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Orifice ionic gelation method for mucoadhesive microcapsules: Predictive tools for achieving the sustained action of Pioglitazone hydrochloride

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Abstract

The present study was aimed to produce microcapsules of Pioglitazone hydrochloride by achieving the sustained action. The microcapsules of pioglitazone hydrochloride were prepared by employing sodium alginate as a cell forming polymer and by using a different bio-adhesive polymers such as carbopol, HPMC and sodium CMC in a various ratios of 1:1, 3:1, 6:1 & 9:1, by orifice ion gelation method. Scanning electron microscope photographs of samples revealed that all prepared microcapsules were almost spherical in shape and have a slightly smooth surface. The *in vitro* release profile of Pioglitazone hydrochloride indicates that all the batches of microcapsules showed controlled and prolonged drug release over an extended period of 10 hr. FT- IR spectra revealed no chemical incompatibility between drug and the polymers.

Key words: Pioglitazone hydrochloride, mucoadhesion, microcapsule, orifice ionic gelation Method.

1.0 Introduction

Pioglitazone hydrochloride is a Thiazolidinedione antidiabetic agent that depends on the presence of insulin for its mechanism of action [1,2]. Pioglitazone hydrochloride decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Pioglitazone hydrochloride is a potent and highly selective agonist for peroxisome proliferator-activated receptor-gamma (PPARg) [3]. PPAR receptors are found in tissues important for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARg nuclear receptors modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism. Eventhough, Pioglitazone hydrochloride is a potent anti-diabetic agent it suffers from major pharmacokinetic drawback, that is it fast metabolism rate. This has resulted in multiple dosing of drug and hence decreased patient compliance.

Therefore control release (CR) products are needed for Pioglitazone hydrochloride to prolong its duration of action and to improve patience compliance; there are few reports [4] on the formulation of Pioglitazone hydrochloride employing coated granules and matrix tablets. Microencapsulation has been accepted as a process to achieve controlled release and drug targeting. The choice of the methods for the preparation of microcapsules depends on many factors such as the drug solubility and short half life 3-5 hour [2] of drugs. Mucoadhesion has been a topic of interest in the design of drug delivery system to prolong the residence time of the dosage form at the site of application or the absorption and to facilitate intimate contact dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs [5-7].

In the present study, an attempt was made to develop sustained release microcapsules to increase half-life of drug concentration in serum by using orifice ionic gelation method. The prepared microcapsules were evaluated for drug content, particle size, surface morphology, muco-adhesive testing and *in vitro* drug release studies.

2.0 Materials and methods

2.1 Materials:

Pioglitazone hydrochloride sample from Ontop Pharmaceuticals LTD (Bangalore, India), Sodium carboxymethylcellulose (sodium CMC), Methyl cellulose (Mc) and Hydroxypropylmethylcellulose (HPMC) was purchase in the market; all the chemicals were of AR Grade.

2.2 Preparation of muco-adhesive microcapsules

Microcapsules were prepared by orifice-ionic gelation method [8,9] by employing the Sodium alginate as a cell forming polymer and Sodium CMC, HPMC and Carbopol as muco-adhesive [10] polymers. All the ingredients were dissolved in purified water in a ratio of 1:1, 1:3 and 6:1 separately to form a homogenous polymer solution. Core material, Pioglitazone hydrochloride (1 gm) was added to polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion is then added manually drop wise into $CaCl_2$ (10% w/v) solution through a syringe with a needle of size no. 18. The added droplets are allowed to stay in $CaCl_2$ solution for 15 min to complete the curing reactions and to produce spherical rigid microcapsule. The microcapsules are collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45˚C for 12 hrs. (Table-1)

2.3 Preformulation studies:

2.3.1 Estimation of Drug

Pioglitazone hydrochloride was estimated by UV spectrophotometric (Shimadzu UV-1700 UV/Vis double beam spectrophotometer) method [11, 12]. Aqueous solutions of Pioglitazone hydrochloride were prepared in phosphate buffer (pH 7.4) and absorbance was

measured on UV spectrophotometer at 269 nm (Figure-1, 2). The method was validated for linearity, accuracy and precision. The method obeys Beer's Law in the concentration range of 1-10 µg/ml.

Formulation code	Composition and ratio	Drug (mg)	Cell forming polymer (mg)	Mucoadhesive Polymer (mg)
MC1	SA: SCMC (1:1)	1000	500	500
MC2	SA: HPMC (1:1)	1000	500	500
MC3	SA: Carbopol (1:1)	1000	500	500
MC4	SA: SCMC (3:1)	1000	750	250
MC5	SA: HPMC (3:1)	1000	750	250
MC6	SA: Carbopol (3:1)	1000	750	250
MC7	SA : SCMC (6:1)	1000	857.14	142.86
MC8	SA : HPMC (6:1)	1000	857.14	142.86
MC9	SA : Carbopol (6:1)	1000	857.14	142.86

Table-1: Composition of different muco-adhesive microcapsules.
Note: Sodium alginate =SA, Sodium CMC = SCMC,

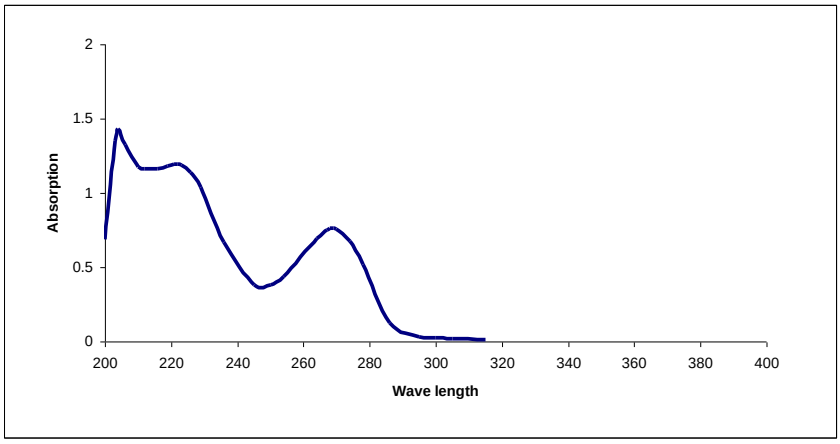

Figure-1: Spectrum of drug (Pioglitazone hydrochloride) in phosphate buffer (pH 7.4)

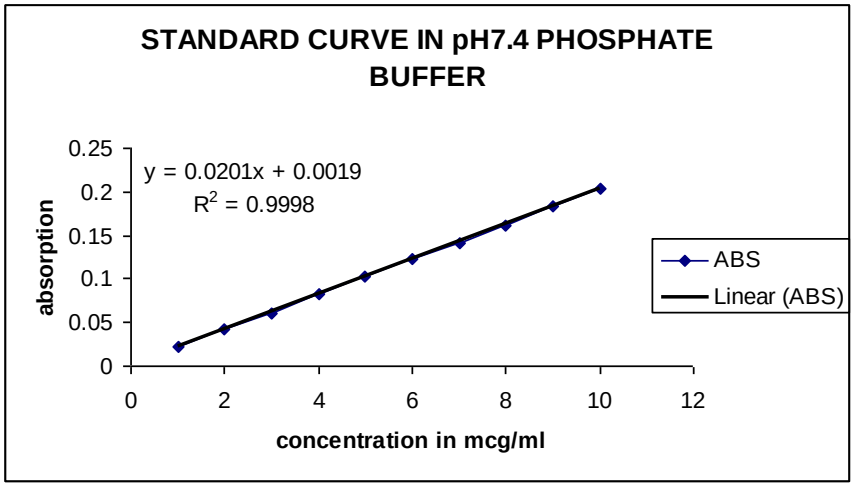

Figure-2: Standard curve in phosphate buffer (pH 7.4)

2.3.2 Fourier transforms infrared spectroscopy:

FT-IR spectra (500-4000 cm $^{-1}$) were obtained on a Nicolet Avatar 37- DTGS FT-IR spectrophotometer (Nicolet) with a resolution of 4 cm $^{-1}$. KBr pellets were prepared by gently mixing 1 mg sample with 200 mg potassium bromide (Table-2).

Sl. No.	Description	Characteristic peaks (Cm-1)
1	Pioglitazone hydrochloride Hydrochloride (28 peaks found)	3416.9, 3366.0, 3084.9, 2927.8, 2741.2, 2616.1, 1991.3, 1885.8, 1743.1, 1689.8, 1613.1, 1551.6, 1510.1, 1461.2, 1396.4, 1314.5, 1241.3, 1150, 1038.1, 930.9, 849.3, 791.3, 739.9, 713.0, 660.1, 584.3, 549.4, 516.5
2	Sodium Alginate (13 peak found)	3770.1, 3432.3, 2927.3, 2363.3, 2151.4, 1612.0, 1417.3, 1309.0, 1093.5, 1031.3, 944.7, 895.3, 818.5
3	Carbopol (10 peaks found)	3102.2, 2959.0, 2362.9, 1713.8, 1451.7, 1411.2, 1247.4, 1173.3, 802.3, 649.6
4	Sodium CMC (09 peaks found)	3408.3, 2921.3, 2363.5, 2143.3, 1602.4, 1422.7, 1327.5, 1060.5, 601.1
5	HPMC (12 peaks found)	3774.4, 3467.5, 2931.4, 2362.8, 2127.2, 1656.4, 1464.0, 1379.3, 1323.1, 1058.3, 944.2, 565.6
6	Drug + Sodium alginate + Carbopol (20 peaks found)	3424.9, 3088.3, 2929.6, 2741.8, 2362.3, 1991.6, 1693.1, 1616.5, 1510.7, 1455.8, 1313.3, 1241.6, 1155.0, 1037.1, 941.9, 826.5, 713.5, 660.3, 584.7, 518.1
7	Drug + Sodium alginate + Sodium CMC (16 peaks found)	3772.1, 3426.3, 2928.1, 2744.5, 2366.7, 2150.5, 1689.1, 1612.7, 1414.8, 1319.2, 1245.8, 1034.0, 944.5, 820.5, 713.5, 519.2
8	Drug + Sodium alginate + HPMC (22 peaks found)	3751.0, 3430.0, 2927.4, 2742.6, 2365.6, 2117.2, 1741.5, 1702.2, 1611.6, 1513.4, 1423.2, 1320.1, 1251.6, 1154.6, 1036.6, 948.6, 900.5, 823.2, 715.7, 660.3, 598.2, 517.5,

Table-2: Characteristic IR peaks of drug, polymer and their physical mixture

2.4 Characterization of microcapsules:

2.4.1 Physical characterization [13,14]

The surface and inner parts of the microspheres were observed through the Scanning Electron microscopy (SEM), (SEM) was performed for surface and inner morphological characterization of microspheres using the scanning electron microscope (SEM- LEICA S430, London, UK). (Figure-4 to 7)

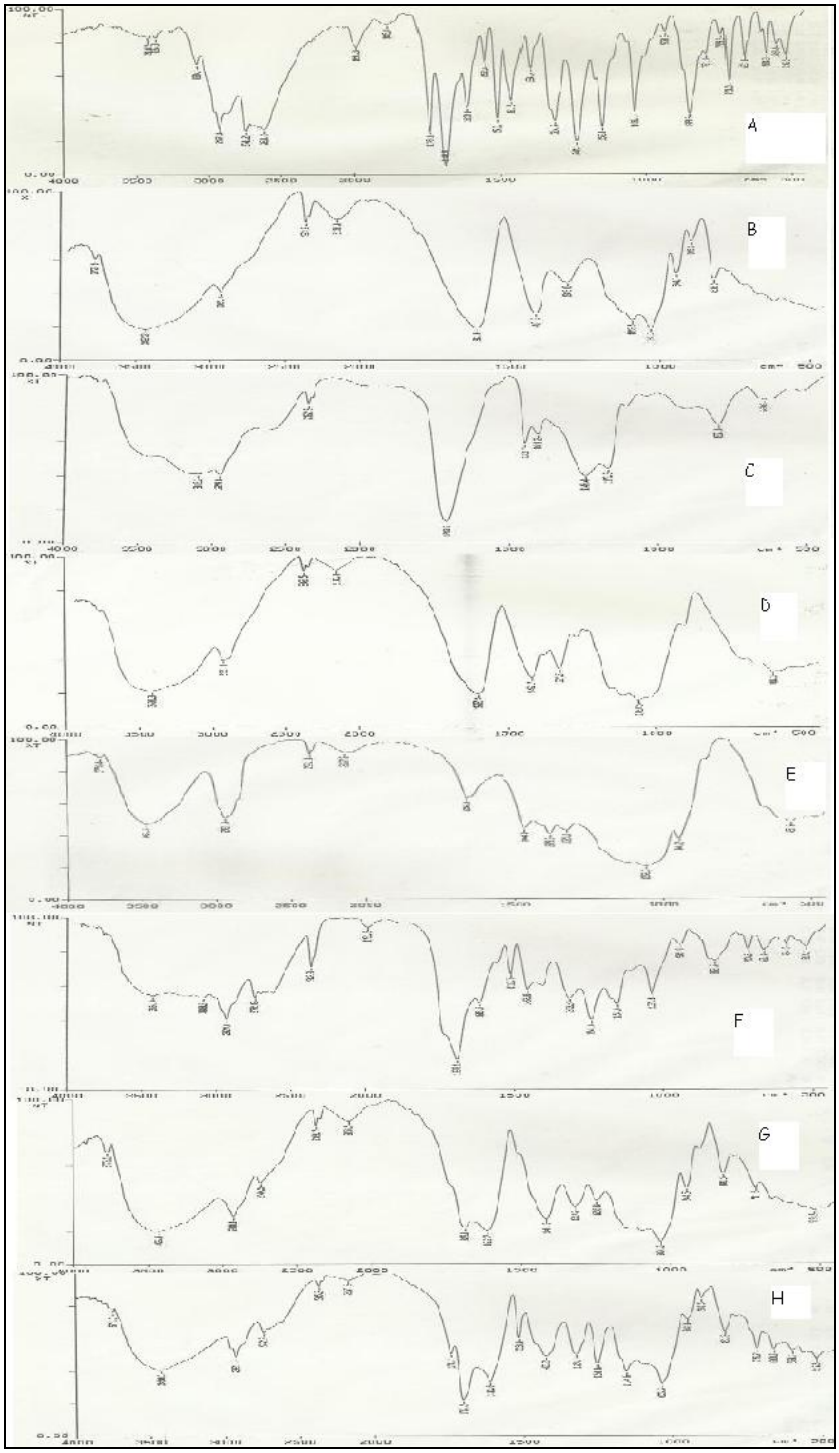

Figure-3: FIR of (A) Pioglitazone hydrochloride , (B) Sodium alginate, (C) Carbopol, (D) Sodium CMC, (E) HPMC, (F) Mixture of A, B and C, (G) Mixture of A, B and D, (H) Mixture of A, B and E

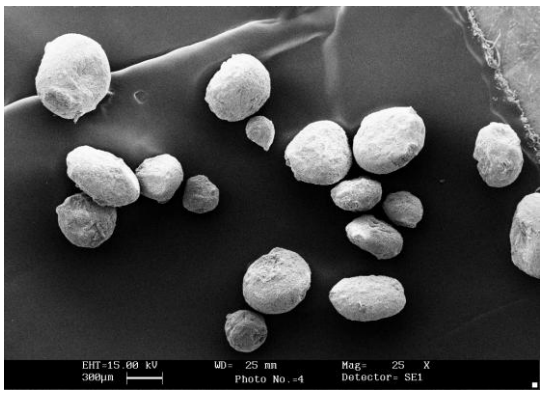

Figure-4: SEM photographs of Pioglitazone hydrochloride microcapsules with size ranging approximately from 300 to 900 µm.

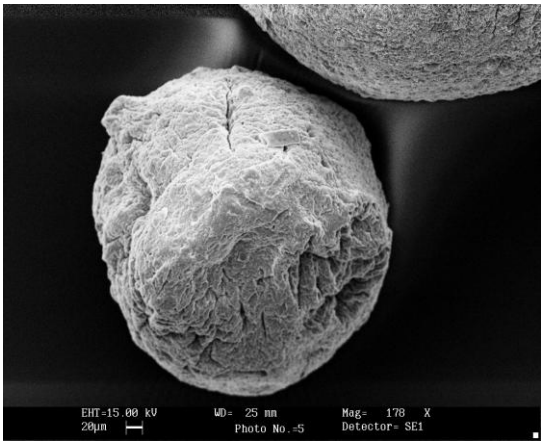

Figure- 5: SEM photographs of Pioglitazone hydrochloride microcapsules

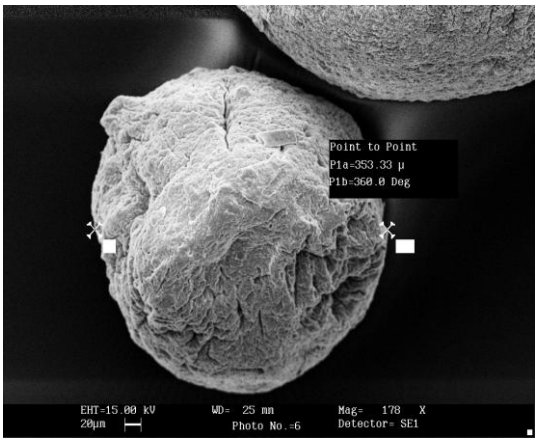

Figure-6: SEM photographs of Pioglitazone hydrochloride microcapsules showing the size 353.33 µm.

2.4.2 Particle Size Distribution

Different sizes of microcapsules in a batch were separated by sieving method using a range of standard sieves (#10, #22, #44, #52 and # 60). The amount retained on different sieves was weighed. From the obtained data, weight percent retained on different sieves and average size of microcapsules were calculated.

2.4.3 Practical yield:

The percentage yield of Pioglitazone hydrochloride in the microencapsulated product was determined by using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of Microcapsules}}{\text{Theortical Weight of drug and polymer}} \times 100$$

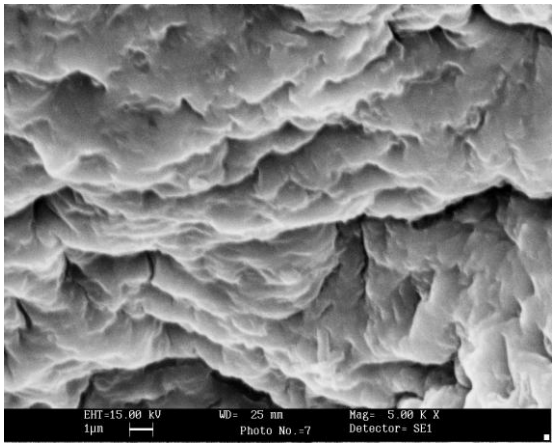

Figure- 7: Surface topography analysis of Pioglitazone hydrochloride hydrochloride microcapsules.

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Formulation code	Composition and ratio	Average size (µm)	% yield	Drug content (mg)	Encapsulation % efficiency
MC1	SA: SCMC (1:1)	350	80.67	185.7	74.28
MC2	SA: HPMC (1:1)	347	83.77	195.425	78.17
MC3	SA: Carbopol (1:1)	389	87.11	169.775	67.91
MC4	SA: SCMC (3:1)	410	89.12	182.8	73.12
MC5	SA: HPMC (3:1)	387	81.27	201.925	80.77
MC6	SA: Carbopol (3:1)	341	86.25	190.625	76.25
MC7	SA : SCMC (6:1)	354	79.87	202.6	81.04
MC8	SA : HPMC (6:1)	372	86.82	198.325	79.33
MC9	SA : Carbopol (6:1)	345	89.77	197.275	78.91

Table-3: Characterization of muco-adhesive microcapsules

2.4.4 Percentage drug content

About 500mg of microcapsules were accurately weighed and transferred in to 1000 ml beaker, which contain 900ml of 7.4 phosphate buffer at 37°C.The phosphate solution was

steered continuously until all the microcapsules were dissolved. Drug loading was determined by U.V Photometric method at 269 nm.
(Microencapsulation efficiency) was calculated by the following formula:

$$\% \text{ Drug content} = \frac{\text{Actual drug content of microcapsules}}{\text{Theoretical weight of drug in microcapsules}} \times 100$$

2.4.4 Encapsulation efficiency:
The encapsulation efficiency of microcapsules was calculated by using the formula:

$$\% \text{ Encapsulation efficiency} = \frac{\% \text{ Drug content}}{\% \text{ theoretical drug Content}} \text{ X } 100$$

2.5 In-vitro drug release
2.5.1 **Release in pH 7.4-phosphate buffer:**
In vitro release rate of Pioglitazone hydrochloride from microcapsules of different samples was determined using single station USP dissolution test apparatus. The dissolution medium consisted of phosphate buffer (pH 7.4) was used, 9gm of SLS [15,16] mixed in the buffer to enhance the solubility of Pioglitazone hydrochloride in the phosphate buffer. Samples of drug, microcapsules equivalent with 100 mg of drug was spread onto the surface of 900 ml of preheated dissolution medium at 37°C. Aliquots of 5 ml were withdrawn at regular intervals of time i.e. (.5, 1, 2, 3 up to 18 hour) and the same is replaced with fresh dissolution medium each time. The samples obtained were filtered through Whatman filter paper no. 1. The filtrate was diluted up to 6 ml with phosphate buffer (pH 7.4). Then the absorbance was measured at 269 nm (Figure-8).

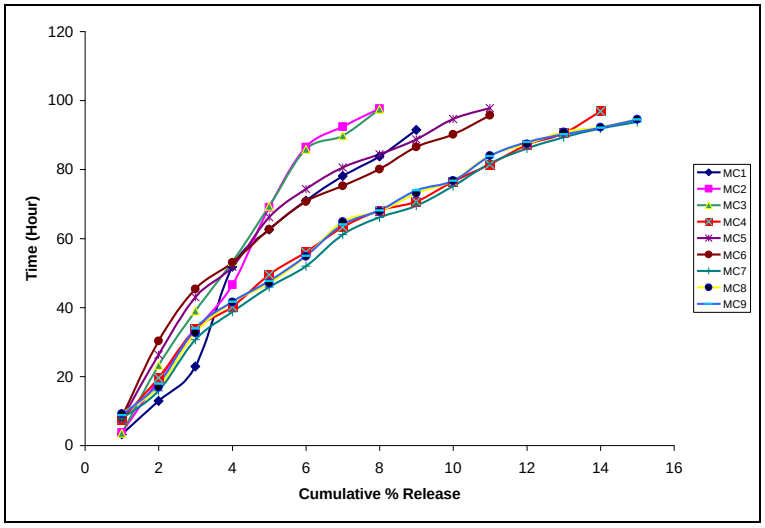

Figure-8: Comparative drug release of various compositions

2.5.2 Drug release kinetics studies:
In order to understand the kinetics and mechanism of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equations like zero order, first order, Weibull model, Korsmeyer -peppas model, Hill equation, Michaelis- Menten model. The

kinetic model that best fits the dissolution data was evaluated by comparing the regression coefficient (r) values obtained in various models.

Formulation code	Regression coefficient (r) value					
	Zero order	First order	Weibull	Korsmeyer-peppas	Hill equation	Michaelis-menten
MC1	0.9516	0.7781	0.9888	0.9600	0.9859	0.9484
MC2	0.9774	0.7993	0.9937	0.8988	0.9404	0.9289
MC3	0.9752	0.7383	0.9871	0.9340	0.9522	0.9041
MC4	0.9650	0.7526	0.9854	0.9645	0.9158	0.9735
MC5	0.9279	0.7015	0.9899	0.9310	0.9443	0.9406
MC6	0.9311	0.6859	0.9786	0.9159	0.9571	0.9296
MC7	0.9611	0.7667	0.9949	0.9732	0.9571	0.9888
MC8	0.9503	0.7664	0.9940	0.9722	0.9538	0.9903
MC9	0.9492	0.7562	0.9951	0.9691	0.9578	0.9889

Table-4: *In vitro* release kinetics studies of Pioglitazone hydrochloride microcapsules

2.6 Mucoadhesive testing:

The mucoadhesive property of the microcapsules was evaluated by wash-off In vitro method. Freshly excised pieces of intestinal mucosa (2 × 2 cm) from sheep were mounted onto glass slides (3 × 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread onto each wet rinsed tissue specimen, and immediately the slides were hung on to the arm of USP tablet disintegrating test apparatus. The tissue specimen was given a slow, regular up-and-down movement in the test fluid at 37°C contained in a 1 L vessel. After definite time interval, the apparatus was stopped and the number of microcapsules still adhering to the tissue was counted. Phosphate buffer (pH 7.4) was used as test fluid. (Table-5)

Formulation code	Percentage of microspheres adhering to tissue at pH7.4				
	1 hour	2 hour	4 hour	6 hour	8 hour
MC1	72.66± 2.08	70.66 ±1.52	54.00 ±1.73	32.00 ±1.2	22.33 ± 3.05
MC2	74.66±1.527	63.57 ±1.3	41.66 ±3.51	25.33 ±1.52	16.00 ± 3.4
MC3	72.33 ±1.52	69.33 ±1.15	45.33±3.05	29.66±1.527	15.00 ± 1.7
MC4	72.33 ±2.08	61.66 ±2.52	49.66 ±1.27	21.66 ±2.08	11.33 ±1.527
MC5	63.66 ± 3.21	56.66±0.57	40.00 ±1.4	11.66 ±2.7	06.70 ± 2.1
MC6	68.52 ± 1.3	54.07 ±2.08	39.72 ± 1.3	29.00±1.52	11.00 ±1.4
MC7	67.22 ±1.8	41.57 ±1.73	35.84±1.15	10.77± 3.4	02.33±1.15
MC8	57.97 ±1.6	33.50 ±1.4	25.36 ±1.02	04.37 ± 1.84	------
MC9	54.52 ± 1.3	32.07 ±2.08	23.72 ± 1.3	06.00±1.52	------

Table-5: Microspheres adherence capacity.

3.0 Results and discussion

3.1 Compatibility Studies:

FTIR studies were done to detect the possible interactions between the drug and the polymers in the microcapsules. Fig.3 Show the IR spectra of drug and the polymers. The characteristic peaks of Pioglitazone hydrochloride and the polymers (Sodium alginate, Carbopol, Sodium CMC, HPMC are given in Table-

2. Comparing the spectra of individual drug and polymers with those of microcapsules prepared by using different methods revealed that there were no differences in the positions of the absorption bands, hence providing evidence for the absence of hydrogen bonding interactions in the solid state between cell forming polymer (Sodium alginate) and Mucoadhesive polymer (Sodium alginate, Carbopol, Sodium CMC, HPMC) with Pioglitazone hydrochloride under investigation. The absence of any significant change in the IR spectral pattern of drug-polymer mixture indicated the absence of any interaction between the drug and the polymer (Figure-3).

3.2 Physical characterization:

The mucoadhesive microspheres of Pioglitazone hydrochloride prepared by the orifice-ionic gelatin method were found to be discrete, spherical, free flowing, and the monolithic matrix type. The microcapsules were uniform in size, with size range of 300 µm. The SEM photographs indicated that microcapsules were spherical and completely covered the coat polymer (Figure- 4-7).

3.3 Particle Size Distribution: The average size of microcapsules in various batches was found to be 350 µm, 347 µm, and 389 µm, 410 µm 387 µm, 341 µm, 354 µm, 372 µm and 345 µm for MC1, MC2, MC3, MC4, MC5, MC6, MC7, MC8 and MC9 respectively.(Table-3)

3.4 Practical yield: The percentage practical yield was found to be in the range of 79.87 to 89.77 %. The maximum percentage practical yield was found to be 89.77% for MC-9. (Table-3)

3.5 Percentage drug content and Encapsulation efficiency: The actual drug content and encapsulation efficiency of all nine formulations is given in Table-3. The encapsulation efficiency ranges from 66.91 to 81.04% for formulation MC1 to MC9. The maximum encapsulation efficiency was found to be 81.04% in MC7.

3.6 In-vitro drug release: The *in vitro* release profiles of nine formulations MC1 to MC9 are shown in Fig.8. It shows the plot of cumulative percent drug released as a function of time for different formulations. The cumulative percentage drug released indicates a controlled and prolonged drug release over an extended period of time. From the *in vitro* drug release profiles, it was observed that the drug release from microcapsules was decreased with an increase in cell forming material in the microcapsules (MC7, MC8, and MC9). The regression coefficient (r) values for formulations MC1 to MC9 are tabulated in Table-4. The model that gave higher 'r' value was considered as best fit model. The regression coefficient 'r' values were found to be higher in the zero order models, Hill equation model, Michaelis menten model, Korsmeyer peppas modal and Weibull model respectively, indicating that the dissolution of Pioglitazone hydrochloride from all formulations followed following above model. The order of release rate observed with all microcapsules was MC8 >MC9>MC7>MC4>MC6>MC5>MC1>MC2>MC3. The drug release from the microcapsules was diffusion controlled.

3.7 Muco-adhesive testing:

Microspheres with a coat consisting of alginate and a mucoadhesive polymer exhibited good muco-adhesive properties in the in vitro wash-off test. From the in vitro wash-off test it was observed that, the drug adherance the microcapsules from tissue was increase with an increase in muco-adhesive material in the microcapsules. The maximum adherence resulted in MC1, MC2 and MC3 respectively and minimum found in MC9 and MC8. (Table-5)

4.0 Conclusion

Sustained release Pioglitazone hydrochloride muco-adhesive microcapsules could be formulated by using cell forming polymer sodium alginate and muco-adhesive polymers (Carbopol, Sodium CMC and HPMC) as a

release retardant by orifice ion gelation method. The Muco-adhesive microcapsules of all the formulated batches were spherical, discrete and free flowing. The drug content was found to be almost uniform in a batch of muco-adhesive microcapsules. Increasing the concentration of cell forming polymer (sodium alginate) in microcapsule formulation decreases the rate of drug release dramatically and the muco-adhesive property increase with increasing the muco-adhesive polymer, best result were found in alginate Carbopol formulations in both cases.

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