CHAPTER 8

Radio Labeling Of Periodontal Formulations

8.1 INTRODUCTION

Radio-labeling of various pharmaceuticals can be done by using various isotopes such as gallium-67 (Ogihara et.al., 1986), Indium-111 (Presne et.al., 1989) and Technetium- 99m (^{99m}Tc) (Barratt et.al., 1984). The extremely favorable physical and radiation characteristics of ^{99m}Tc made it suitable to be used in nearly 80% of all the pharmaceuticals used in nuclear medicine. The 6hr physical half life and the little amount of electron emission permit the administration of millicurie amounts of ^{99m}Tc radioactivity without significant radiation dose to the patient. In addition the monochromatic 140 KeV photons are readily collimated to give images of superior spatial resolution. Furthermore, ^{99m}Tc is readily available in a sterile pyrogen-free and carrier-free state from ^{99}Mo-^{99m}Tc generators.

8.2 CHEMISTRY OF TECHNETIUM

In the periodic table, technetium is placed as a transition metal of silvery grey color belonging to VIIB group, (Mn, Tc and Re) with atomic number 43 without any stable isotope. The ground state ^{99m}Tc has a half life of 2.1×10^5 years. The electronic structure of the neutral technetium atom is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^6 4d^6 5s^1$. Technetium can exist in 8 oxidation states namely -1 to +7, which results from the loss of a given number of electrons from the 4d and 5s orbital or gain of an electron to the 4d orbital. The stability of these oxidation states depends on the type of ligand and chemical environment. The +7 and +4 states are the most stable and are represented in oxides, sulphides, halides and pertechnetates. The lower oxidation states -1, +1, +2 and +3 are normally stabilized by complexation with ligands. For example Tc^{+1}, complexed with six isonitrile groups in ^{99m}Tc- sestamibi. Otherwise they are oxidized to +4 state and finally to the +7 state (Saha, 1993).

8.3 REDUCTION OF $^{99M}TCO_4^-$

The chemical form of ^{99m}Tc available from the Molybdenum generator is sodium pertechnetate (^{99m}Tc-$NaTcO_4^-$). The pertechnetate ion, $^{99m}TcO_4^-$, having the oxidation state +7 for the ^{99m}Tc, resembles the permanganate ion MnO_4^-, and the pertechnetate ion ReO_4^-. Chemically $^{99m}TcO_4^-$is a rather non- reactive species and does not label any compound by direct addition. In ^{99m}Tc- labeling of many compounds, prior reduction of ^{99m}Tc from +7 states to a lower oxidation state is required (Shah, 1993). Various reducing systems that has

been used are stannous chloride ($SnCl_2$. $2H_2O$), stannous citrate, stannous tartarate, concentrated HCl, sodium borohydride ($NaBH_4$), dithionite and ferrous sulphate. Among these, stannous chloride is the most commonly used reducing agent in acidic medium in most of the ^{99m}Tc labeled preparations.

8.4 LABELING WITH REDUCED TECHNETIUM

The reduced ^{99m}Tc species are chemically reactive and combine with a wide variety of compounds, which usually donates lone pair of electrons to form coordinate covalent bonds with ^{99m}Tc. Compounds bearing chemical groups such as $-COO^-$, $-OH^-$, $-NH_2$ and –SH are eligible for labeling with technetium.

8.5 HYDROLYSIS OF REDUCED TECHNETIUM AND TIN

There is a possibility that reduced ^{99m}Tc may undergo hydrolysis in aqueous solution. In this case, the reduced ^{99m}Tc reacts with water to form various hydrolyzed species depending on pH, duration of hydrolysis and presence of other agents. Some species of this category are $^{99m}TcO_2$, $^{99m}Tc^{2+}$ and $^{99m}TcOOH^+$. This hydrolysis competes with the chelation process of the desired compound and this reduces the yield of the ^{99m}Tc-chelate.

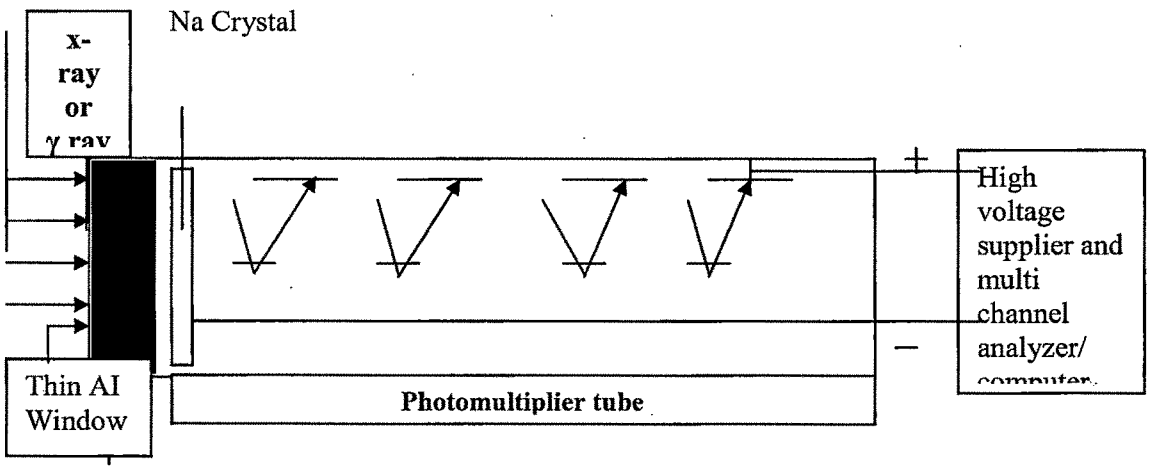
The use of stannous chloride has a disadvantage in that it also readily undergoes hydrolysis in aqueous solution at approximately pH 6 to 7 and forms insoluble colloids. These colloids bind to reduced ^{99m}Tc and thus compromise the labeling yield. To prevent this colloid formation, an acid is added to prevent the hydrolysis of Sn^{2-} before the reduction of technetium.

8.6 PRINCIPLE OF WORKING OF GAMMA SCINTILLATION COUNTER AND GAMMA CAMERA

Scintillation counters are commonly used for X-rays and gamma rays. The name suggests that the working principle for scintillation counters is based on light emission. The gamma scintillation counter consists of a sodium iodide crystal which is enclosed in a stainless steel casing. It also contains 0.5 mole percent of thallium iodide as an activator. This is optically coupled to photomultiplier tube which is connected to a pre-amplifier. The pre-amplifier is

connected to a linear amplifier, pulse height analyzer or single channel analyzer which amplifies the pulses linearly. The amplified pulse can be then recorded using a scaler. The output pulses from a scintillation counter are proportional to the energy of the radiation. Photons striking a sodium iodide (NaI) crystal, which contains 0.5 mole percent of thallium iodide (TII) as an activator, cause the emission of a short flash of light in the wave length range of 3300-5000 A^{o} (in the UV region). The light flashes are detected by a photomultiplier tube, which gives a pulse corresponding to the light intensity. These pulses are measured by a multi channel counter. In case of gamma camera, the scaler is substituted by a camera which can noninvasively detect the presence of the radioactivity in the body.

Figure 8.01



The Key components of a Typical Scintillation counter

8.7 MATERIALS

Stannous chloride dihydrate ($SnCl_2.2H_2O$) was purchased from Sigma Chemical Co., St. Louis, M.O.; sodium pertechnetate was separated from molybdenum-99 by solvent extraction method was procured from Regional center for Radiopharmaceutical division (Northen Region), Board of Radiation and Isotope Technology, Delhi, India.

8.8 RADIO LABELING OF MINOCYCLINE HYDROCHLORIDE/ CLINDAMYCIN PHOSPHATE AND THEIR FORMULATIONS

The radio labeling of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations (MnHCl, MGF02, MSF27, MMF01, ML14, $ClPO_4$, CGF54, CSF27, CMF01 and CL14) with reduced ^{99m}Tc were carried out as per the procedure given below;

All the periodontal formulations (MnHCl, MGF02, MSF27, MMF01, ML14, $ClPO_4$, CGF54, CSF27, CMF01 and CL14) were labeled with ^{99m}Tc by direct labeling method as described earlier (Richardson et. al., 1997). Briefly 1ml of ^{99m}Tc (2mci/ml) was mixed with 100µl of stannous chloride solution in 10% acetic acid (2mg/ml) for all the periodontal formulations. The pH was adjusted to 5.5-6 using 0.5 M sodium bicarbonate solutions. To this mixture, 1ml of minocycline hydrochloride/ clindamycin phosphate solution (1mg/ml) or the minocycline hydrochloride/ clindamycin phosphate loaded formulations (MnHCl, MGF02, MSF27, MMF01, ML14, $ClPO_4$, CGF54, CSF27, CMF01 and CL14) (1%w/w strength) was added. The radio chemical purity of labeled complex was estimated by ascending thin layer chromatography using 100% acetone or 0.9% sodium chloride as developing solvent (Theobald, 1990).

8.8.1 Labeling efficiency

\The labeling efficiency of all the periodontal formulations (MnHCl, MGF02, MSF27, MMF01, ML14, $ClPO_4$, CGF54, CSF27, CMF01 and CL14) was estimated by ascending instant thin layer chromatography (ITLC) using silica gel coated fiber sheets (1 × 12 cm) (Gelman Sciences. Inc., Ann Arbor, MI), as stationary phase and 100% acetone or 0.9% saline as mobile phase. Approximately 2µl of the radio labeled complex was applied at a point 1cm from one end of an ITLC-SG strip. The strip was developed in the mobile phase till the solvent front reaches about 8cm from the origin. The strip was cut horizontally into two halves and the radio activity in each segment was determined in a well type Gamma ray counter (Gamma Ray Spectrometer, Type GRS23C, Electronics Corporation of India Ltd., Mumbai). The free pertechnetate that moved with the solvent (R_f= 0.9) was determined. The reduced/ hydrolyzed (R/ H) technetium along with the labeled complex remained at the point

of application. The amount of reduced/ hydrolyzed (R/ H) technetium was determined using pyridine: acetic acid: water (3:5:1.5 v/v) as mobile phase. The reduced/ hydrolysed (R/ H) technetium remained at the point of application while both the free pertechnetate and the labeled complex moved away with the solvent front. By subtracting the activity moved with the solvent front using either acetone or saline from that using pyridine: acetic acid: water as a mixture, the net amount of ^{99m}Tc labeled complex was calculated.

8.8.2 Stability study of ^{99m}Tc labeled complex

The in vitro stability of radio labeled complex was tested in physiological saline (Chauhan et. al., 1993). The study was performed by addition of 0.1ml of labeled complex with 0.9ml of saline at room temperature. ITLC was performed using 100% acetone as mobile phase. Strips were dried and cut into two pieces and radioactivity in both the pieces was counted in well type Gamma ray counter. Any increase in pertechnetate percentage was considered as the degree of degradation of the labeled complex.

8.8.3 DTPA challenging test

The binding affinity of the labeled complex was conformed by transchelation using DTPA. The stability and strength of the binding of ^{99m}Tc with all the periodontal formulations (MnHCl, MGF02, MSF27, MMF01, ML14, $ClPO_4$, CGF54, CSF27, CMF01 and CL14) was examined by challenging with various concentrations of DTPA, which includes addition of 0.5ml of 1.0mM solutions of DTPA in saline with 0.1ml of the radio labeled periodontal formulations in a 5ml vial separately. A mixture of 0.5ml of saline with 0.1ml of labeled preparation was taken as control. After brief mixing the effect of DTPA on the labeling efficiency was determined using ITLC-SG strip using normal saline (Eckelman et. al., 1989) as mobile phase, which allowed the separation of free pertechnetate and DTPA complex (R_f = 0.8-1.0) from the 99mTc-drug/ formulation complex, which remained at the point of application (R_f = 0).

8.9 OPTIMIZATION OF RADIO LABELING OF MINOCYCLINE HYDROCHLORIDE/ CLINDAMYCIN PHOSPHATE AND THEIR PERIODONTAL FORMULATIONS

Minocycline hydrochloride/ clindamycin phosphate and their formulations (mucoadhesive periodontal thermoreversible gel, strip, microspheres and liposomal gel) were labeled with Technetium- 99m by simple reduction method (Richardson et. al., 1977). The pertechnetate used for the study was first reduced to its lower valence state using stannous chloride dihydrate and then pH was adjusted to neutral using 0.5M sodium bicarbonate before mixing with the minocycline hydrochloride/ clindamycin phosphate and their formulations. Basing on three factors such as; stannous chloride dihydrate concentration, pH of the complex formed and incubation time, the radio labeling was optimized. The effect of stannous chloride dihydrate concentration on the labeling efficiency was studied to obtain the optimum concentration required for maximum labeling as shown in table no.8.01 and 8.02 respectively. The radio labeling efficiency was studied at varying pH (4-7) keeping other variables constant and the results obtained are given in table no.8.03 and 8.04 and figure no.8.02 and 8.03. The effect of incubation time on the radio labeling efficiency of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were studied by incubating the samples for various time periods. The results are shown in table no. 8.05 and 8.06 and figure no. 8.04 and 8.05. The in vitro stability study of the radio labeled complex was carried out in saline and the results are shown in table no.8.07 and 8.08. The radio labeling procedure of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations is as follows;

1ml of 99mTc (2mci/ml) was mixed with required amount of stannous chloride (2mg/ml) solution and then the pH was adjusted suitably using 0.5 M sodium bicarbonate solutions. To this mixture, 1ml of minocycline hydrochloride/ clindamycin phosphate solution (1mg/ml) or the minocycline hydrochloride/ clindamycin phosphate loaded formulations (1%w/w strength) was added. The radio chemical purity of labeled complex was estimated by ascending thin layer chromatography using 100% acetone or 0.9% sodium chloride as developing solvent (Theobald, 1990).

Table No. 8.01: Effect of $SnCl_2.2H_2O$ concentration on radio labeling efficiency of plain minocycline hydrochloride and its periodontal formulations

$SnCl_2.2H_2O$ in µg	MnHCl			MGF02			MSF27			MMF01			ML14		
	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free
25	60.5	0.1	39.4	65.1	0.1	34.8	63.4	0.1	36.5	61.4	0.16	38.44	60.2	0.21	39.59
50	73.9	0.3	25.8	82.5	0.4	17.1	81.7	0.2	18.1	80.1	0.2	19.7	81.3	0.39	18.31
100	85.7	1.0	13.3	89.7	0.2	10.1	87.9	0.6	11.5	87.03	0.91	12.06	88.1	0.17	11.73
200	97.3	1.1	1.6	99.1	0.1	0.8	98.6	0.9	0.5	98.2	1.0	0.8	97.6	0.9	1.5
400	93.7	4.4	1.9	95.8	2.8	1.4	95.1	0.2	4.7	94.6	4.2	1.2	95.4	1.6	3.0

Table No. 8.02: Effect of $SnCl_2.2H_2O$ concentration on radio labeling efficiency of plain clindamycin phosphate and its periodontal formulations

$SnCl_2.2H_2O$ in µg	$ClPO_4$			CGF54			CSF27			CMF01			CL14		
	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free	% Labeled	% colloid	% Free
25	65.1	0.1	34.8	69.3	0.3	30.4	67.1	0.5	32.4	66.3	1.1	32.6	65.1	0.29	34.61
50	77.6	0.4	22.0	85.1	0.6	14.3	83.9	0.3	15.8	82.5	0.5	17.0	83.2	0.31	16.49
100	89.3	0.2	10.5	92.3	1.2	6.5	90.5	1.0	8.5	89.7	0.9	9.4	90.1	0.13	9.77
200	98.6	0.1	1.3	99.6	0.06	0.34	99.4	0.1	0.5	99.1	0.6	0.3	98.7	0.7	0.6
400	94.8	3.8	1.4	96.4	2.6	1.0	95.8	2.1	2.1	95.2	1.8	3.0	96.3	1.3	2.4

Table No. 8.03: Effect of pH on radio labeling efficiency of minocycline hydrochloride and its periodontal formulations

pH range	Percentage Radio labeled (± S.D.)				
	MnHCl	MGF02	MSF27	MMF01	ML14
4-4.5	73.9±3.21	76.4±2.63	75.3±1.75	74.1±2.15	72.7±1.78
4.5-5	84.5±3.14	83.5±2.51	85.6±2.36	84.7±2.36	83.2±2.01
5-5.5	89.3±2.68	93.1±3.61	92.3±3.16	91.2±3.27	90.8±2.11
5.5-6	99.3±2.54	99.4±2.13	99.3±2.95	99.1±2.72	98.78±1.93
6-6.5	91.1±1.87	90.6±2.67	89.4±1.63	92.3±2.31	91.6±1.76
6.5-7	87.6±1.36	88.3±3.15	86.5±2.34	90.6±3.16	87.3±2.29

Figure No. 8.02: Effect of pH on radio labeling efficiency of minocycline hydrochloride and its periodontal formulations

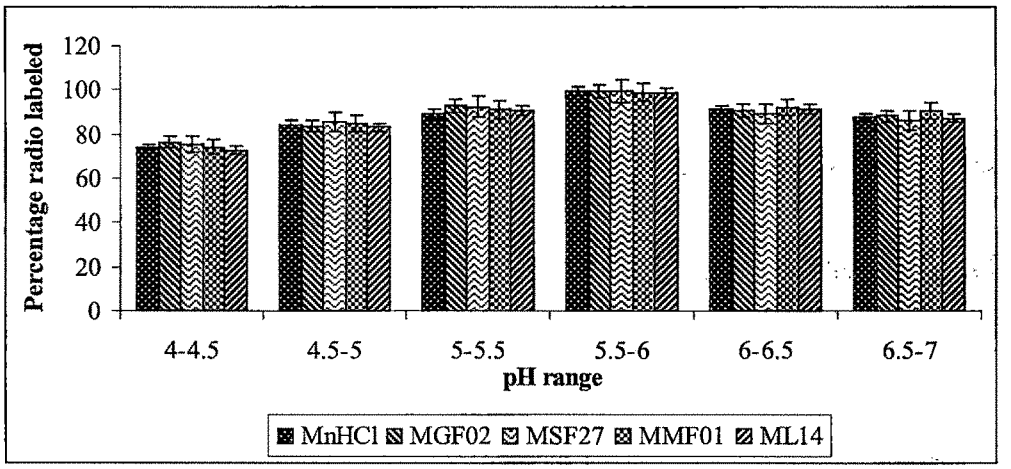


Table No. 8.04: Effect of pH on radio labeling efficiency of clindamycin phosphate and its periodontal formulations

pH range	Percentage Radio labeled (± S.D.)				
	$ClPO_4$	CGF54	CSF27	CMF01	CL14
4-4.5	70.2±2.17	71.9±1.95	70.3±3.25	72.3±2.37	69.4±2.15
4.5-5	83.5±2.36	84.6±2.69	85.1±3.78	84.9±3.41	82.1±1.97
5-5.5	87.5±3.19	86.8±3.09	88.3±2.31	89.2±2.78	87.6±1.38
5.5-6	98.9±3.04	99.1±1.87	99.5±2.41	99.3±2.45	98.3±1.87
6-6.5	90.9±1.63	91.3±2.29	90.5±1.95	89.6±2.18	86.7±1.22
6.5-7	85.5±2.39	84.6±3.04	85.3±2.36	83.9±1.39	79.8±1.79

Figure No. 8.03: Effect of pH on radio labeling efficiency of clindamycin phosphate and its periodontal formulations

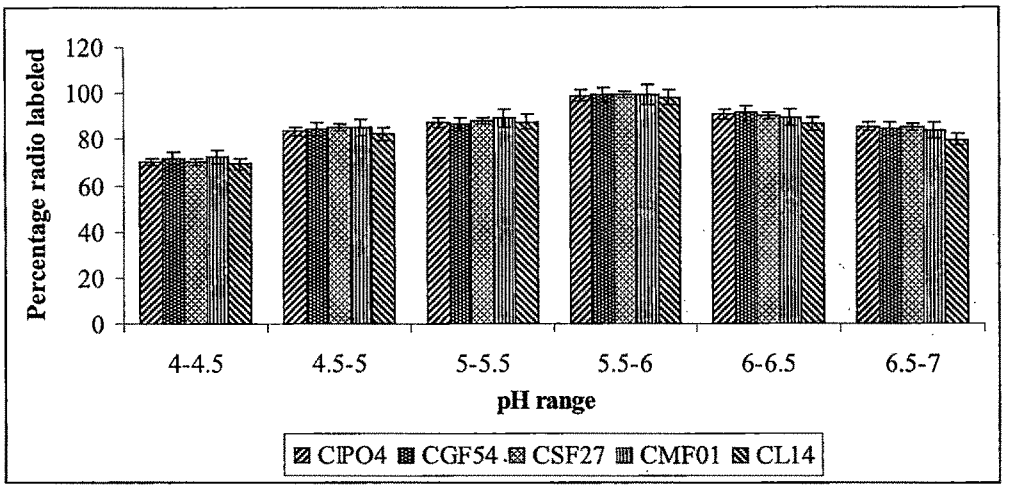


Table No. 8.05: Effect of incubation time on radio labeling efficiency of minocycline hydrochloride and its periodontal formulations

Incubation time (min.)	Percentage Radio labeled (± S.D.)				
	MnHCl	MGF02	MSF27	MMF01	ML14
0	99.8±2.16	99.9±3.63	99.3±2.86	99.5±1.39	99.6±1.19
5	98.7±3.21	98.1±2.86	98.4±2.35	97.9±3.51	98.1±2.17
10	81.5±1.24	85.8±2.04	83.3±3.21	82.2±2.63	83.6±2.33
15	70.2±1.86	75.6±3.15	78.5±3.86	71.3±3.16	72.7±1.78
20	65.9±2.15	68.4±2.17	67.5±1.76	59.3±2.45	68.1±1.92

Figure No. 8.04: Effect of incubation time on radio labeling efficiency of minocycline hydrochloride and its periodontal formulations

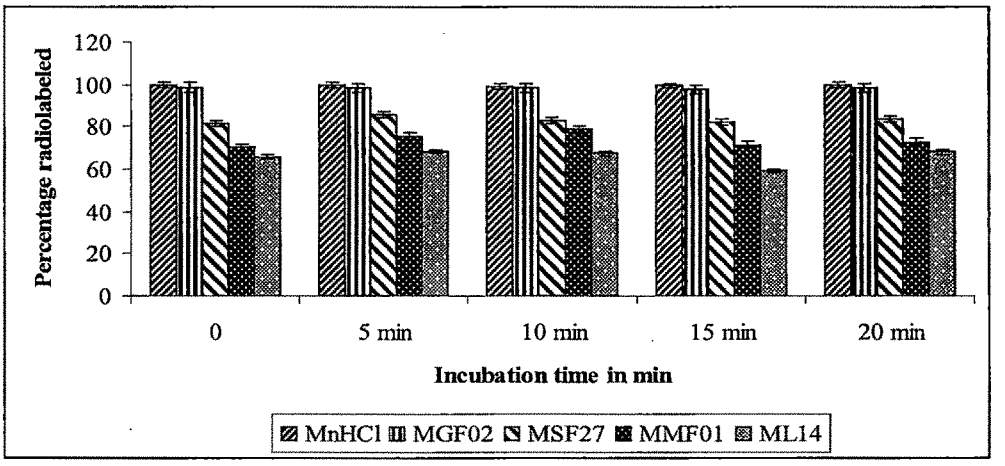


Table No. 8.06: Effect of incubation time on radio labeling efficiency of clindamycin phosphate and its periodontal formulations

Incubation time (min.)	Percentage Radio labeled (± S.D.)				
	$ClPO_4$	CGF54	CSF27	CMF01	CL14
0	99.1±1.39	99.9±2.81	99.3±1.35	99.7±3.26	99.3±3.21
5	98.1±2.45	97.6±3.26	97.3±1.89	98.3±2.37	98.2±2.37
10	85.1±2.63	83.6±2.84	84.3±1.34	86.2±5.21	85.7±1.77
15	75.2±3.58	76.5±3.17	77.8±2.71	73.7±2.35	74.6±1.89
20	68.9±1.76	67.4±3.54	63.7±2.67	66.5±3.42	65.3±2.36

Figure No. 8.05: Effect of incubation time on radio labeling efficiency of clindamycin phosphate and its periodontal formulations

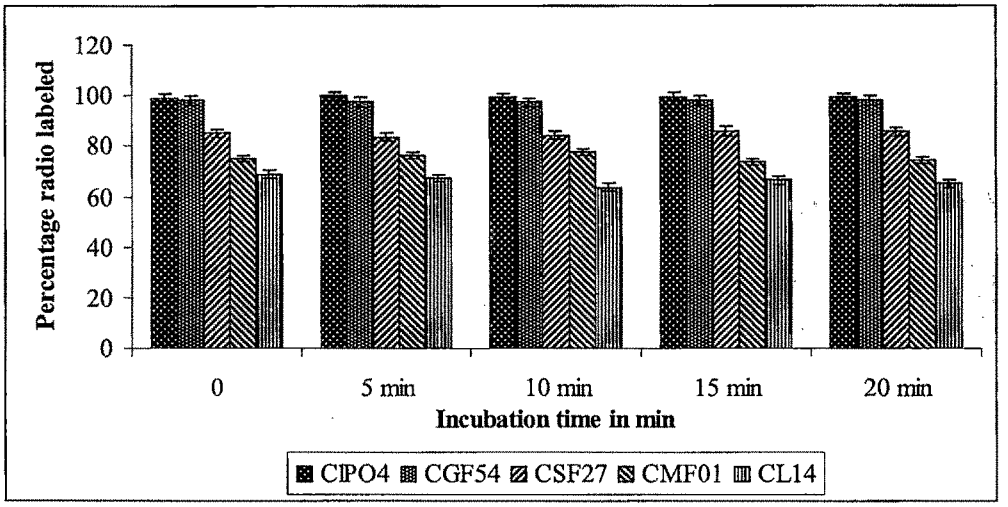


Table No. 8.07: In vitro stability studies of radio labeled minocycline hydrochloride and its periodontal formulations in saline

Formulation Code	Percentage Radio labeled (± S.D.)						
	0 min	30 min	1hr	2hr	4hr	6hr	24hr
MnHCl	98.63± 1.35	98.46± 2.36	98.32± 1.67	97.38± 3.27	96.54± 2.63	98.13± 1.96	98.78± 2.39
MGF02	99.43± 2.36	99.15± 2.15	98.57± 2.44	98.34± 1.69	97.25± 2.47	98.67± 3.47	99.13± 2.87
MSF27	98.79± 3.15	98.62± 3.26	97.36± 3.16	98.19± 2.38	96.75± 3.15	97.71± 2.45	98.48± 3.27
MMF01	98.42± 2.61	98.26± 1.35	97.39± 2.68	98.47± 2.45	98.89± 3.26	96.45± 2.15	98.17± 1.36
ML14	98.67± 2.17	98.39± 1.58	98.03± 2.38	97.58± 2.11	98.11± 1.67	97.67± 1.85	97.89± 1.91

Table No. 8.08: In vitro stability studies of radio labeled clindamycin phosphate and its periodontal formulations in saline

Formulation Code	Percentage Radio labeled (± S.D.)						
	0 min	30 min	1hr	2hr	4hr	6hr	24hr
$ClPO_4$	98.63± 1.36	98.46± 1.76	98.32± 1.68	97.38± 1.94	96.54± 3.17	98.13± 3.91	98.78± 1.98
CGF54	99.43± 3.45	99.15± 2.76	98.57± 2.46	98.34± 2.37	97.25± 3.28	98.67± 2.38	99.13± 2.87
CSF27	98.79± 2.18	98.62± 3.78	97.36± 2.95	98.19± 2.87	96.75± 2.57	97.71± 2.65	98.48± 3.14
CMF01	98.42± 2.47	98.26± 2.95	97.39± 2.36	98.47± 2.47	98.89± 2.37	96.45± 1.79	98.17± 2.58
CL14	99.19± 2.36	98.42± 1.74	98.71± 2.37	99.02± 1.63	97.86± 1.59	98.15± 1.68	98.66± 2.15

Table No. 8.09: Stability studies of radio labeled minocycline hydrochloride and its periodontal formulations in human serum at 37^0C

Formulation Code	Percentage Radio labeled (± S.D.)						
	0 min	30 min	1hr	2hr	4hr	6hr	24hr
MnHCl	97.14± 2.15	96.89± 1.36	96.13± 2.46	95.46± 3.16	94.14± 1.47	97.69± 2.46	97.63± 1.68
MGF02	98.3± 3.66	98.75± 3.45	97.28± 2.54	97.84± 2.93	95.91± 1.47	96.13± 1.62	98.52± 2.14
MSF27	98.13± 2.73	97.69± 2.47	97.24± 1.49	93.69± 1.37	96.46± 1.73	97.18± 3.96	97.11± 1.38
MMF01	98.83± 1.47	97.36± 3.62	96.32± 1.47	94.37± 3.15	95.67± 2.93	95.35± 1.46	97.57± 2.37
ML14	99.15± 1.48	98.67± 2.12	98.05± 1.68	98.37± 1.79	99.1± 2.32	98.78± 2.11	98.57± 1.63

Table No. 8.10: Stability studies of radio labeled clindamycin phosphate and its periodontal formulations in human serum at 37^0C

Formulation Code	Percentage Radio labeled (± S.D.)						
	0 min	30 min	1hr	2hr	4hr	6hr	24hr
$ClPO_4$	98.1± 3.27	98.68± 1.36	98.79± 3.73	98.3± 2.46	98.54± 1.46	98.83± 1.35	98.15± 2.86
CGF54	98.5± 1.36	97.1± 3.47	97.5± 3.65	95.8± 1.35	96.3± 1.57	96.7± 3.25	98.1± 3.51
CSF27	98.1± 1.37	98.5± 1.36	97.12± 2.94	96.5± 3.47	95.9± 3.51	97.16± 1.38	97.34± 1.96
CMF01	98.14± 1.36	97.27± 1.57	97.69± 1.45	95.37± 2.75	96.48± 2.53	97.86± 3.66	98.3± 3.82
CL14	98.37± 1.76	98.78± 1.69	98.21± 2.11	97.67± 2.36	98.35± 2.17	98.43± 1.84	98.57± 1.93

Table No. 8.11: DTPA challenging test of radio labeled minocycline hydrochloride and its periodontal formulations

Concentration of DTPA in mg	Percentage Transchelation (± S.D.)				
	MnHCl	MGF02	MSF27	MMF01	ML14
2	2.38± 0.30	3.15± 0.38	2.79± 0.41	2.86± 0.38	2.79±0.23
4	3.25± 0.28	3.63± 0.28	3.47± 0.39	3.57± 0.29	3.21±0.37
6	4.79± 0.37	5.36± 0.31	4.15± 0.18	4.86± 0.35	4.76±0.19
8	6.63± 0.19	6.71± 0.29	7.36± 0.26	6.74± 0.29	5.97±0.27
10	8.31± 0.26	8.79± 0.15	8.96± 0.37	9.13± 0.16	9.02±0.36

Table No. 8.12: DTPA challenging test of radio labeled clindamycin phosphate and its periodontal formulations

Concentration of DTPA in mg	Percentage Transchelation (± S.D.)				
	$ClPO_4$	CGF54	CSF27	CMF01	CL14
2	2.67± 0.15	3.19± 0.43	2.96± 0.28	2.19± 0.17	2.48±0.21
4	3.63± 0.21	3.71± 0.37	3.83± 0.32	2.94± 0.11	3.12±0.19
6	5.12± 0.18	5.45± 0.26	4.28± 0.38	3.29± 0.24	3.76±0.31
8	7.16± 0.37	7.34± 0.38	7.54± 0.27	4.68± 0.61	5.93±0.37
10	8.93± 0.41	9.10± 0.19	8.68± 0.28	8.91± 0.42	8.79±0.42

8.10 RESULT AND DISCUSSION

8.10.1 Radio labeling of minocycline hydrochloride/ clindamycin phosphate and their various mucoadhesive periodontal formulations

Minocycline hydrochloride/ clindamycin phosphate and their various mucoadhesive periodontal formulations were radio labeled with high efficiency by the direct labeling technique using reduced ^{99m}Tc. The radio labeling efficiency of ^{99m}Tc with minocycline hydrochloride/ clindamycin phosphate and their formulations was studied by ascending instant thin layer chromatography using ITLC- SG strips. As shown in table no.8.05 and 8.06, the radio labeling was optimized by taking two factors into account, i.e. pH of the complex and stannous chloride dihydrate concentration.

8.10.1.1 pH of the complex

The radio labeling was carried out at various pH range from 4 to 7 and the labeling efficiency was calculated (table no. 8.03 and 8.04 and figure no. 8.02 and 8.03). It was

found that pH plays an important role in determining the labeling efficiency. As the pH increases the radio labeling also increases. Hence the optimum pH for labeling was found to be within the range 5.5-6 where excellent labeling took place.

8.10.1.2 Incubation time

As shown in table no. 8.05 and 8.06 and figure no. 8.04 and 8.05 all the formulations were radio labeled effectively without any incubation period. Hence no incubation time is given to any of the formulations or minocycline hydrochloride/ clindamycin phosphate.

8.10.1.3 Stannous chloride dihydrate concentration

The concentration of $SnCl_2.2H_2O$ was found to be very critical in optimization of radio labeling process. At low concentration of $SnCl_2.2H_2O$, the labeling of the compound was not complete. This led to the presence of free ^{99m}Tc, which was assessed by instant thin layer chromatography using 100% acetone or 0.9% saline as mobile phase. The table 8.01 and 8.02 represents the effect of various concentrations of $SnCl_2.2H_2O$ on labeling efficiency. By varying the concentration of $SnCl_2.2H_2O$ from 25-400 mcg and keeping the other factors constant (pH 5.5-6), the influence on labeling yield was found to be significant. The labeling efficiency increased from 25-200mcg with increase in $SnCl_2.2H_2O$ concentration. Further increase in the concentration of $SnCl_2.2H_2O$ led to a reduction in yield and increased in concentration of reduced/ hydrolyzed ^{99m}Tc. Thus the optimum concentration of $SnCl_2.2H_2O$ was found to be 200µg for efficient labeling.

8.10.1.4 In vitro stability studies of 99mTc- labeled drug and their mucoadhesive periodontal formulations

In vitro stability of the ^{99m}Tc labeled complex with time was studied in saline and are shown in table no.8.07 and 8.08 and in human serum in table no. 8.09 and 8.10. The experimental data revealed that there was hardly any detachment of radioisotope from the complex. Even after a period of 24hrs, the presence of more than 95% labeled compound and only 1-2% decrease of the labeled product signifies the high stability of the radio labeled product and its suitability for the in vivo use. The data obtained from percentage of radio activity in serum confirm their use in vivo. As obtained from table no. 8.11 and 8.12, the percent chelation of the ^{99m}Tc minocycline hydrochloride/ clindamycin phosphate and their formulations was found to be nearly equal to 10mg.

8.11 CONCLUSION

From the radiolabelling study of various mucoadhesive periodontal formulations to be administered locally into the periodontal cavity, it was found that the procedure adopted for the labeling of the minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were resulted in highly stable radio labeled complexes with high labeling efficiency at no incubation time period. Hence the above method can be adopted for the radiolabelling of the periodontal formulations.

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