeation of the minocycline hydrochloride and clindamycin phosphate was significantly different in formulations containing the PAA ($P>0.001$) compared to the plain pluronic F127 thermoreversible gels. Presence of PAA results in very rapid dissolution of the drug due to swelling and dissolution of PAA. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. As seen from the results in presence of 25% w/w pluronic F127

drug release is less compared to the 20% w/w and 23% w/w pluronic F127 containing formulations this may be due to the formations of larger concentrations of the micelles. Addition of the PAA increases the drug permeation compared to the plain pluronic F127 formulations , this may be due to increase in concentrations of ionized carboxyl group to a level require to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized PAA. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of PAA not only increase in the Ca^{++} binding site but also increase the interaccessibility of Ca^{++} binding sites due to relaxation of polymer network.

The investigation of in vitro release and permeation data showed that the diffusion is the mechanism of drug release and followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Results of the stability study showed stable during the storage period of 6 months, and their chemical and mechanical property does not change significantly.

It may be concluded mucoadhesive polymer PAA increases mucoadhesive, physico-chemical and mechanical properties than compared to the plain periodontal thermoreversible gels. Thermoreversible gel formulations maintained a satisfactory residence time in the periodontal cavity and ensured zero order of release of the drug over relatively longer period, which made them good candidate for drug delivery system through periodontal route for the treatment of infectious periodontal diseases.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability formulations containing 0.50 % PAA along with 20% and 23 % w/w pluronic F127 were found to be best for periodontal thermoreversible gel delivery of minocycline hydrochloride and clindamycin phosphate.

4.13 SUMMERIZATION

4.13.1 Conclusion of the various mucoadhesive thermoreversible periodontal gel formulations containing minocycline hydrochloride/ clindamycin phosphate

Various thermoreversible gel formulations for periodontal administration was prepared using different concentrations of pluronic F127 along with various mucoadhesive polymers polycarbophil, HPMC, HEC, PVP, carbopol 934P, PVA and poly acrylic acid by incorporating antibiotics minocycline hydrochloride/ clindamycin phosphate. Gel formulations containing minocycline hydrochloride/ clindamycin phosphate studied, existed as a free flowing viscous liquid at storage temperature (4°C), formed a semisolid gel at experimental temperature (i.e. 37 °C), and return to the liquid state upon cooling below gelation temperature. All the thermoreversible gel formulations were screened on the basis of the sol-gel transition temperature, mucoadhesive strength and gel strength. Rheological behavior of all the formulations was measured.

Considering the rheological behavior, gelling temperature, mucoadhesive property, syringeability and effective permeability, the gel formulations containing 1% w/w minocycline hydrochloride prepared with 20 % w/w pluronic F127 along with 0.2% polycarbophil (MGF02) and 20 % w/w pluronic F127 with 0.5 % w/w poly acrylic acid (MGF82) were found to be the best. Similarly formulations containing 1% w/w clindamycin phosphate prepared with 20 % w/w pluronic F127 along with 0.2% polycarbophil (CGF02) and 20 % w/w pluronic F127 with 0.2 w/w carbopol 934P (CGF54) were found to be the best for localized periodontal delivery. The compositions of the optimized periodontal thermoreversible gel formulations are given in Table No4.145.

Table No 4.145: Composition of various optimized periodontal thermoreversible gel formulations

Compositions in % w/w	Formulation Code			
	MGF02	MGF82	CGF02	CGF54
MnHCl	1.00	1.00	-	-
$ClPO_4$	-	-	1.00	1.00
Pluronic F127	20.00	20.00	20.00	20.00
Polycarbophil	0.20	-	0.20	-
Poly acrylic acid	-	0.50	-	
Carbopol 934P	-	-	-	0.20
Sodium metabisulphite	0.50	0.50	-	-
PEG 1000	15.00	15.00	15.00	15.00
0.5% NaOH	2.00ml	2.00ml	2.00ml	2.00ml
Purified water	qs	qs	qs	qs

It is evident from the data that the presence of mucoadhesive polymers polycarbophil and carbopol 934P lowered the gelation temperature. The addition of increasing concentration of mucoadhesive polymer further lowered the gelation temperature which might be partly due to the increased viscosity after dissolution of mucoadhesive polymer on exposure to water where the uncoiling of the polymer begins generating an increase in viscosity and gel formation resulted due to polymer swelling and elastic gel formation.

The gel strength of the formulations in terms of force required to penetrate shows that the pluronic F127 preparations possess stiffness properties that increase with addition of mucoadhesive polymer which may be due to bond formation between pluronic F127 and mucoadhesive polymer.

Mucoadhesive strength in terms of detachment stress showed that the pluronic F127 preparations possess adhesive properties that increase with addition of mucoadhesive polymer. From the study it was evidenced that the availability of the carboxyl groups or hydroxylic group determines the mucoadhesion. Presence of mucoadhesive polymer polycarbophil, poly acrylic acid and carbopol 934P having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins resulting in prolonged retention and increased absorption across mucosal tissues.

Gel formulations containing the mucoadhesive polymers possess the higher syringeability force compared to the plain pluronic F127 gel formulations; which may be due to the increase in the viscosity of the formulation after addition of the mucoadhesive polymer. Syringeability for formulations prepared using 20%w/w and 23%w/w pluronic F127 along with different concentration of mucoadhesive polymer increases significantly ($P< 0.001$) with respect to plain pluronic F127 gel.

In vitro release and permeation study showed a sustain release of the drug for a period of 8 hours compared to plain drugs. The in vitro release study showed that highest release of the minocycline hydrochloride occurs from the formulation prepared with 20 %w/w pluronic F127 along with 0.2 %w/w polycarbophil and 0.5%w/w poly acrylic acid (MGF02, MGF82). The release of clindamycin phosphate from the formulation prepared with 20 %w/w pluronic F127 along with 0.2 %w/w polycarbophil and 0.2 %w/w carbopol 934P (CGF02, CGF54) was found to be highest. The preliminary study shows that the formulations MGF02 and MGF82 possess the comparatively low viscosity than other minocycline hydrochloride loaded gel formulations. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in case of MGF02 and MGF82. Similarly study also shows that the formulations CGF02 and CGF54 had low viscosity among all the clindamycin phosphate loaded gel formulations. Hence, the diffusion of the drug will be easier in case of MGF02, MGF82, CGF02 and CGF54, thereby possess more available waters to diffuse consequently showing more diffusion through the membrane.

It is evident from the results that effective permeability coefficient for minocycline hydrochloride and clindamycin phosphate are significantly lower for polycarbophil, carbopol 934P and poly acrylic acid containing pluronic F127 thermoreversible gels than the pure drug solution. Since the pluronic F127 gels are viscous isotropic liquid crystals containing micelles, it may be hypothesized that the drug is released by diffusion through the extra micellar water channels of the gel matrix. Presence of polycarbophil, carbopol 934P and poly acrylic acid results in very rapid dissolution of the drug due to swelling and dissolution of the mucoadhesive polymer. However, presence of pluronic F127 in the gel retards the drug release rate slightly due to reduction in dimension of the water channels resulting in enhanced micellar structures. Addition of the polycarbophil, carbopol 934P and poly acrylic

acid increases the drug permeation compared to the plain pluronic F127 formulations, this may be due to increase in concentrations of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of ionized carboxylic group results in decoiling of polymer chain resulting in the relaxation of the polymer network. At this point drug is rapidly dissolved and released from the gels due to very high swelling or fast dissolution of the ionized mucoadhesive polymers. Increase in the permeation of the drug from the formulations can be further explained on the basis that the presence of polycarbophil, carbopol 934P and poly acrylic acid not only increase in the Ca^{++} binding site but also increase the interaccessibility of Ca^{++} binding sites due to relaxation of polymer network.

The investigation of in vitro release and permeation data showed that the diffusion is the mechanism of drug release and followed zero order release model. The main advantages of this formulation is that it contains a lower drug dose, sufficient for the therapeutic effect as it is located directly on the site of the periodontal infection, compared to traditional systemic therapies. Moreover periodontal thermoreversible gel is comfortable because it is non-irritant, biodegradable and may be preferred over other dosages forms in terms of easy application and capability to protect the inflamed surface. Results of the stability study showed stable during the storage period of 6 months, and their chemical and mechanical property does not change significantly.

It may be concluded that the periodontal thermoreversible gel to be placed locally into the periodontal pocket are the promising drug delivery systems against the infectious periodontal diseases. The mucoadhesive polymer polycarbophil, carbopol 934P and poly acrylic acid increases mucoadhesive, physico-chemical and mechanical properties than compared to that of the plain periodontal thermoreversible gels. The selected thermoreversible gel formulations maintained a satisfactory residence time in the periodontal cavity and ensured zero order of release of the drug over relatively longer period, which made them good candidate for drug delivery system through periodontal route for the treatment of infectious periodontal diseases.

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