CHAPTER 6

Mucoadhesive Periodontal Microspheres

6.1 INTRODUCTION

One of the primary objectives in the design of novel drug delivery systems is the controlled delivery of the pharmacological agent to its site of action at a therapeutically optimal rate and dosage regimen (Kreuter, 1992). This site-specific or targeted delivery combined with delivery at an optimal rate would not only improve the efficacy of a drug but would also reduce the possibility of unwanted toxic side effects of the drug, thus improving the therapeutic index (Youssef et al, 1988). Microspheres are made of synthetic or natural polymers. The use of these polymers is often restricted by their bioacceptability. Due to biocompatibility, there has been number of studies reported using ethyl cellulose as a drug carrier. Ethyl cellulose is very much suitable as a drug delivery carrier due to its mucoadhesive potential and permeation enhancing material. Mucoadhesive microspheres are one of the potential periodontal drug delivery systems whereby many of the limitations of periodontal drug delivery like lower residence time could be solved by modulation of microspheres. Therefore we attempted to formulate and optimize ethyl cellulose microspheres incorporating minocycline hydrochloride/ clindamycin phosphate for periodontal drug delivery.

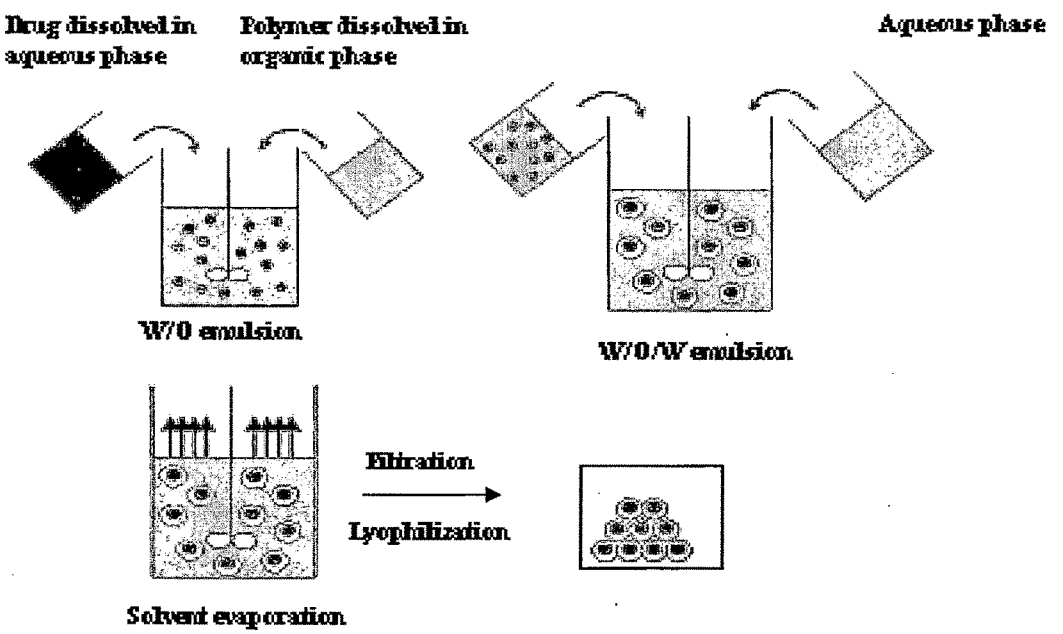
Factorial experimental design, multiple regression analysis, and desirability function have been proven to be the useful approach for the optimization of the formulation. It was found from the preliminary studies that the amount of ethyl cellulose, amount of solvent and the drug loading has significant influence on the properties of microspheres.

The objective of this chapter was to formulate and optimize mucoadhesive periodontal microspheres of ethyl cellulose incorporating minocycline hydrochloride/ clindamycin phosphate, which can be used as the periodontal drug delivery system for the treatment of infectious periodontal diseases. Experimental design and desirability function were applied for the optimization. As part of the optimization process, the main effects like interaction effect and quadratic effect of amount of concentration of ethyl cellulose, drug loading and volume of organic solvent on drug entrapment, particle size, mucoadhesive strength and effective permeability were investigated.

6.2 EXPERIMENTAL

6.2.1 Preparation of periodontal microspheres containing ethyl cellulose by multiple emulsion solvent diffusion method

Periodontal microspheres were prepared by multiple emulsion solvent diffusion method (Dhawan et al, 2004). In this method, the organic internal phase contains designed amount of ethyl cellulose (2.50 % w/v, 5.00 % w/v, 7.50 % w/v, 10.00 % w/v, 12.50 % w/v, 15.00 % w/v) in 20 ml dichloromethane (good solvent for the polymer). To the organic phase 5 ml aqueous solution of minocycline hydrochloride/ clindamycin phosphate (10.00 % w/v) was emulsified using span 80 as the emulsifying agent to prepare the primary emulsion. The primary emulsion was gradually added drop wise into a glass beaker containing 500 ml saturated drug solution in distilled water containing 1.00% (w/v) of poly vinyl alcohol (PVA) as emulsifying agent. The mixture was stirred at 500 rpm for 12 h, at room temperature to remove dichloromethane from the beaker. The formed microspheres were separated by centrifugation and washed with distilled water till it completely removed the excess PVA and unentrapped minocycline hydrochloride/ clindamycin phosphate. The obtained microspheres were lyophilized to obtain the finished product. The finished product was stored in desiccators till further use.



6.2.2 Actual drug content and encapsulation efficiency of periodontal microspheres

The weighed samples of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres (30 mg) was dispersed in 100 ml of 50:50 ratio of dichloromethane to water and sonicated for 30 min at room temperature. The samples were filtered using 0.2 micron membrane filter and absorbance of samples was read at 246nm and 210nm respectively against blank using spectrophotometer. The actual drug content and encapsulation efficiency were calculated as given below. All the analysis was carried out in triplicate.

Actual drug content (%) = Mact / Mms × 100

Encapsulation efficiency (%) = Mact / Mthe × 100

Where Mact is the actual minocycline hydrochloride/ clindamycin phosphate content in weighed quantity of periodontal microspheres, Mms is the weighed quantity of powdered periodontal microspheres and Mthe is the theoretical amount of minocycline hydrochloride/ clindamycin phosphate in periodontal microspheres calculated from the quantity added in the process.

6.2.3 Morphology and particle size studies of periodontal microspheres

Particle size analysis was performed on minocycline hydrochloride/ clindamycin phosphate loaded periodontal microsphere formulations by Malvern Mastersizer (Malvern Instruments, Mastersizer 2000, UK). The results are the average of three analyses. For all formulations, the values (D50) were expressed as mean size range. For morphology and surface characteristics, prepared microspheres were coated with gold palladium under an argon atmosphere at room temperature and then the surface morphology of the microspheres was studied by scanning electron microscopy (SEM) using a JEOL JXA 840A (USA).

6.2.4 Determination of Zeta Potential of periodontal microspheres

The zeta potential, representative of particle charge, was measured by electrophoresis method, using a Malvern Zetasizer nanoZS apparatus. 1%w/v PVA was used as environment. The periodontal microspheres were suspended in 1%w/v PVA by ultra-sonication for 2-3 minutes to get the concentration of the suspension 2%w/v. The cell was filled with a measured amount of sample and inserted with its integral gold electrodes

close to the lid. Single-factor analysis of variance (ANOVA) using MS Excel was performed to determine the difference in zeta potential owing to different methods.

6.2.5 Characterization of pore structure of periodontal microspheres

Porosity parameters of periodontal microspheres such as intrusion extrusion isotherms, pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density were determined by using mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA). Incremental intrusion volumes were plotted against pore diameters that represented pore size distributions. The pore diameter of periodontal microspheres was calculated by using Washburn equation (Washburn, 1921);

$$D = -4\gamma \cos \theta / P$$

Where D is the pore diameter (μm); γ is the surface tension of mercury (485 dyn cm^{-1}); θ is the contact angle (130^0); and P is the pressure (psia).

Total pore area (Atot) was calculated by using equation;

$$A_{tot} = 1 / \gamma \cos \theta \int_0^{Vtot} P \cdot dV$$

Where P is the pressure (psia); V the intrusion volume (ml g^{-1}); V_{tot} is the total specific intrusion volume (ml g^{-1}).

The average pore diameter (Dm) was calculated by using equation;

$$Dm = 4V_{tot} / A_{tot}$$

Envelope (bulk) density (ρse) of the microspheres was calculated by using equation;

$$\rho se = Ws\ V_p - V_{Hg}$$

Where Ws is the weight of the microspheres sample (g); V_p the empty penetrometer (mL); V_{Hg} is the volume of mercury (ml).

Absolute (skeletal) density (ρsa) of microspheres was calculated by using equation;

$$\rho sa = W_s /\ V_{se} - V_{tot}$$

Where, Vse is the volume of the penetrometer minus the volume of the mercury (mL).

The percent porosity of the sample was found from

$$\text{Porosity (\%)} = (1 - \rho se/ \rho sa) \times 100$$

Pore morphology was characterized from the intrusion extrusion profiles of mercury in the periodontal microspheres as described by (Orr, 1969).

6.2.6 Syringeability of periodontal microspheres

Syringeability of the periodontal microsphere formulations was measured using the Universal Testing Machine (UTM) at room temperature with the 6 mm diameter probe (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 3.00 ml glass syringe up to 2 ml 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 syringe containing the sample 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 a maximum of 40 mm. Force recorded was the mean of three readings.

6.2.7 Evaluation of the mucoadhesive strength of periodontal microspheres

The mucoadhesive strength of the microspheres were determined by measuring the force required to detach the formulation from mucosal tissue using a modified method described by Jones et al (Jones et. al, 2000). Briefly, mucosal tissues were carefully removed from the cheek of the sheep obtained from the local slaughter house. Tissues were immediately used after separation. At that time of testing a section of cheek tissue was secured, keeping the mucosal side out, to upper probe using a cyanoacrylate adhesive. Upper probe was attached to the precalibrated force transducer (UTM, Model, LF Plus, Lloyd Instruments, UK) connected to data acquisition system. The surface area of each exposed mucosal membrane was kept constant (0.80 cm^2). At room temperature, fixed amount of sample was uniformly spread on lower probe using double sided adhesive tape, Upper probe was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 2 min to ensure intimate contact between the tissues and the sample. The probe was then moved upwards at a constant speed of 0.1 mm/s and the force in terms of detachment stress in dynes/cm^2, was determined from the weight required to detach the tissues from the surface of each formulation, which was determined as the peak value in the resultant force-time plot, using the following equation (Chang et. al., 1985);

Detachment stress (dyne/ cm^2) = m.g/ A

Where, m is the weight added in gram, g is acceleration due to gravity taken as 980cm/ sec^2 and A is the area of tissue exposed.

6.2.8 Effect of initial contact time on mucoadhesive strength of periodontal microspheres

Effect of the initial contact time (1min, 2min, 3min, 5min, and 10min) on mucoadhesive strength was investigated for periodontal microsphere formulations to optimize the initial contact time. In brief, formulations were allowed to be in contact with mucosa for varying contact time and the mucoadhesive force was determined as discussed above. Contact time that resulted maximum mucoadhesive strength was selected as optimum contact time required for adequate adhesion.

6.2.9 In vitro release of minocycline hydrochloride/ clindamycin phosphate from the periodontal microspheres

As described by Park et. al. in 2005, the microspheres (50 mg) were placed in phosphate buffer pH 6.75 at 37 ^{0} C and were shaken at 15 rpm. The buffer (2 ml) was removed at predetermined intervals and replenished with fresh phosphate buffer (pH 6.75). The amount of minocycline hydrochloride/ clindamycin phosphate released into the buffer was measured spectroscopically at 246nm and 210nm respectively after appropriate dilution to quantitate the amount of minocycline hydrochloride/ clindamycin phosphate released respectively.

6.2.10 In vitro permeation studies of drug loaded formulations of periodontal microspheres

In vitro permeation studies were done as described by many research groups (Caschel et al, 2000; Pisal et al, 2004). Cheek mucosal tissues were carefully removed from the oral mucosal cavity of sheep obtained from the local slaughter house. Fresh sheep cheek mucosal membrane was fixed onto the Franz Diffusion cell. The 500 mg of microspheres 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 ± 1^0C 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 h. were spectroscopically estimated to quantitate the amount of minocycline hydrochloride/ clindamycin phosphate permeated.

6.2.11 Data analysis of permeation studies of drug loaded periodontal microspheres

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.

6.2.12 FTIR studies

FTIR spectral measurements were performed using a Shimadzu FTIR spectrometer. Periodontal mucoadhesive microspheres were grounded with KBR and FTIR spectra was taken in the range 4500-500cm^{-1}.

6.2.13 Stability study

The optimized batch was subjected to stability studies. Formulation was stored in tightly closed vials at room temperature and at 4^0C for three months. The change in percentage entrapment efficiency and particle size was determined after 3 months.

6.3 RESULTS AND DISCUSSION

6.3.1 Preparation of periodontal microspheres containing ethyl cellulose by multiple emulsion solvent diffusion method

In emulsion solvent diffusion method, the formation of the microspheres could be described in the following processes: the formation of emulsion droplets, the diffusion of the ethyl alcohol and the solidification of the droplets. Since the polymer was insoluble in water, the rapid diffusion of dichloromethane into the aqueous medium might reduce the solubility of the polymer in the droplets. The instant mixing of the dichloromethane and water at the interface of the droplets induced precipitation of the polymer, thus forming a shell enclosing the ethanol and the dissolved drug. The counter diffusions of dichloromethane and water through the shell promoted further crystallization of the drug in the droplets from the surface inwards. The finely dispersed droplets of the polymer solution of the drug were solidified in the aqueous phase via diffusion of the solvent (R´e and Biscans, 1999). In this respect, the effect of the dichloromethane amount on the formation of microspheres and the effects of speed on the formation of the microspheres were also investigated.

6.3.1.1 Effect of volume ratio of water: oil phase on periodontal microspheres

Three different ratios of volume of water: oil phase, 1:0.01, 1:0.05 and 1:0.1 was considered. The microspheres were aggregated in the case of 1:0.01 ratios and were found to be discrete in case of other three volume ratios. Increase in the concentration of the dichloromethane, the reduction of the particle size was observed, which may be resulted due to the slow diffusion of the internal dichloromethane into the external phase. It was also found that as the volume fraction of the dichloromethane increases in the internal phase, diffusion of the internal dichloromethane into the external phase slows down. A significant difference in the particle size was observed, whereas no significant difference in the entrapment of drug was observed. However, with further increase in the concentration of dichloromethane (in case of 1:0.1 water: oil ratio) formation of bigger

size particles were observed. Hence 1:0.05 was selected as an optimum ratio of volume of the water: oil phase.

6.3.1.2 Effect of stirring speed on periodontal microspheres

To study the effect of stirring speed on the entrapment efficiency and particle size, the microspheres were prepared using three different stirring speeds viz. 300 rpm, 500 rpm and 750 rpm. As shown in the table no 6.01 and 6.02 with an increase in the stirring speed from 300 rpm to 500 rpm, there was a decrease in the particle size with more uniform distribution was observed. With further increase in the stirring speed to 750 rpm, there was no significant decrease in the particle size. Thus 500 rpm was selected as an optimum stirring speed. The stirring speed does not have any influence on the entrapment efficiency of the microspheres.

Table No 6.01: Effect of stirring speed on the encapsulation efficiency and particle size of minocycline hydrochloride loaded ethyl cellulose microspheres

Drug : Polymer (Formulation Code)	Stirring Speed in rpm					
	300		500		750	
	EE ± SD (%)	PS ± SD (µm)	EE ± SD (%)	PS ± SD (µm)	EE ± SD (%)	PS ± SD (µm)
1:1 (MMF01)	73.98±1.4	62.373 ±0.550	75.33±1.3	51.707 ± 0.609	75.90±1.5	52.721 ±0.619
1:2 (MMF02)	77.17±0.8	63.406 ±0.523	78.31±1.0	52.740 ± 0.632	78.77±1.1	53.744 ±0.627
1:3 (MMF03)	79.90±1.6	64.439 ±0.575	81.27±1.5	53.773 ± 0.579	81.82±1.2	54.774 ±0.581
1:4 (MMF04)	81.27±0.8	65.443 ± 0.575	82.64± 0.8	54.776 ±0.580	83.32±0.7	55.778 ±0.580
1:5 (MMF05)	84.46±1.6	66.445 ± 0.575	85.60±1.2	55.780 ± 0.579	86.05±0.7	56.777 ±0.577
1:6 (MMF06)	87.24±1.7	67.448 ± 0.574	88.24± 1.0	56.782 ± 0.580	88.65±0.8	57.447 ±0.580
2:1 (MMF07)	64.47±1.4	68.450 ± 0.573	67.20±1.3	57.78367 ± 0.579	67.84±0.7	58.784 ±0.583
3:1 (MMF08)	45.39±1.3	69.453 ± 0.575	48.16±1.2	58.78633 ± 0.569	50.13±0.8	59.786 ±0.579

* EE: Encapsulation Efficiency

PS: Particle Size

Table No 6.02: Effect of stirring speed on the encapsulation efficiency and particle size of clindamycin phosphate loaded ethyl cellulose microspheres

Drug : Polymer / (Formulation Code)	Stirring Speed in rpm					
	300		500		750	
	EE ± SD (%)	PS ± SD (µm)	EE ± SD (%)	PS ± SD (µm)	EE ± SD (%)	PS ± SD (µm)
1:1 (CMF01)	69.31±0.7	63.616 ±0.935	70.59± 0.7	52.437 ± 0.575	71.11±0.9	53.406 ± 0.521
1:2 (CMF02)	72.73±2.0	64.622 ±0.939	73.67± 1.4	53.407 ± 0.524	74.27±1.4	54.411 ± 0.528
1:3 (CMF03)	78.07±0.6	65.643 ±0.984	78.93± 1.0	54.443 ± 0.572	79.35±1.3	55.441 ± 0.575
1:4 (CMF04)	80.00±1.3	66.647 ±0.983	81.28 ±1.2	55.446 ± 0.580	81.79±1.2	56.446 ± 0.574
1:5 (CMF05)	81.32±1.3	67.653 ±0.989	82.60± 1.3	56.445 ± 0.577	83.07±1.4	57.446 ± 0.577
1:6 (CMF06)	83.97±1.5	68.663 ±0.991	85.25± 1.4	57.445 ± 0.578	85.68±1.5	58.444 ± 0.583
2:1 (CMF07)	54.36±1.3	69.69 ± 0.530	58.33±1.3	58.448 ± 0.577	59.12±1.1	59.451 ± 0.572
3:1 (CMF08)	44.20±1.3	70.695 ± 0.529	48.44±1.6	59.447 ± 0.580	50.25±1.7	60.446 ± 0.582

* EE: Encapsulation Efficiency

PS: Particle Size

6.3.2 Actual drug content and encapsulation efficiency of periodontal microspheres

For determination of the actual drug content and entrapment efficiency, it was necessary to break the matrix of the microsphere so that the drug association with the microspheres could be determined. For breaking, microspheres were dissolved in dichloromethane and were diluted appropriately in phosphate buffer pH 6.75, then filtered to remove the ethyl cellulose residues. Aliquots were spectroscopically estimated to quantitate the amount of minocycline hydrochloride/ clindamycin phosphate. Results were tabulated in Table no 6.03 and 6.04 respectively.

Table No 6.03: Actual Drug Content and Encapsulation Efficiency of Various Microspheres Containing Minocycline Hydrochloride

Formulation Code	Actual drug content (mg/g)	Encapsulation efficiency ± SD (%)
MMF01	376.675 ± 6.83	75.335 ± 1.36
MMF02	261.047 ± 3.47	78.315 ± 1.04
MMF03	203.187 ± 3.94	81.275 ± 1.57
MMF04	165.282 ± 1.57	82.641 ± 0.78
MMF05	142.668 ± 1.97	85.601 ± 1.18
MMF06	126.060 ± 1.46	88.242 ± 1.02
MMF07	448.026 ± 4.33	67.204 ± 1.29
MMF08	361.269 ± 2.92	48.170 ± 1.167

Table No 6.04: Actual Drug Content and Encapsulation Efficiency of Various Microspheres Containing Clindamycin Phosphate

Formulation Code	Actual drug content (mg/g)	Encapsulation efficiency ± SD (%)
CMF01	352.991 ± 3.70	70.598 ± 0.74
CMF02	245.584 ± 4.70	73.675 ± 1.41
CMF03	197.329 ± 2.44	78.931 ± 0.97
CMF04	162.564 ± 2.56	81.282 ± 1.28
CMF05	136.678 ± 2.24	82.606 ± 1.34
CMF06	121.794 ± 2.11	85.256 ± 1.47
CMF07	382.888 ± 4.34	58.333 ± 1.30
CMF08	370.833 ± 4.03	49.444 ± 1.61

As it could be seen from the table no 6.01 and 6.02, the drug entrapment was found to be significantly affected by the drug to polymer ratio. The increase in drug entrapment with an increase in polymer concentration may be attributed to increase in viscosity, which resulted in the formation of the large microspheres thus increasing incorporation. Similar results were obtained by studies conducted by Denkbas et. al, in 2002. The method adopted for the preparation of microspheres could be a suitable method for the preparation of minocycline hydrochloride/ clindamycin phosphate microspheres with higher encapsulation efficiency.

6.3.3 Morphology and particle size studies of periodontal microspheres

The morphology of the microspheres prepared by emulsion solvent diffusion method and entrapment were investigated by scanning electron microscope (SEM). Samples were mounted onto aluminum stubs using double-sided adhesive tape and then sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were

scanned with an electron beam of 1.2 kV acceleration potential, and images were collected in secondary electron mode. The representative SEM photographs of the minocycline hydrochloride/ clindamycin phosphate loaded microspheres are shown in Figure 6.01(a), (b) and (c) and 6.02 (a), (b) and (c) respectively. It was observed by SEM analysis that the microspheres were finely spherical and uniform.

From the results of particle size shown in table 6.01 and 6.02 it can be concluded that the effect of the polymer concentration and percentage drug loading on the particle size were found statistically significant ($p< 0.05$). The results showed that the largest microspheres were obtained at the high level of polymer concentration, followed by increasing drug loading. The increase in the particle size observed with an increase in the drug: polymer concentration could be attributed to an increase in the relative viscosity of the medium, which may have caused an increase in the interfacial tension. This results in the formation of larger globules during emulsification. Similar results were obtained by studies conducted by Denkbas et al, (2002).

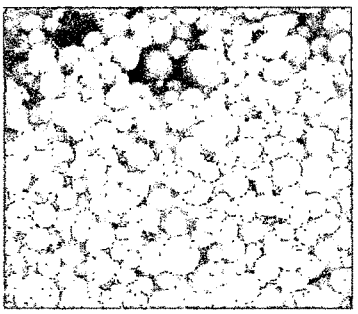


Figure 6.01(a): SEM photograph of whole image of formulation MMF01 at 694X.

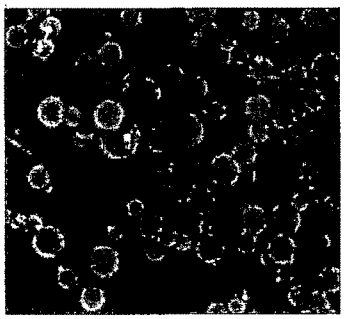


Figure 6.01(b): SEM photograph of whole image of formulation MMF02 at 694X.

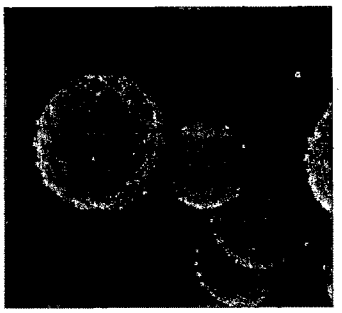


Figure 6.01(c): SEM photograph of whole image of formulation MMF03 at 694X.

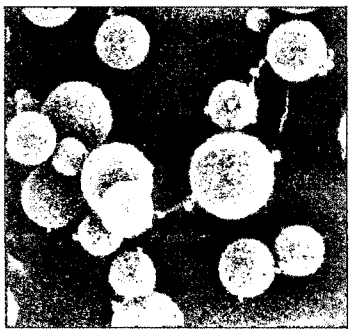


Figure 6.02(a): SEM photograph of whole image of formulation CMF01 at 694X.

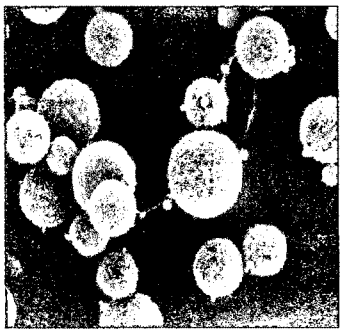


Figure 6.02(b): SEM photograph of whole image of formulation CMF02 at 694X.

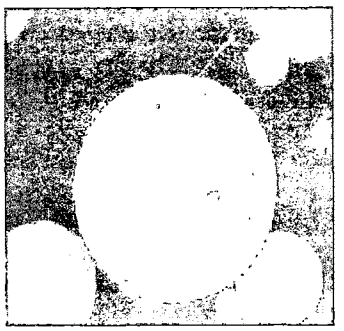


Figure 6.02(c): SEM photograph of whole image of formulation CMF03 at 694X.

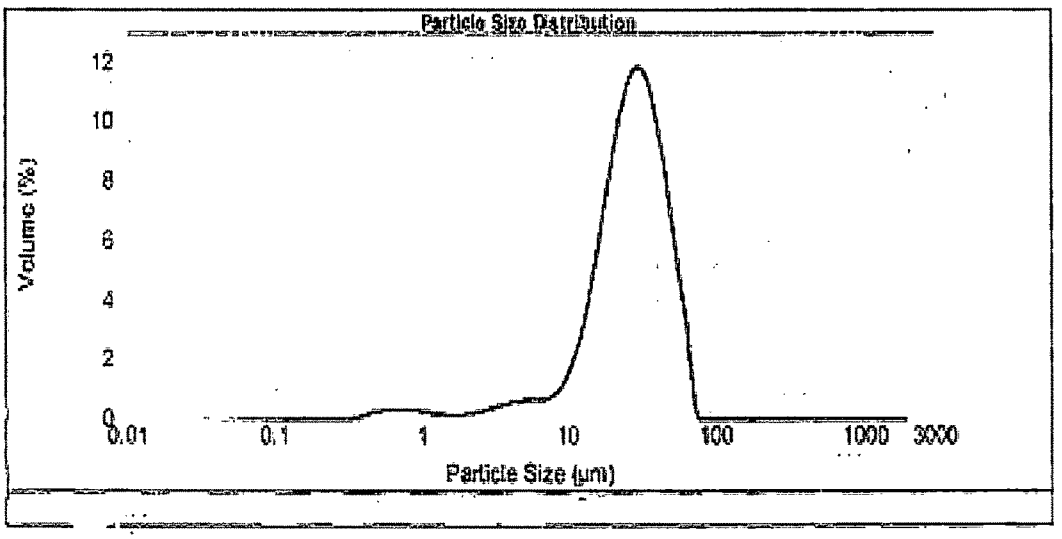


Figure 6.03: Particle size distribution of minocycline hydrochloride loaded ethyl cellulose microspheres

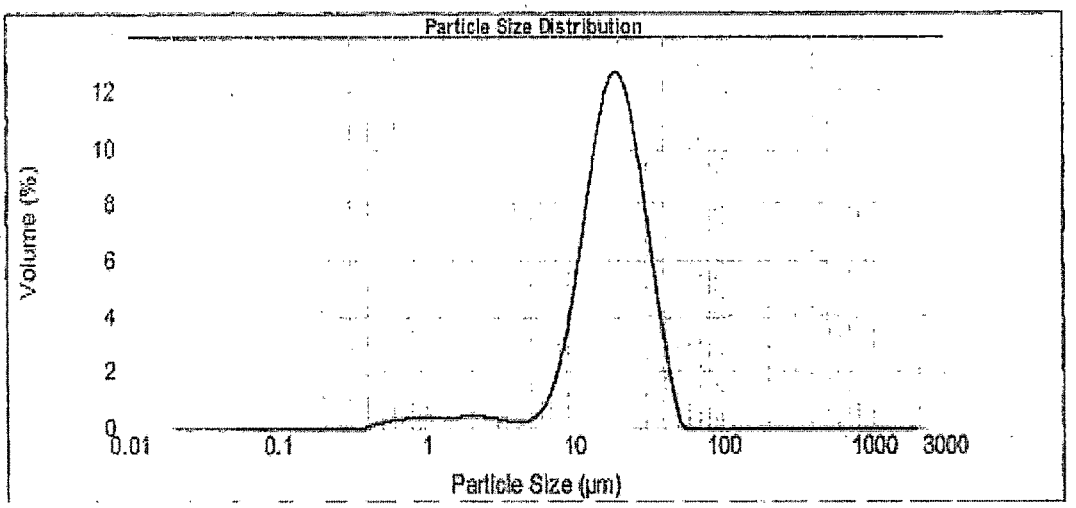


Figure 6.04: Particle size distribution of clindamycin phosphate loaded ethyl cellulose microspheres

6.3.4 Determination of Zeta Potential

The zeta potential of various minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres was performed with a Malvern Zetasizer nano ZS apparatus. Phosphate buffer with pH 6.75 was used as environment. The results of the investigation for minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres is given in table no.6.05 and 6.06 respectively, which showed that the increase in polymer concentration leads to increase in the zeta potential.

6.3.5 Characterization of pore structure of periodontal microspheres

Emulsion solvent diffusion method could fuel interest in the preparation of microspheres, thus providing a high percent of porosity (Kawashima et al, 1992). Intrusion volume of mercury is a function of total porosity (Webb and Orr, 1997; Mehta et al, 2001; Mattsson and Nystrom, 2001; Rahman and Sablani, 2003). According to intrusion and extrusion curves, the majority of the pores presented were of spherical type. The percent of porosity of MMF01 to MMF03 ranged between 71.83 to 77.41% ($p > 0.05$), of MMF04 to MMF06 was 60.91 and 65.24% and the difference between the formulations was statistically significant ($p < 0.05$). Similarly the percent of porosity of CMF01 to CMF03 ranged between 73.93 to 79.21%, of CMF04 to CMF06 was 61.75 and 67.54% ($p > 0.05$) and the difference between these formulations was statistically significant ($p < 0.05$). The data indicated that as the drug: polymer ratio increased, the average pore diameter decreased.

Table No. 6.05: Characteristics of minocycline hydrochloride loaded mucoadhesive periodontal microspheres

Characterization	Formulation Code					
	MMF01	MMF02	MMF03	MMF04	MMF05	MMF06
Zeta Potential (mV)	- 4.036	-6.85	- 8.78	- 9.52	- 11.85	- 15.75
Average pore diameter* (µm)	0.31 ± 0.06	0.29 ± 0.05	0.28 ± 0.04	0.26 ± 0.05	0.24 ± 0.03	0.21 ± 0.02
Envelope (bulk) density* (g/mL)	0.29 ± 0.05	0.31 ± 0.03	0.34 ± 0.04	0.41 ± 0.09	0.44 ± 0.06	0.48 ± 0.08
Apparent (skeletal) density** (g/mL)	1.31 ± 0.15	1.29 ± 0.18	1.27 ± 0.17	1.26 ± 0.13	1.24 ± 0.11	1.23 ± 0.14
Total pore area**(m^2/g)	31.25 ± 0.37	29.68 ± 0.47	28.09 ± 0.35	26.94 ± 0.42	25.27 ± 0.39	24.01 ± 0.43
Porosity* (%)	77.41 ± 1.85	75.93 ± 2.25	71.83 ± 2.05	65.24 ± 2.01	62.56 ± 1.94	60.91 ± 2.11

* Significant differences between formulations ($p < 0.05$)

** No significant differences between formulations ($p > 0.05$)

n=3

Table No. 6.06: Characteristics of clindamycin phosphate loaded mucoadhesive periodontal microspheres

Characterization	Formulation Code					
	CMF01	CMF02	CMF03	CMF04	CMF05	CMF06
Zeta Potential (mV)	- 5.029	-7.36	- 9.21	- 10.05	- 12.19	- 16.31
Average pore diameter* (µm)	0.33± 0.06	0.31 ± 0.02	0.30 ± 0.03	0.28 ± 0.07	0.25 ± 0.04	0.22 ± 0.05
Envelope (bulk) density* (g/mL)	0.27 ± 0.05	0.32 ± 0.02	0.33 ± 0.04	0.42 ± 0.04	0.45± 0.05	0.49 ± 0.07
Apparent (skeletal) density** (g/mL)	1.34 ± 0.14	1.33 ± 0.19	1.31 ± 0.16	1.30 ± 0.15	1.29 ± 0.13	1.27 ± 0.17
Total pore area**(m^2/g)	32.35 ± 0.43	30.72 ± 0.49	29.29 ± 0.52	27.74 ± 0.47	26.38 ± 0.42	25.07 ± 0.23
Porosity* (%)	79.21 ± 1.98	77.43 ± 2.29	73.93 ± 1.34	67.54 ± 1.87	63.86 ± 2.21	61.75 ± 1.97

* Significant differences between formulations ($p < 0.05$)

** No significant differences between formulations ($p > 0.05$)

n=3

6.3.6 Syringeability of periodontal microspheres

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 particle size of the microspheres. The prepared microspheres possess a particle size ranging from 51.707 to 69.453 µm and 52.437 to 70.695 µm respectively, for minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres, which enabled their easy injection into periodontal pockets; microspheres above 200 µm clogged the injection needle. It was found that the mean particle size increased with the increase in the polymer amount. The results of the syringeability shown in table no 6.07 is the mean of three observations.

Table No. 6.07: Determination of syringeability of various minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres

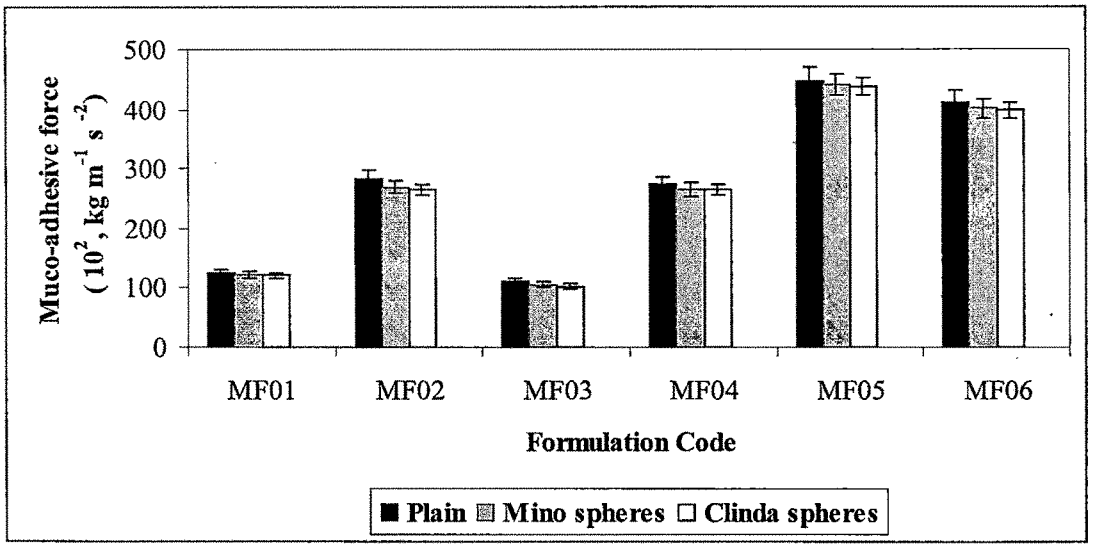
Formulation Code	Syringeability (gf)
MMF01	22.00 ± 0.50
MMF02	23.33 ± 0.21
MMF03	25.73 ± 0.65
MMF04	27.83 ± 0.50
MMF05	30.06 ± 0.66
MMF06	31.63 ± 0.51
CMF01	20.76 ± 0.25
CMF02	21.66 ± 0.49
CMF03	24.20 ± 0.26
CMF04	26.60 ± 0.46
CMF05	28.76 ± 0.32
CMF06	30.70 ± 0.53

6.3.7 Mucoadhesive strength of periodontal microspheres

Mucoadhesive studies were carried out to ensure the adhesion of the formulation to the mucosa for a prolonged period of time at the site of absorption. The model used for mucoadhesive strength measurement was validated by studying effect of initial contact time of the tissues with formulation. About 2 min was found to be the optimum time to achieve maximum detachment stress. At lower contact time, formulations did not have sufficient time to interact with mucosal membrane where as increase in contact time greater then 2 min did not affect mucoadhesive strength further. Mucoadhesive studies indicated that (figure no. 6.03) the mucoadhesive strength was found to be significantly affected by the polymer concentration. Increase in the polymer concentration would result in increased migration of polymer to the surface. This would increase the probability of

hydroxyl groups for binding with sialic acid residues at the mucosal membrane resulting in increased mucoadhesive strength.

Figure No. 6.05: Mucoadhesive strength of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres



6.3.8 In vitro release of minocycline hydrochloride/ clindamycin phosphate from the drug loaded periodontal microspheres

The release studies were carried out in phosphate buffer pH 6.75 at 37 ^{0} C and shaken at 15 rpm. After the release test, the microspheres were observed under optical microscope, the microspheres were found intact.

The in vitro release studies indicated that the main factors affecting release of minocycline hydrochloride/ clindamycin phosphate from the microspheres were the polymer concentration as shown in table no 6.08 and 6.09. There was a decrease in the drug release with an increase in the concentration of the ethyl cellulose was observed. The microspheres prepared using 1:6 drug: ethyl cellulose ratio, released the drug slowly compared to those with the microspheres prepared using 1:2 drug: ethyl cellulose. It was observed that the initial release varies from 3% to 15% in case of minocycline hydrochloride and from 4% to 20% in case of clindamycin phosphate. There is a significant difference ($p< 0.05$) in the drug release rates of the microspheres with increasing polymer concentration was resulted. The microspheres prepared with higher drug to polymer ratio showed significantly low release rates than that of the low drug to polymer ratio microspheres. With an increase in the ethyl cellulose concentration, more

amount of drug is associated with the microsphere matrix and thus less at the surface. The reason behind this finding may be that the microspheres prepared using 15% ethyl cellulose when comes in contact with the dissolution medium, produced a more rigid microdrop and hence slow release of the drug. The release pattern is in similar to the release of various drugs from hydroxy propyl methyl cellulose matrix, a commonly used hydrophilic delivery system (Shao et al, 2001; Shah et al, 1997; Ritger and Peppas 1987).

The release of minocycline hydrochloride/ clindamycin phosphate has been examined keeping the ethyl cellulose content constant. The release rate increases with increase in the drug loading as shown in the table no 6.08 and 6.09 respectively. However a burst effect was observed in all the formulations. This burst effect increased with the increase in the drug loading, this effect is attributed to the drug crystals present on the surface of the microspheres. Similar results were obtained by previous workers (Muvaffak et al, 2004). It was observed that the initial burst varies from 15 to 26% in case of minocycline hydrochloride while it was from 20 to 30% in case of clindamycin phosphate. There is a significant difference ($p< 0.05$) in the drug release rates of the microspheres with increasing drug loading. The higher drug loaded microspheres showed significantly higher release rates than that of the low drug loaded microspheres. The reason behind this finding may be the formation of larger porous network and pore size with increase in the drug loading.

Table no. 6.08: In vitro release studies of minocycline hydrochloride from the drug loaded periodontal microspheres

Time in (h)	Cumulative percentage release					
	MMF01	MMF02	MMF03	MMF04	MMF05	MMF06
1	15.185 ± 0.160	12.054 ± 0.016	10.048 ± 0.160	8.122 ± 0.160	4.911 ± 0.160	2.985 ± 0.160
2	25.141 ± 0.167	20.112 ± 0.016	17.057 ± 0.167	14.888 ± 0.167	12.183 ± 0.167	8.730 ± 0.167
4	39.185 ± 0.174	31.781 ± 0.161	27.155 ± 0.029	23.854 ± 0.174	21.200 ± 0.174	16.480 ± 0.174
6	49.128 ± 0.181	42.627 ± 0.024	36.529 ± 0.174	31.811± 0.036	27.209 ± 0.181	21.816 ± 0.181
8	57.285 ± 1.693	49.706 ± 0.169	42.884 ± 0.037	37.576 ± 0.037	31.670 ± 0.187	25.262 ± 0.187
12	65.172 ± 0.132	58.057 ± 0.176	50.089 ± 0.182	43.293 ± 0.182	36.595 ± 0.194	30.905 ± 0.194
24	75.173 ± 0.138	67.082 ± 0.038	58.092 ± 0.189	50.554 ± 0.189	42.962 ± 0.201	37.549 ± 0.201

Table no. 6.09: In vitro release studies of clindamycin phosphate from the drug loaded periodontal microspheres

Time in (h)	Cumulative percentage release					
	CMF01	CMF02	CMF03	CMF04	CMF05	CMF06
1	20.185 ± 1.506	14.160 ± 1.506	11.599 ± 0.150	10.394 ± 0.150	6.628 ± 1.506	3.916 ± 0.150
2	30.083 ± 1.570	23.048 ± 0.214	19.474 ± 0.157	16.410 ± 0.157	13.538 ± 0.214	10.259 ± 0.157
4	46.391 ± 1.634	36.054 ± 0.221	31.429 ± 0.163	27.483 ± 0.163	22.839 ± 0.221	18.221 ± 0.163
6	56.560 ± 0.342	48.067 ± 0.227	40.394 ± 0.169	34.631± 0.169	29.801 ± 0.227	25.147 ± 0.169
8	64.823 ± 0.349	54.955 ± 0.233	46.371 ± 0.176	41.131 ± 0.176	36.115 ± 0.233	30.378 ± 0.176
12	71.836 ± 0.355	64.163 ± 0.240	55.397 ± 0.182	48.301 ± 0.182	41.141 ± 0.240	34.432 ± 0.182
24	81.301 ± 1.717	74.426 ± 0.246	64.278 ± 0.189	56.913 ± 0.189	49.032 ± 0.246	42.073 ± 0.189

Table no. 6.10: Release kinetics parameters of minocycline hydrochloride loaded mucoadhesive periodontal microspheres

Batch Code	Correlation coefficient				N (Release exponent)	K (Release rate constant)
	Zero order	Higuchi	First order	Peppas		
MMF01	0.780	0.925	0.908	0.767	0.202	0.160
MMF02	0.793	0.932	0.888	0.818	0.189	0.167
MMF03	0.796	0.934	0.871	0.824	0.165	0.190
MMF04	0.790	0.931	0.853	0.825	0.144	0.216
MMF05	0.769	0.919	0.826	0.761	0.126	0.239
MMF06	0.814	0.946	0.861	0.732	0.115	0.243

Table no. 6.11: Release kinetics parameters of clindamycin phosphate loaded mucoadhesive periodontal microspheres

Batch Code	Correlation coefficient				N (Release exponent)	K (Release rate constant)
	Zero order	Higuchi	First order	Peppas		
CMF01	0.751	0.907	0.900	0.709	0.207	0.160
CMF02	0.794	0.934	0.911	0.779	0.206	0.154
CMF03	0.803	0.940	0.892	0.759	0.179	0.177
CMF04	0.814	0.946	0.886	0.670	0.159	0.207
CMF05	0.795	0.935	0.954	0.791	0.143	0.213
CMF06	0.797	0.936	0.851	0.768	0.128	0.227

Figure No. 6.06: Cumulative percentage release profile of minocycline hydrochloride in mcg/cm^2 from periodontal microspheres

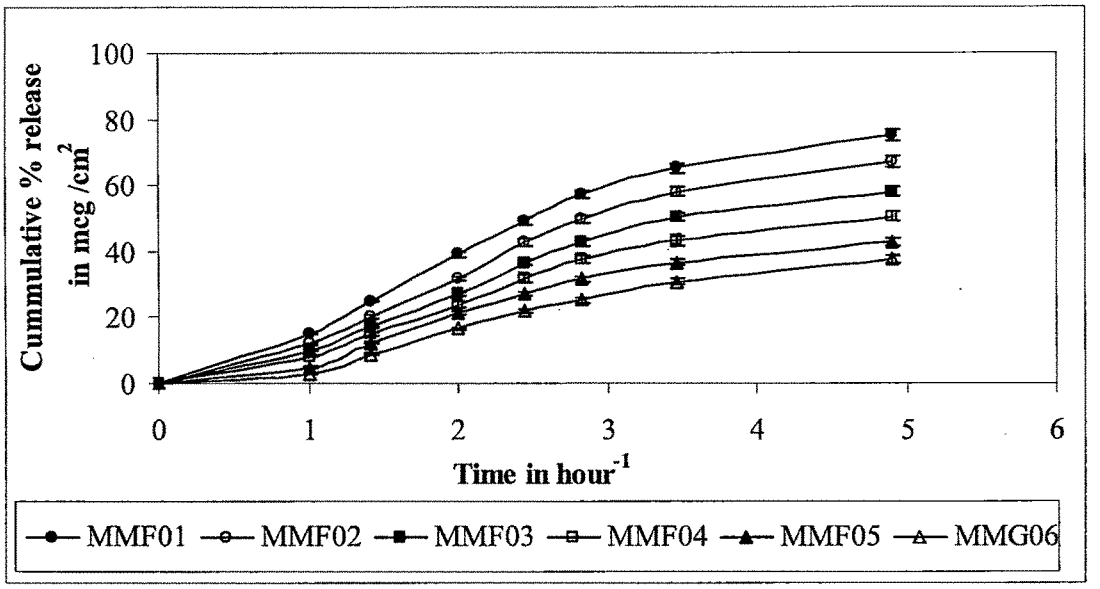
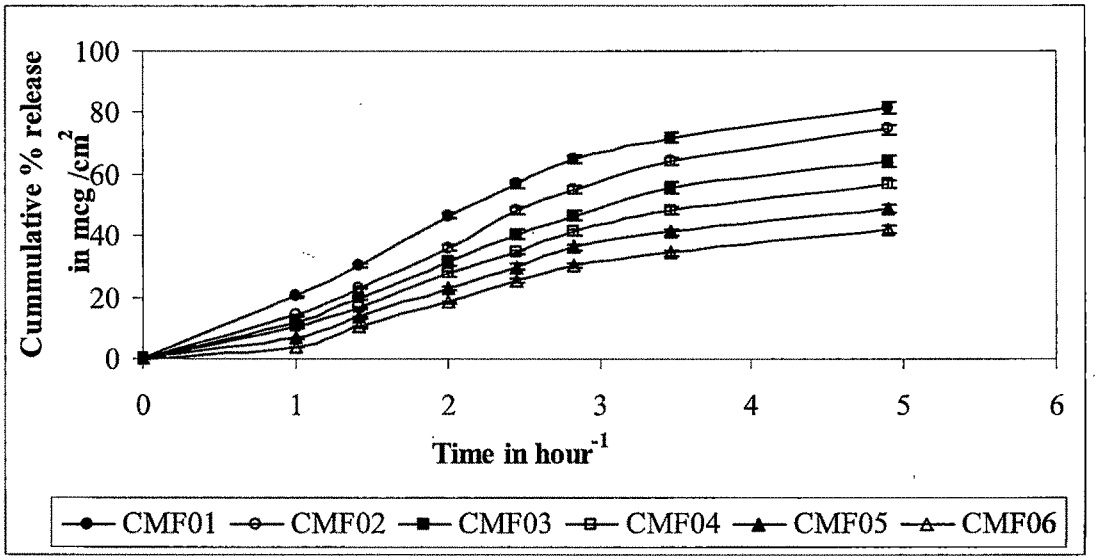


Figure No. 6.07: Cumulative % release profile of clindamycin phosphate in mcg/cm^2 from periodontal microspheres



To examine the kinetics of drug release and mechanism, the release data were fitted to models representing zero order, first order, Higuchi's square root of time (Sankar and Mishra, 2003) and korsemeyer and peppas model. The coefficient of correlation values (calculated from the plot of Q vs t for zero order, Log (Qo-Q) vs t for first order and Q vs $t^{1/2}$ for Higuchi model, Log (Q/Qα) vs log t for peppas model where Q is the amount of drug release at time t, Qα is the amount of drug release at time α and Qo-Q is the amount

of drug remaining after time t) were as shown in table no 6.08 and 6.09 respectively. Thus it can be concluded that the minocycline hydrochloride/ clindamycin phosphate release from the microspheres is best explained by Higuchi model. The mechanism of the drug release is further investigated by the well-known exponential equation, which is often used to describe the drug release behavior from polymeric systems;

$$Mt / M\alpha = kt^n$$

Where $Mt/M\alpha$ is the fractional drug release at time t; k is a constant incorporating the properties of the macromolecular polymeric systems and the drug and n is a kinetic constant which depends on and is used to characterize the transport mechanism. When n 0.5, this indicates a quasi diffusion mechanism, when $n > 0.5$, an anomalous non-fickian diffusion is observed, when $n = 1$ indicates a zero order release (Sankar and Mishra, 2003). This values of n and k was obtained from the plot of Q vs $t^{1/2}$. The values of n obtained for all the batches are less than 0.5, which indicates that the drug is released by quasi fickian diffusion.

From the in vitro release data of minocycline hydrochloride/ clindamycin phosphate loaded ethyl cellulose microspheres it may be concluded that the main factor affecting the drug release from the microspheres are may be the concentrations of ethyl cellulose and the amount of drug loading in the microspheres. The formulations prepared using 1:1, 1:2 and 1:3 drug to polymer ratio is selected for the further study.

6.3.9 Ex vivo permeation study of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres

6.3.9.1 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 2 hours. The temperature of the solution was maintained at 25^0C 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 106.994 mg ml^{-1} and 103.900 mg ml^{-1} respectively.

6.3.9.2 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.

6.3.9.3 Measurement of thickness of sheep cheek mucosal membrane

The mucosal thickness of sheep cheek mucous membrane was measured microscopically in the similar manner as given earlier. The average thickness was found to be 1.52 ± 0.325 $\times 10^{-2}$ μm, which is the mean of three measurements.

In vitro permeation studies of various minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal microspheres were done as described by Caschel et al in 2000 and Pisal et. al in 2004. From the results of permeation it is evidenced that all the periodontal microsphere formulations posses sustain release of drug, which may be due to low surface wetting ability and swelling of the periodontal microspheres. The swelling of the polymers was affected by the ionic strength and pH (Park and Robinson, 1985).

Cumulative amount of drug permeated as function of inverse of time is given in figure 6.08. Different drug permeation kinetics is presented in table no 6.13. It was evidenced that cumulative amount of drug permeated with time was reduced for all the formulations compared to the pure drug solution. Effective permeability (Permeability coefficient) of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres was found to be within 5.281 to 3.680 which is much more less than that of pure drug.

Table no. 6.12: In vitro permeation study of minocycline hydrochloride/ clindamycin phosphate loaded microspheres

Time in (h)	Cumulative percentage permeated					
	MMF01 (1:1)	MMF02 (1:2)	MMF03 (1:3)	CMF01 (1:1)	CMF02 (1:2)	CMF03 (1:3)
1	28.900± 0.321	21.284± 0.321	15.185 ±0.160	33.000± 0.321	24.400± 0.321	19.100± 1.506
2	44.600± 0.334	33.747 ±0.334	25.141 ±0.167	49.500 ±0.334	39.800 ±0.334	30.083 ±1.570
4	62.881 ±0.348	51.400 ±0.348	39.185 ±0.174	67.122 ±0.348	57.400 ±0.348	46.391 ±1.634
6	71.853 ±0.362	62.300 ±0.362	49.128 ±0.181	76.741 ±0.362	66.450 ±0.362	57.400 ±0.342
8	78.800 ±0.375	68.600 ±0.375	57.285 ± 1.693	83.282 ± 0.375	72.800 ±0.299	64.823 ±0.349

Figure No. 6.08: In vitro permeation study of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal microspheres

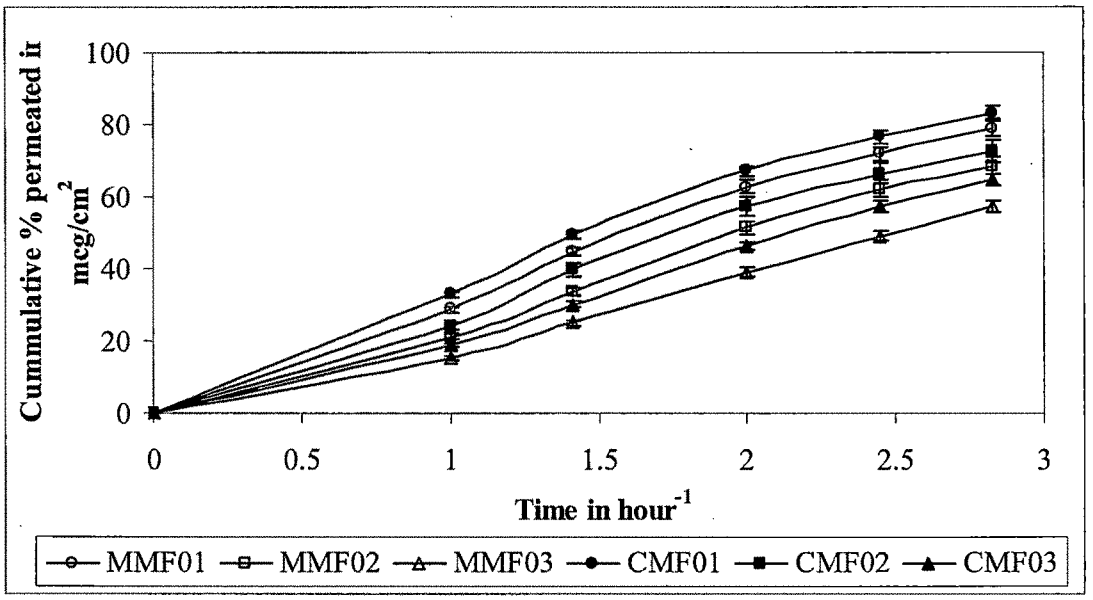


Table no. 6.13: Permeation kinetics parameters of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal microspheres

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 $(P\times10^{7}cm.sec^{-1})$
MMF01	0.194	0.26	4.114	5.036
MMF02	0.169	0.60	1.783	4.402
MMF03	0.141	0.94	1.138	3.680
CMF01	0.198	0.15	7.130	5.281
CMF02	0.179	0.41	2.608	4.788
CMF03	0.146	0.56	1.910	3.959

6.3.10 Infrared spectra

FTIR spectra of ethyl cellulose loaded minocycline/ clindamycin phosphate microspheres are given in figure no. 6.09 and 6.10 respectively.

From the FTIR spectral studies of mincycline phosphate loaded ethyl cellulose microspheres, the characteristic bands of two important functional groups of the pure drug as well as that of the polymer were identified. The FTIR spectra showed that the characteristic bands of minocycline hydrochloride were not altered after the formulation. No change in the positions of the important functional groups reveals that there is absence of any chemical interaction occurred among minocycline hydrochloride, polymer and the formulation additives.

The interpretation of IR spectra of the functional groups reveals as follows: IR (KBr) cm^{-1}: 3479.08 (OH Str.), 3349.81 (NH Str.), 2875.54, 2978.1 (CH_2 Str.), 1648.07 (C=O Str.), 1445.59 (CH=CH Str.), 1312 (CN Str.), 882.23 (CH of Ar-H).

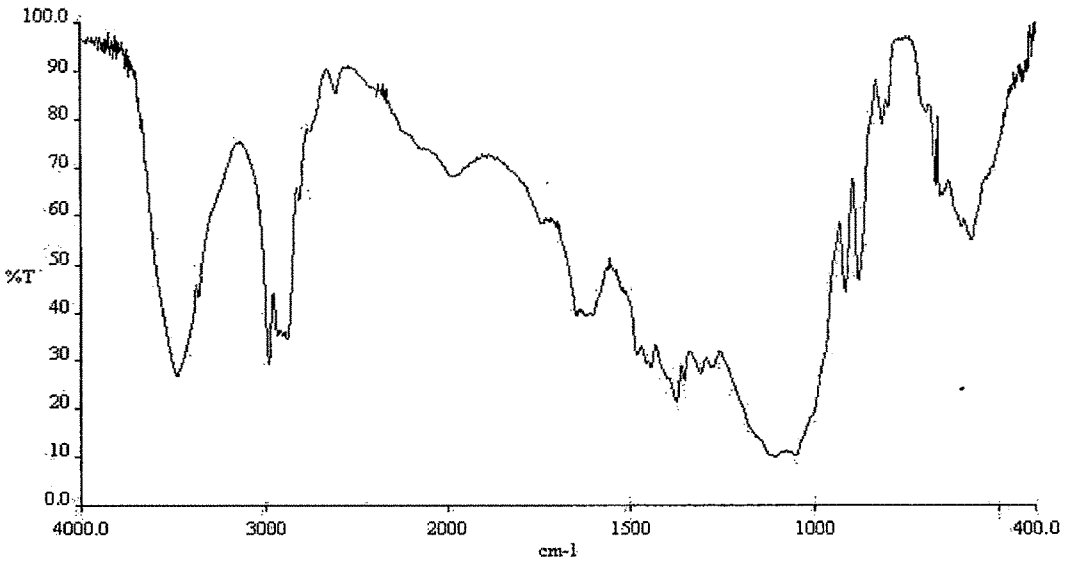
The above data is compared with the standard peaks of the minocycline hydrochloride and the polymer and interpretation of all these in our spectra satisfies and agreed with the above conditions indicating no chemical interaction between the minocycline hydrochloride and the polymer.

From the FTIR spectral studies of clindamycin phosphate loaded ethyl cellulose microspheres, the characteristic bands of two important functional groups of the pure drug as well as that of the polymer were identified. The FTIR spectra showed that the characteristic bands of the clindamycin phosphate were not altered after the formulation. No change in the positions of the important functional groups confirms the absence of any chemical interaction among the drug and the polymer.

The interpretation of IR spectra of the functional groups reveals as follows: IR (KBr) cm^{-1}: 3481.81 (OH Str.), 3386.69 (NH Str.), 2931.20, 2977.33 (CH_2 Str.), 1637.65, 1703.93 (C=O Str.), 1445.12-1487.19 (CH=CH Str.), 1313.15 (CN Str.), 671.74 (C-Cl Str.).

The above data is compared with the standard peaks of the drug and polymer and interpretation of all these in our spectra satisfies and agreed with the above conditions indicating no chemical interaction between the drug and polymer has occurred.

Figure No. 6.09: FTIR Data of MnHCl-ethyl cellulose Periodontal Microsphere



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Description: MSU
Comments: KBr pellet

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End: 400.00
Interval: -1.000000

Ordinate: (%T)
Maximum: 100.00
Minimum: 10.00
Points: 3601

Instrument Model: Spectrum One
Instrument Serial Number: 69888
Instrument Software Version: CPU32-Main 00.02
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Detector: LiTaO3
Source: MIR
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Resolution: 4.00 cm-1
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Beam type: Ratio
Phase Correction: Background
OPD Velocity: 0.20 cm/sec.
I-Stop Size: 8.94 mm
IR Accessory: Slide Holder
Igram Type: Double
Scan Direction: Combined
Filterwheel Position: 1
IR Type: FT
IR Laser Wavenumber: 15798.00 cm-1

Accessories:
Manufacture: P
Part Number: L1200301
Description: Standard sample ready kit
Scan Range / cm-1: 4000 450
Temperature / C: Not Specified

MEC pk

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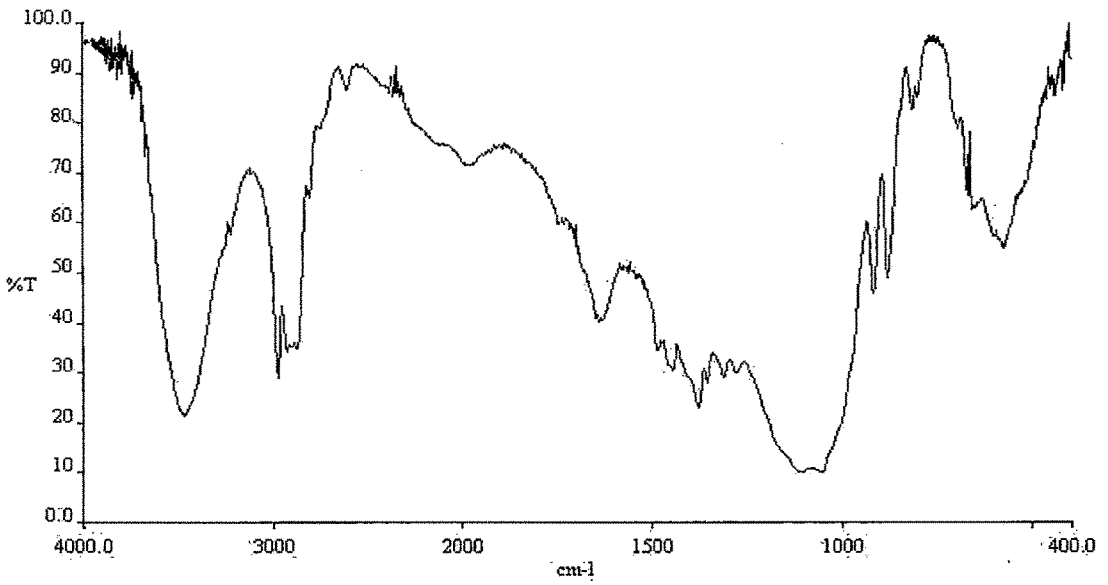
3743.28 90.00 3735.23 88.91 3479.08 78.13 3349.81 26.74 2978.10 29.51
2875.54 34.38 2810.27 63.49 2611.04 85.53 2382.63 84.90 2357.27 84.98
1981.16 68.14 1648.07 39.30 1445.59 28.41 1378.43 21.52 1355.85 25.78
1312.06 27.23 1111.05 9.99 918.17 44.45 882.23 46.40 817.17 78.87
697.33 81.11 672.10 66.26 660.84 64.00 575.96 54.68 456.36 86.31
436.06 87.63 428.05 89.52 423.95 89.56 420.08 90.54 411.87 92.93
403.93 96.16

Figure No. 6.10: FTIR Data of $ClPO_4$-ethyl cellulose Periodontal Microsphere



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Interval: -1.000000

Ordinate: (%T)
Maximum: 100.00
Minimum: 10.00
Points: 3601

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Instrument Serial Number: 69388
Instrument Software Version: CPU32 Main 00.02
Accumulations: 8
Detector: LiTaO3
Source: MIR
Beamsplitter: Opt.KBr
Resolution: 4.00 cm-1
Apodization: Weak
Spectrum Type: Spectrum
Beam type: Ratio
Phase Correction: Background
OPD Velocity: 0.20 cm/sec
J-Stop Size: 8.94 mm
IR Accessory: Slide Holder
Igram Type: Double
Scan Direction: Combined
Filterwheel Position: 1
IR Type: FT
IR-Laser Wavenumber: 15798.00 cm-1

Accessories:
Manufacturer: P
Part Number: L1200301
Description: Standard sample reader kit
Scan Range / cm-1: 4000 450
Temperature / C: Not Specified

CEC.pk

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3841.95 91.42 3824.94 91.48 3820.00 89.04 3815.16 91.00 3810.39 92.86
3805.47 91.27 3800.39 89.52 3778.75 92.79 3768.90 91.69 3764.07 91.63
3755.92 88.99 3748.03 85.95 3743.22 84.77 3738.97 85.37 3735.19 85.44
3721.60 87.96 3691.18 80.31 3678.78 76.31 3674.08 73.56 3481.81 75.69
3386.69 21.54 2977.33 29.21 2931.20 34.19 2809.66 65.26 2609.99 86.63
2383.07 85.95 2365.01 85.52 2347.87 86.07 2325.52 85.31 1968.77 71.57
1703.93 56.94 1637.65 40.06 1487.19 34.44 1445.12 30.41 1378.94 23.04
1355.92 27.98 1313.15 29.30 1280.88 30.02 1056.70 9.99 919.75 45.79
881.60 49.14 816.77 82.52 699.81 79.00 671.74 65.46 660.94 62.37
576.70 54.84 456.09 87.04 444.24 85.38 440.40 85.30 419.82 86.12
408.13 94.00

6.3.11 Stability study

The results of the short term stability at room temperatures for 3 months were done for the optimized formulations indicated that the prepared formulations are highly stable. The changes in the percentage entrapment efficiency of minocycline hydrochloride/ clindamycin phosphate loaded microspheres are given in table no. 6.14. In the similar manner on storage at 4^0C, a slight change in the particle size was also observed as shown in table no. 6.15. From the results it is evident that there was no significant change observed in percentage entrapment efficiency or particle size of the microspheres on storage at room temperature and at 4^0C for 3 months. Formulations were remaining in the form of free flowing powder which accounts for their stability.

Table no. 6.14: Actual Drug Content and Encapsulation Efficiency of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal microspheres after 180 days storage at room temperature

Formulation Code	Actual drug content (mg/g)	Encapsulation efficiency ± SD (%)
MMF01	372.431 ± 5.89	73.500 ± 1.18
MMF02	444.047 ± 3.49	65.461 ± 1.08
MMF03	359.187 ± 2.98	46.375 ± 1.01
CMF01	348.861 ± 1.57	68.683 ± 0.78
CMF02	380.642 ± 1.97	56.324 ± 1.12
CMF03	368. 649 ± 4.19	47.244 ± 1.02

Table no. 6.15: Actual Drug Content and Encapsulation Efficiency of minocycline hydrochloride/ clindamycin phosphate loaded mucoadhesive periodontal microspheres after 180 days storage at 4° C

Formulation Code	Actual drug content (mg/g)	Encapsulation efficiency ± SD (%)
MMF01	373.341 ± 5.89	73.361 ± 0.93
MMF02	443.407 ± 3.49	65.146 ± 0.89
MMF03	358.817 ± 2.98	46.105 ± 1.01
CMF01	347.681 ± 1.57	68.368 ± 0.91
CMF02	381.462 ± 1.97	56.109 ± 0.86
CMF03	369. 469 ± 1.46	47.204 ± 0.95

6.4 CONCLUSION

The preparation methods of microspheres are limited in the means of complexity and cost. This method seems to be promising for the preparation of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres with being easy, reproducible, and has an advantage of avoiding solvent toxicity. The increase in the concentration of the dichloromethane reduced the particle size, which may be due to the slow diffusion of the internal dichloromethane into the external phase. Basing on the particle size and entrapment efficiency of the drug loaded periodontal microspheres, the stirring speed of 500rpm resulted in a decreased particle size with improved entrapment efficiency. However, all the optimized formulations showed better drug entrapment with smaller particle size. The sequence remained same for both the drugs.

The actual drug content of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres expressed in mg/g varied between 448.026 –126.060 mg/g for minocycline hydrochloride microspheres and 382.888 to 121.794 mg/g for clindamycin phosphate microspheres with a significant difference between the periodontal microsphere formulations ($p < 0.05$). On increasing the amount of polymer, the actual drug content of microspheres decreased. The encapsulation efficiency was found to be between 88.242 to 48.170 and 85.256 to 49.444 respectively for minocycline hydrochloride and clindamycin phosphate loaded periodontal microspheres. The relatively high drug content and encapsulation efficiency of microspheres also indicated that the method was suitable for preparing the microsphere formulations.

The morphology of the periodontal microspheres was investigated by SEM. From the SEM photographs of the minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres it was observed that the periodontal microspheres were finely spherical and uniform throughout with no entire drug under visual observation.

The prepared periodontal microspheres had an average diameter of 65.28 to 80.26 μm, which enabled their easy injection into periodontal pockets. Smaller microcapsules, below 50 μm diameter would be removed from the periodontal pocket due to a high flow rate of gingival fluid (20 μl h^{-1}), while the microspheres above 200 μm clogged the injection needle. It was observed that the mean particle size increased with the increase in the polymer amount.

Porosity of the microspheres possess significant effect on the release and other physiochemical property of the microspheres, for the measurement of the porosity of the microspheres mercury displacement method was used, where the intrusion volume of mercury is a function of total porosity. According to intrusion and extrusion curves, the majority of the pores present were of spherical type. The data indicated that as the drug: polymer ratio increased, the average pore diameter decreased.

The syringeability data indicated that the increase in polymer concentration increases the particle size of the microspheres thereby improving the syringeability of the periodontal microsphere formulations. The addition of drug (minocycline hydrochloride/ clindamycin phosphate) to the plain periodontal microsphere formulations resulted in decrease in mucoadhesive strength of the formulations, which may be due to the increased migration of polymer to the surface.

The in vitro release data of minocycline hydrochloride/ clindamycin phosphate loaded periodontal microspheres indicated that the increase in the polymer concentration decreases the drug release significantly. An initial burst of release was caused by the minocycline hydrochloride/ clindamycin phosphate at the surface of the periodontal microspheres which was followed by a release rate approaching higuchi order. The release rates of minocycline hydrochloride/ clindamycin phosphate after the initial burst was varying between 2.985 to 15.185 $\mu g\ ml^{-1}\ h^{-1}$ and from 3.916 to 20.185 $\mu g\ ml^{-1}\ h^{-1}$ respectively, indicating maintenance of a concentration above its minimum inhibition concentration. The kinetics parameters conforms the release of drug following higuchi order of release. So these formulations can be taken for the further study.

In vitro permeation data of minocycline hydrochloride and clindamycin phosphate loaded periodontal microspheres showed that as the polymer concentration increases, lag time, permeability coefficient and diffusion coefficient decreases, this may be because of increase of the path of the drug molecule.

Results of the stability study indicated that the microspheres containing the minocycline hydrochloride/ clindamycin phosphate showed stability for 180 days in room temperature and at 4^0C. Morphology of the microspheres does not change in the accelerated storage conditions.

From the above study this can be concluded that the controlled release of minocycline hydrochloride/ clindamycin phosphate over a period of 24hrs may be achieved by using ethyl cellulose as a matrix forming polymer with biocompatibility. Therefore, the minocycline hydrochloride/ clindamycin phosphate loaded microspheres of ethyl cellulose are suggested as an effective therapeutic modality for the treatment of periodontitis.

6.5 REFERENCES

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