FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF PIOGLITAZONE HYDROCHLORIDE USING A NATURAL POLYMER

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ABSTRACT

The objective of the present investigation was to design a controlled release dosage form for a thiazolidinedione oral hypoglycemic drug i.e., pioglitazone hydrochloride employing a natural polymer. The present study was also aimed to increase the biological half-life by developing it in the form of sustained release microspheres. The present study aimed at employing a natural polymer in formulating the mucoadhesive microspheres and estimate its effect over the controlled release of the drug from the formulation. The microspheres of pioglitazone hydrochloride were prepared by employing sodium alginate as a cell forming polymer and by using a natural bio-adhesive polymer viz. goru gum in the ratios of 1:1, 1:1.5 and 1:2, by orifice ion gelation method with varying concentrations of calcium chloride. Six batches of microspheres (MS1 – MS6) were prepared. The microspheres were evaluated for various micromeritic properties and it was observed that all the batches exhibited free-flowing properties. Scanning electron microscopy results showed that the microspheres were almost spherical in shape and discrete. The FTIR results showed that there were no interactions between the drug and the excipients. The *in vitro* release profile indicated that all the batches of microspheres showed controlled and prolonged drug release over an extended period, with acceptable release kinetics. The work demonstrated that among all the formulations of microspheres, the microspheres of the formulation MS4 are promising candidates for the sustained release of pioglitazone hydrochloride.

KEYWORDS: Pioglitazone hydrochloride, Goru gum, Microspheres, Orifice ion gelation.

INTRODUCTION:

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm or 1mm). Microspheres are sometimes referred to as microparticles. Microspheres are defined as “the monolithic spheres or therapeutic agents distributed throughout the matrix either as a molecular dispersion of particles”. These can also be defined as “the structures made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level”.

According to IUPAC, microspheres are defined as “the microparticles of spherical shape without membrane or any distinct outer layer”. The absence of outer layer forming a distinct phase is important to distinguish microspheres from microcapsules because it leads to first-order diffusion phenomena, whereas diffusion is zero order in the case of microcapsules.

The currently available slow release oral dosage forms, such as enteric coated/double-layer tablets which release the drug for 12-24 hours still result in inefficient systemic delivery of the drug and potential gastrointestinal irritation. Microencapsulation for oral use has been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided.[1-5] Microencapsulation is used to modify and retard drug release. Due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa.[6-10]

Pioglitazone is a thiazolidinedione antidiabetic agent that depends on the presence of insulin for its mechanism of action. Pioglitazone is a potent and highly selective agonist for peroxisome proliferator-activated receptor-gamma (PPARγ). Therefore control release (CR) products are needed for pioglitazone to prolong its duration of action and to improve patience compliance; there are few reports on the formulation of pioglitazone 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 its short half-life 3-5 hour and is eliminated rapidly.

MATERIALS AND METHODS

Materials

Pioglitazone hydrochloride gifted by Ranbaxy Ltd., India;Sodium alginate gifted by Finar Chemicals, India; Calcium chloride gifted by Virat Lab, India; and goru gum, which was obtained by milling the whole pods of Guar plant were used. Goru gum is an off white to pale greenish yellow colored powder obtained by grinding the beans and whole pods of Guar plant (*Cyamopsis tetragonoloba*).Goru gum, like the conventionally used guar gum, is considered a galactomannan, but differs from guar gum in the source or parts of the plant used for obtaining the gum. Guar gum is obtained by dehusking, milling and screening the guar seeds, but goru gum was obtained by milling the entire pods of the plant. Not much is known about goru gum, but it is considered to possess the same properties as those of guar gum. All the chemicals and reagents used, except goru gum, were of A.R. grade, procured commercially and used as received.

Calibration curve of pioglitazone hydrochloride

About 100 mg of pioglitazone hydrochloride was accurately weighed and dissolved in a little amount of phosphate buffer (pH 7.4). The volume was made up to 100 ml with phosphate buffer (pH 7.4) to obtain the primary stock solution, containing 1000µg/ml concentration of the drug. 10 ml of the primary stock solution was taken in a 1000 ml volumetric flask. It diluted up to 1000 ml with phosphate buffer (pH 7.4) to obtain the secondary stock solution, containing 10µg/ml concentration of the drug. The λ_{max} of pioglitazone hydrochloride in phosphate buffer (pH 7.4) was estimated using a U.V. visible spectrophotometer and it was found to have maximum absorbance at 269nm. Using this data, the standard curve of pioglitazone hydrochloride was obtained by analysing various diluted samples of the secondary stock solution of pioglitazone hydrochloride along with the pre-existing 10µg/ml solution i.e. 2µg/ml, 4µg/ml, 6µg/ml and 8µg/ml in a U.V. visible spectrophotometer.

Preparation of pioglitazone hydrochloride microspheres by orifice ion gelation technique

The mucoadhesive microspheres of pioglitazone hydrochloride were formulated by orifice ion gelation technique using sodium alginate as a cell forming polymer and by using a natural bio-adhesive polymer *viz.* goru gum in varying ratios with varying concentrations of calcium chloride solution.

Table 1. Formulae of the pioglitazone hydrochloride microspheres

Formulation code	Composition and ratio	Drug (mg)	Cell forming polymer (mg)	Mucoadhesive polymer (mg)	Concentration of calcium chloride solution used (w/v)
MS1	Pioglitazone: Goru gum (1:1)	1000	5000	1000	5%
MS2	Pioglitazone: Goru gum (1:1.5)	1000	5000	1500	5%
MS3	Pioglitazone: Goru gum (1:2)	1000	5000	2000	5%
MS4	Pioglitazone: Goru gum (1:1)	1000	5000	1000	10%
MS5	Pioglitazone: Goru gum (1:1.5)	1000	5000	1500	10%
MS6	Pioglitazone: Goru gum (1:2)	1000	5000	2000	10%

Sodium alginate was made into a solution and was mixed with varying concentrations of the natural mucoadhesive polymer i.e. goru gum. The core material i.e. pioglitazone hydrochloride (1 gm) was then added to polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (5% w/v and 10% w/v) solution through a syringe with a needle of size no. 21. The added droplets were kept dispersed in the calcium chloride solution for 15 minutes to complete the curing reactions and to produce spherical rigid microcapsules. The microcapsules were collected by decantation, and the product thus separated, was washed repeatedly with water and dried at 45˚C for 12-48 hrs.

Evaluation of the formulated microspheres

1.Micromeritic studies

The microspheres were evaluated for micromeritic properties such as bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio.

a. Bulk density

Bulk density is the ratio of the weight of the powder and the volume it occupies. It is expressed in gms/ml. Bulk density is imparted in determining the size of the container needed for handling and processing. A weighed quantity of the microspheres (W) was carefully taken into a graduated measuring cylinder and the volume occupied by it (V_0) was measured. The bulk density was calculated using the formula

$$Bulk\ density\ (B.D.) = \frac{Weight\ of\ the\ microspheres\ (W)}{Initial\ volume\ occupied\ by\ the\ microspheres\ (V_0)}$$

b. Tapped density

Tapped density is the ratio of the weight of the powder and the volume occupied by it after a specified compaction process, usually involving vibration of the container. It is obtained by mechanically tapping a graduated cylinder containing the powder until a little change from the initial volume is observed. It is expressed in gms/ml. A weighed quantity of the microspheres (W) was carefully taken into a graduated measuring cylinder and its initial volume (V_0) is noted. The measuring cylinder was closed with a lid and the bulk density apparatus was set for 100 tappings. After the tappings were done, the final volume (V_f) was measured and the procedure was continued till the consecutive readings were equal. The tapped density was measured by the formula

$$Tapped\ density\ (T.D.) = \frac{Weight\ of\ the\ microspheres\ (W)}{Final\ volume\ occupied\ by\ the\ microspheres\ (V_f)}$$

c. Carr's compressibility index

The Carr's compressibility index is indirectly related to flow rate, cohesiveness and particle size of a powder. It is a simple, fast and popular method of predicting powder flow characteristics. It was estimated from the bulk density and tapped density of the powder using the formula

$$Carr's\ compressibility\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density}\ x\ 100$$

d. Hausner's ratio

Hausner's ratio is an indirect measure of the flow property of a powder. It is estimated by the following formula

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density}$$

e. Angle of repose

Angle of repose is the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The frictional forces in the loose powder can be measured by angle of repose. The tangent of the angle of repose is equal to the coefficient of friction (μ) between the particles. Hence, the rougher and more irregular the surface of the particles, the greater will be the angle of repose. The angle of repose of the microspheres was determined by the funnel method. Accurately weighed quantity of microspheres were taken in a funnel and the height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the microspheres inside. The microspheres were allowed to flow through the funnel freely onto the surface. The diameter of the pile of the microspheres was measured and the angle of repose was calculated using the following equation:

$$\tan\theta = \frac{h}{r}$$

$$\theta = \tan^{-1}\frac{h}{r}$$

where, θ = angle of repose, h = height of the heap (in cms) and r = radius of the base (in cms).

2. Drug – excipients compatibility studies

The infrared absorption spectrum of pioglitazone hydrochloride, excipients and the formulation were evaluated using an FTIR spectrophotometer, wherein 2 – 4 mg of drug sample was used. The resultant spectrum of the drug was compared with the reference spectrum of pioglitazone hydrochloride.

3. Physical characterization

The surface and inner part of the microspheres were observed through the scanning electron microscopy (SEM). It was performed for surface and inner morphological characterization of the microspheres. The samples were prepared by lightly sprinkling the microspheres' powder on a double-side adhesive tape which was already shucked on aluminium stubs. The stubs were then placed into a fine coat ion sputter for gold coating. After gold coating, the samples were randomly scanned for particle size and surface morphology.

4. Percentage yield:

The percentage yield of the mucoadhesive pioglitazone hydrochloride microspheres was determined by using the formula:

$$Percentage\ yield = \frac{Weight\ of\ the\ microspheres\ obtained}{Total\ weight\ of\ the\ drug\ and\ excipients}\ x\ 100$$

5. Percentage drug content:

An empty hard gelatin capsule shell was taken and it was filled with the microspheres. The amount of microspheres that can be encapsulated were determined i.e. 200mg. This amount was first crushed and dissolved in 10ml of phosphate buffer (pH 7.4). The resulting solution was then filtered and the filtrate was diluted to 100ml using phosphate buffer (pH 7.4). The solutions were assayed using a UV-visible spectrophotometer at the wavelength of 269nm. Dilutions were prepared, if necessary and those were assayed again. The percentage drug content was then calculated using the formula:

$$Percentage\ drug\ content = \frac{1000\ x\ Absorbance\ of\ test\ solution\ x\ Concentration\ of\ an\ absorbance}{Absorbance\ of\ the\ particular\ concentration\ x\ 900}\ x\ 100$$

6. Drug entrapment efficiency:

The drug entrapment efficiency of the microspheres was calculated by using the following formula:

$$Drug\ entrapment\ efficiency\ (DEE) = \frac{Actual\ drug\ content}{Theoritical\ drug\ content}\ x\ 100$$

7. Percentage drug loading:

The percentage drug loading for the microspheres was calculated by using the following formula:

$$Percentage\ drug\ loading = \frac{Actual\ drug\ content}{Weight\ of\ the\ microspheres\ taken}\ x\ 100$$

8. *In vitro* wash-off test for microspheres:

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test. A 4cm x 4cm piece of goat intestinal mucosa was tied onto the paddle bottom of a USP dissolution test apparatus - II using a thread. A specified number of microspheres, i.e. 100 microspheres were spread onto the wet, rinsed tissue specimen. The dissolution test apparatus was operated such that the tissue specimen was rotated at a speed of 25 rpm in phosphate buffer (pH 7.4). At the end of 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. The percentage mucoadhesion of the microspheres was determined using the following formula:

$$Percentage\ mucoadhesion = \frac{Number\ of\ microspheres\ still\ adhering}{Number\ of\ microspheres\ applied}\ x\ 100$$

9. Swelling study:

An accurately weighed amount of microspheres were placed in phosphate buffer (pH 7.4) and allowed to swell to a constant weight. The microspheres were removed, blotted with filter paper and the changes in their weight were measured at an interval period of 10 minutes and recorded. The degree of swelling was then calculated from the formula

$$Swelling\ index = \frac{Weight\ after\ swelling\ (W_f) - Initial\ weight\ (W_0)}{Initial\ weight\ (W_0)}\ x\ 100$$

10. *In vitro* drug release studies:

In vitro drug release studies were carried out through dissolution using USP type-I (basket type) apparatus. The release of pioglitazone hydrochloride from the microspheres was studied using phosphate buffer (pH 7.4) in a dissolution apparatus with a rotating basket stirrer at a stirring speed of 100 rpm and a temperature of 37 ± 1°C.

200mg of microspheres were used in each test and these were placed within each basket by encapsulating them in empty hard gelatin capsule shells. Samples of the dissolution fluid were withdrawn at different time intervals and replaced with 5ml of fresh dissolution medium. The withdrawn samples were assayed at 269 nm for pioglitazone hydrochloride content using a UV visible spectrophotometer. Three trials were carried out for all the formulations. From this, percentage drug release was calculated and plotted against the function of time to study the pattern of the drug release.

11. *In vitro* drug release kinetics[11]:

Drug release data were fitted to kinetic models including the zero order, first order, Weibull, Higuchi matrix, Hixson – Crowell and Korsmeyer – Peppas release equations to find the equation with the best fit.

RESULTS AND DISCUSSION

Calibration curve of pioglitazone hydrochloride

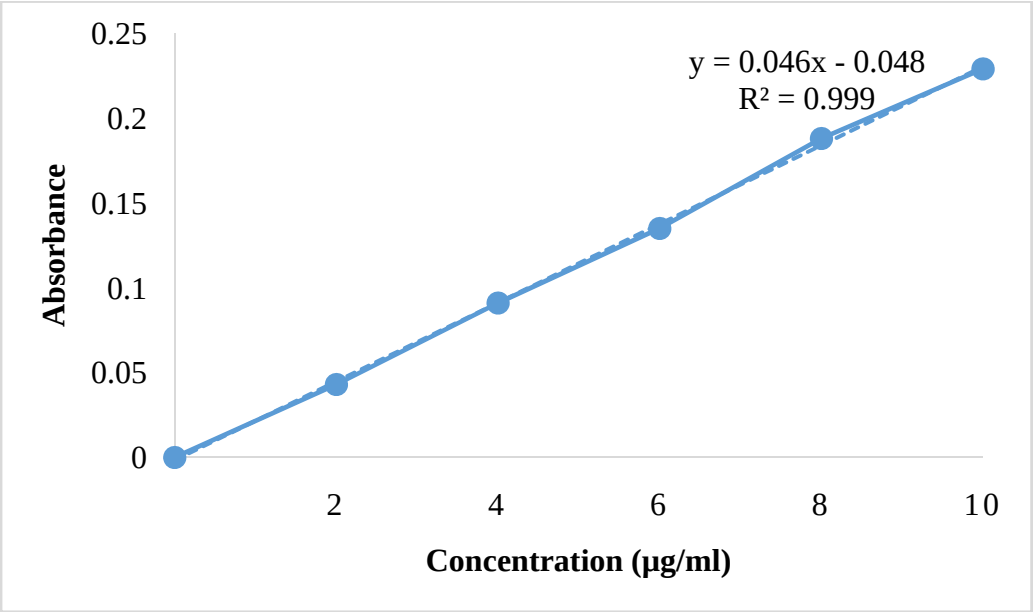

Fig. 1. Calibration curve of pioglitazone hydrochloride in phosphate buffer (pH 7.4) at 269 nm.

Micromeritic studies

The flow properties of pioglitazone hydrochloride mucoadhesive microspheres were estimated by studying their bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose. All the batches of microspheres were found to be free flowing with good flow properties as shown in the Table 2.

Table 2. Micromeritic properties for pioglitazone hydrochloride microspheres

Formulation code	Angle of repose (°)	Bulk density (gms/ml)	Tapped density (gms/ml)	Carr's index (%)	Hausner's ratio
MS1	23.52	0.571	0.666	14.26	1.166
MS2	24.79	0.584	0.685	14.74	1.172
MS3	26.50	0.564	0.676	16.56	1.198
MS4	23.11	0.545	0.615	11.38	1.128
MS5	24.49	0.571	0.656	12.95	1.148
MS6	23.51	0.574	0.666	13.81	1.160

Drug – excipients compatibility studies

FTIR studies were done to detect the possible interactions between the drug and the polymers in the microspheres. The spectrum of pioglitazone hydrochloride, sodium alginate, goru gum and the formulation were recorded using an FTIR spectrophotometer as shown in the Figures 2, 3, 4 and 5. By comparing the spectra of the individual drug and polymers with those of the microspheres, it was 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 the mucoadhesive polymer (goru gum) with pioglitazone hydrochloride under investigation. The absence of any significant change in the IR spectral pattern of the drug-polymer mixture indicated the absence of any interaction between the drug and the polymer.

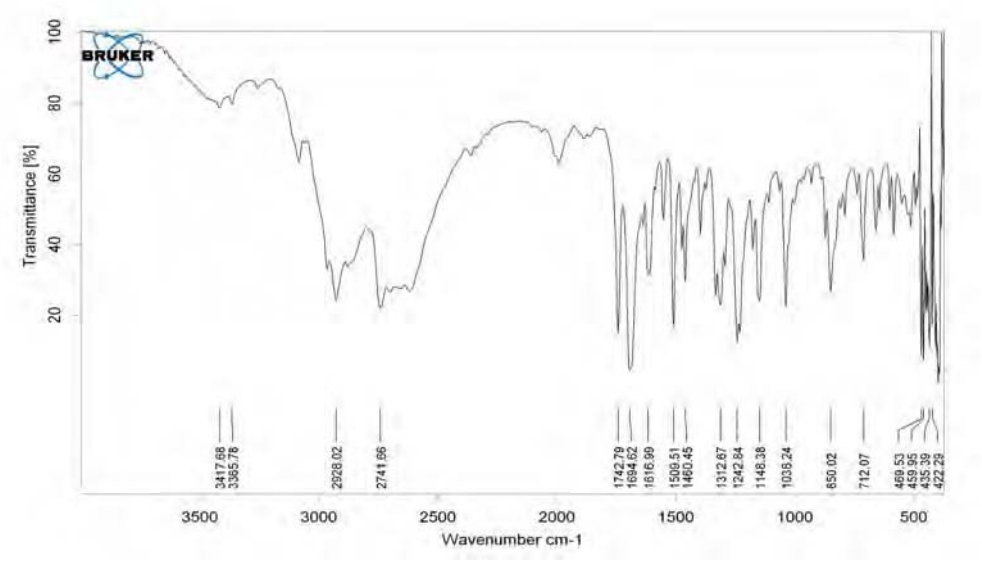

Fig. 2. FTIR spectrum of pioglitazone hydrochloride

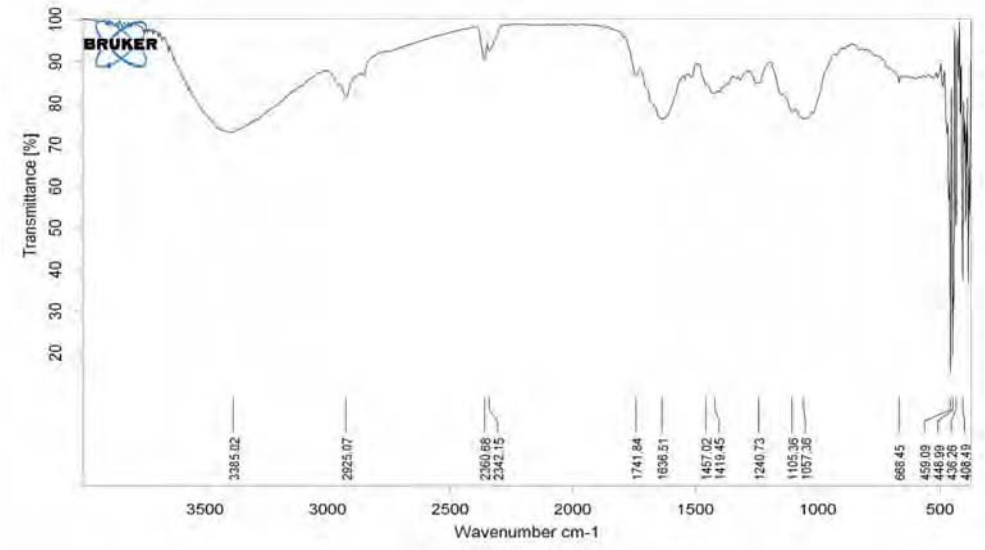

Fig. 3. FTIR spectrum of sodium alginate

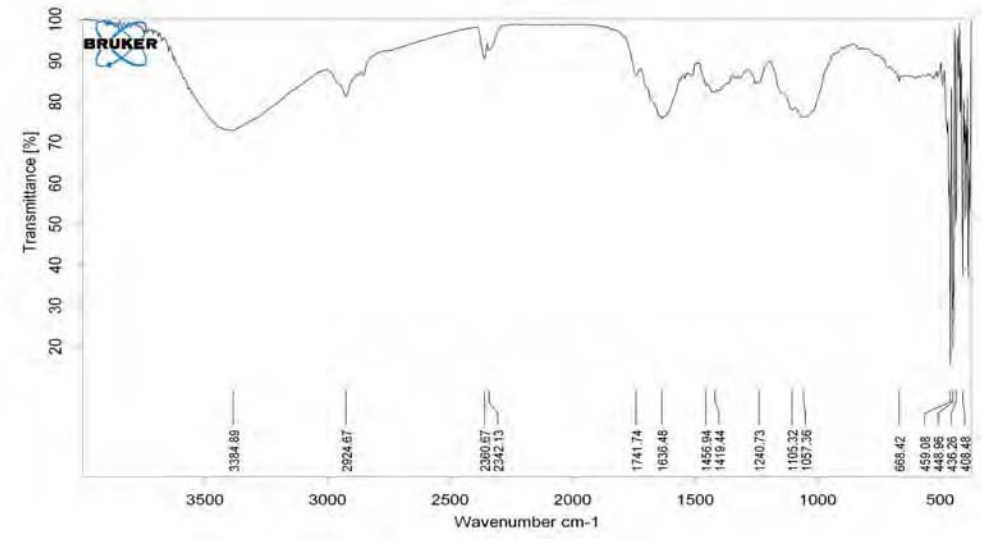

Fig. 4. FTIR spectrum of goru gum

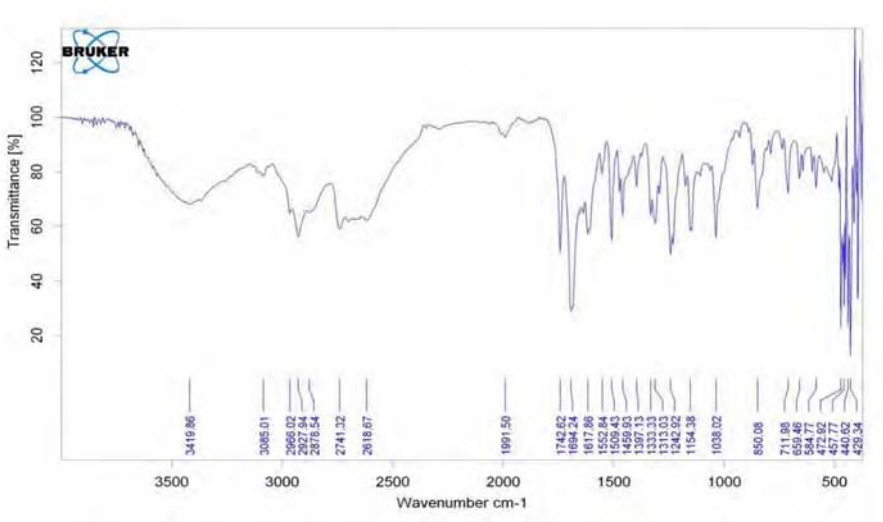

Fig. 5. FTIR spectrum of formulation

Physical characterization:

The mucoadhesive microspheres of pioglitazone prepared by the orifice-ionic gelatin method were found to be discrete, spherical, free flowing, and of the monolithic matrix type. The microspheres were uniform in size, with size range of 500 µm. The SEM photographs shown in Figure 6 indicated that microcapsules were spherical and completely covered the coat polymer.

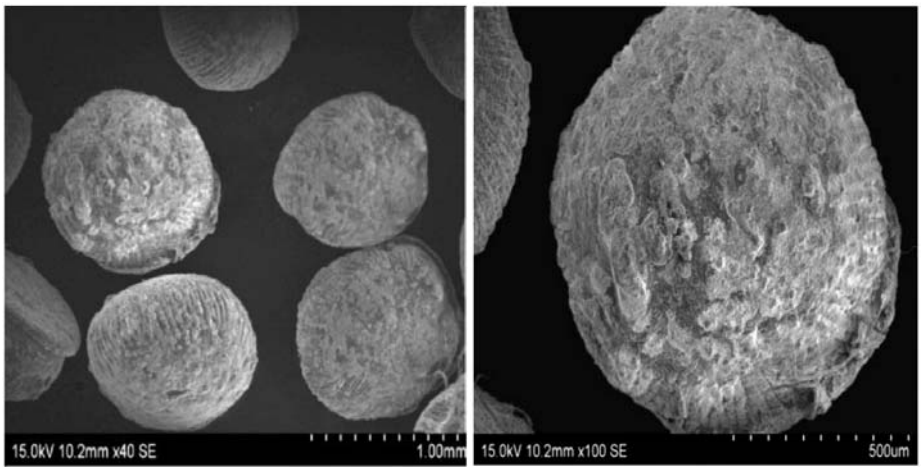

Fig. 6. SEM photographs of pioglitazone hydrochloride microspheres

Percentage yield, percentage drug content and drug entrapment efficiency

The percentage yield, percentage drug content and drug entrapment efficiency (DEE) of the pioglitazone hydrochloride mucoadhesive microspheres were estimated and are shown in Table 3.

Table 3. Percentage yield, percentage drug content and drug entrapment efficiency for MS1 – MS6

Formulation code	Total weight of the microspheres	Percentage yield	Percentage drug content	Drug entrapment efficiency	Percentage drug loading
MS1	6.37 gms	91.0%	2.17%	69.13%	10.85%
MS2	6.41 gms	91.5%	2.33%	74.67%	11.65%
MS3	7.24 gms	90.5%	2.03%	73.49%	10.15%
MS4	6.38 gms	91.1%	2.07%	66.04%	10.35%
MS5	6.55 gms	87.3%	2.01%	65.83%	10.05%
MS6	7.19 gms	89.8%	2.25%	80.90%	11.25%

The percentage yield ranged from 87.3% to 91.5%. The percentage drug content was found to be ranging from 2.01% to 2.33%, while the drug entrapment efficiency ranged from 65.83% to 80.90%. The percentage drug loading was found to be 10.05% to 11.65%.

***In vitro* wash-off test for microspheres:**

The *in vitro* wash-off test was performed for the all the formulations. The test was carried out for a period of 8 hrs. The results obtained are shown in the Table 4.

Table 4. *In vitro* wash-off test for the formulations MS1 – MS6

Time (hrs)	Initial no. of microspheres attached	No. of microspheres remaining						Percentage mucoadhesion					
		MS1	MS2	MS3	MS4	MS5	MS6	MS1	MS2	MS3	MS4	MS5	MS6
0	100	100	100	100	100	100	100	100%	100%	100%	100%	100%	100%
1		97	97	98	98	97	98	97%	97%	98%	98%	97%	98%
2		93	95	96	95	95	96	93%	95%	96%	95%	95%	96%
3		90	91	94	93	93	94	90%	91%	94%	93%	93%	94%
4		88	89	90	91	91	92	88%	89%	90%	91%	91%	92%
5		85	87	88	88	89	89	85%	87%	88%	88%	89%	89%
6		83	84	84	86	87	86	83%	84%	84%	86%	87%	86%
7		80	81	82	83	85	84	80%	81%	82%	83%	85%	84%
8		77	79	80	81	81	82	77%	79%	80%	81%	81%	82%

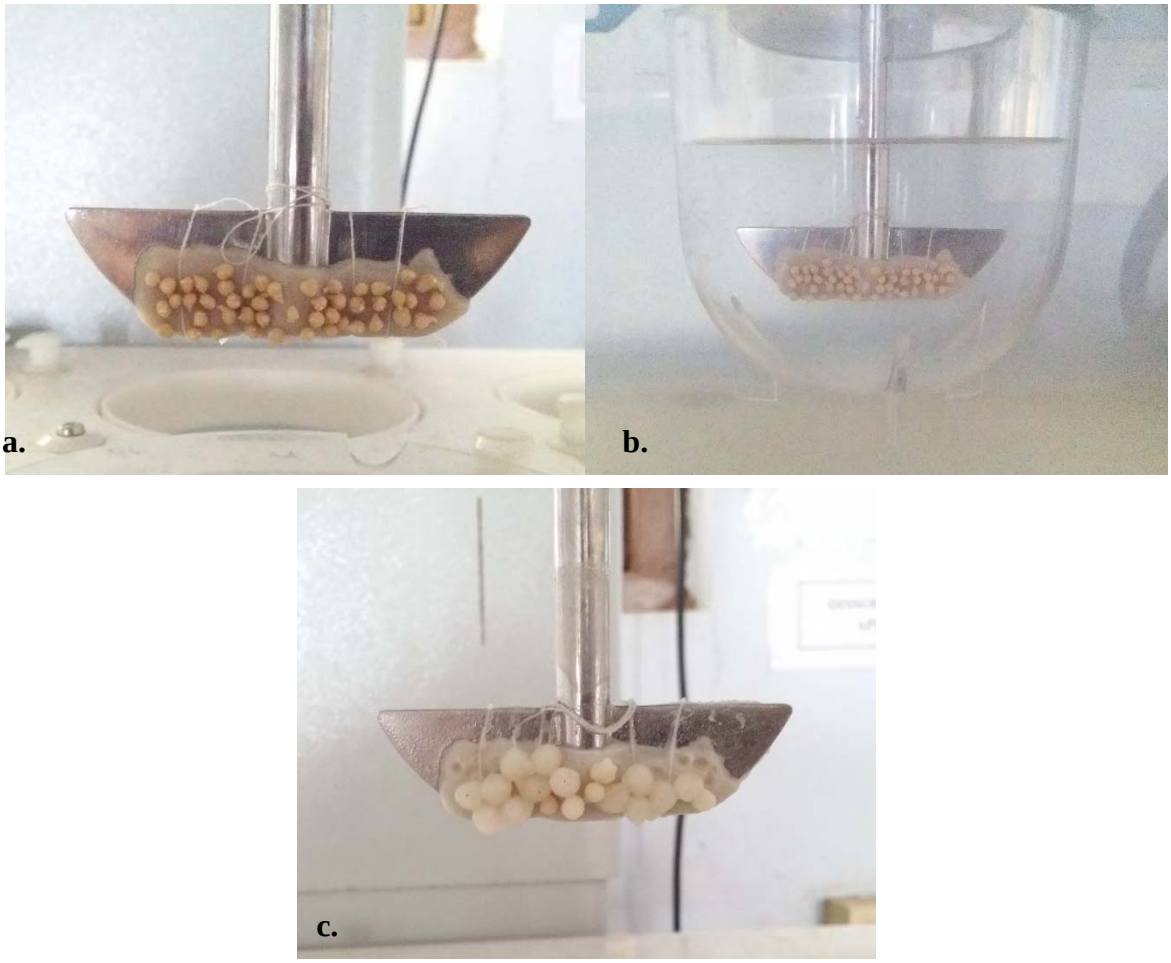

Fig. 7. *In vitro* wash-off test of microspheres
a. Initially at 0 hrs; b. Microspheres during the test; c. At 8 hrs.

The results indicated that all the formulations exhibited a percentage mucoadhesion ranging from 77% to 82%, with MS1 showing the lowest mucoadhesion at 77% and MS6 showing the highest mucoadhesion at 82%, followed by MS4 and MS5 at the end of 8 hrs. The results also indicated that as the concentration of the polymer was increased, the percentage of mucoadhesion also got increased.

Swelling index

The swelling index was also performed for all the formulations. The test was carried out for a period of 8 hrs. The results are shown in the Table 5.

Table 5. Swelling index for the formulations MS1 – MS6

Formulation code	Initial weight of the microspheres (W_0)	Weight of the microspheres after swelling (W_f)	Swelling index
MS1	100 mg	423 mg	76.35%
MS2	100 mg	438 mg	77.16%
MS3	100 mg	479 mg	79.12%
MS4	100 mg	510 mg	80.39%
MS5	100 mg	516 mg	80.62%
MS6	100 mg	520 mg	80.76%

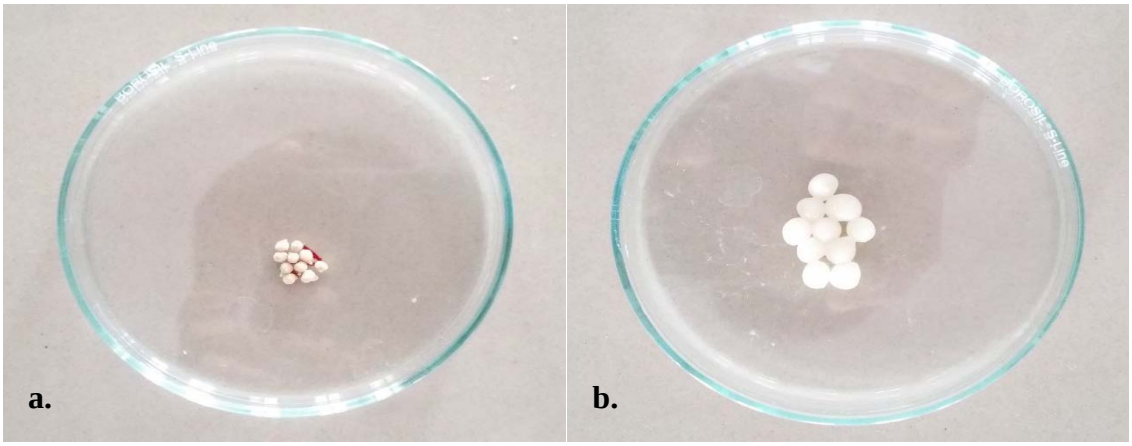

Fig. 8. Swelling index of microspheres
a. Initially at 0 hrs; b. At 8 hrs.

The results indicated that all the formulations exhibited a swelling index ranging from 76.35% to 80.76%. MS1 exhibited the lowest swelling index of 76.35%, while MS6 exhibited the highest swelling index of 80.76%, followed closely by MS5 and MS4. The results also clearly indicated that as the concentration of the mucoadhesive polymer in the formulations got increased, the swelling index also increased.

***In vitro* drug release and its kinetics:**

The *in vitro* drug release data and its kinetics for all the batches of pioglitazone hydrochloride mucoadhesive microspheres in phosphate buffer (pH 7.4) were estimated and these are shown in Tables 6 - 7 and Figures 9 - 13.

Table 6. *In vitro* drug release data of various batches of microspheres

Time (mins)	Percentage drug release of various batches of microspheres					
	MS1	**MS2**	**MS3**	**MS4**	**MS5**	**MS6**
0	0	0	0	0	0	0
5	3.31±0.68	3.21±0.25	3.17±0.67	3.26±0.56	3.41±0.38	3.16±0.47
10	6.51±0.34	6.34±0.78	6.29±0.98	6.48±0.28	6.54±0.12	6.28±0.65
15	7.91±0.44	7.87±0.94	7.71±0.62	7.93±0.15	7.91±0.35	7.71±0.35
20	9.81±0.75	9.71±0.56	9.64±0.45	9.65±0.18	9.53±0.65	9.68±0.94
25	10.43±0.14	10.05±0.64	9.87±0.81	10.29±0.94	10.34±0.57	9.87±0.50
30	11.78±0.37	11.34±0.31	11.14±0.77	11.52±0.64	11.47±0.61	11.01±0.62
35	12.75±0.81	12.32±0.27	12.19±0.65	12.41±0.27	12.31±0.75	11.99±0.93
40	15.61±0.65	14.56±0.65	14.35±0.30	14.98±0.35	13.09±0.29	13.98±0.72
45	16.01±0.72	15.21±0.40	15.07±0.12	15.34±0.85	14.98±0.64	15.07±0.18
50	16.97±0.68	16.15±0.25	15.91±0.67	16.42±0.56	16.21±0.38	16.02±0.47
55	17.51±0.34	16.84±0.78	16.63±0.98	16.98±0.28	16.55±0.12	16.11±0.65
60	17.98±0.44	17.35±0.94	17.14±0.62	17.63±0.15	17.23±0.35	17.03±0.35
65	18.80±0.75	18.02±0.56	17.84±0.45	18.81±0.18	18.25±0.65	17.88±0.94
70	20.41±0.14	19.31±0.64	19.03±0.81	20.44±0.94	19.61±0.57	18.92±0.50
75	21.92±0.37	20.22±0.31	20.05±0.77	21.98±0.64	20.89±0.61	20.01±0.62
80	23.61±0.81	21.91±0.27	21.78±0.65	22.20±0.27	21.97±0.75	21.31±0.93
85	25.61±0.65	23.63±0.65	23.48±0.30	24.81±0.35	24.01±0.29	23.27±0.72
90	26.71±0.72	25.88±0.40	24.57±0.12	25.91±0.85	26.08±0.64	25.34±0.18
95	27.07±0.68	26.94±0.25	25.59±0.67	26.63±0.56	26.54±0.38	26.37±0.47
100	28.31±0.34	27.01±0.78	26.23±0.98	28.25±0.28	27.11±0.12	26.88±0.65
105	29.25±0.44	27.56±0.94	26.95±0.62	29.31±0.15	28.08±0.35	27.04±0.35
110	30.41±0.75	27.98±0.56	27.34±0.45	30.67±0.18	27.99±0.65	27.11±0.94
115	31.25±0.14	28.01±0.64	27.88±0.81	31.27±0.94	29.87±0.57	27.87±0.50
120	32.37±0.37	28.43±0.31	28.01±0.77	32.50±0.64	30.63±0.61	28.03±0.62
150	33.53±0.81	30.22±0.27	29.31±0.65	33.97±0.27	31.98±0.75	29.73±0.93
180	35.36±0.65	32.73±0.65	31.56±0.30	35.42±0.35	33.64±0.29	31.88±0.72
210	37.97±0.72	36.01±0.40	34.97±0.12	38.01±0.85	36.98±0.64	35.88±0.18
240	39.98±0.68	38.91±0.25	37.75±0.67	40.46±0.56	39.54±0.38	38.02±0.47
270	42.88±0.34	42.51±0.78	41.95±0.98	42.97±0.28	42.08±0.12	41.96±0.65
300	45.38±0.44	46.11±0.94	44.67±0.62	45.41±0.15	45.39±0.35	45.35±0.35
330	48.42±0.75	48.08±0.56	46.11±0.45	48.92±0.18	47.86±0.65	47.68±0.94

*All the values are expressed as mean ± S.D., where n = 3

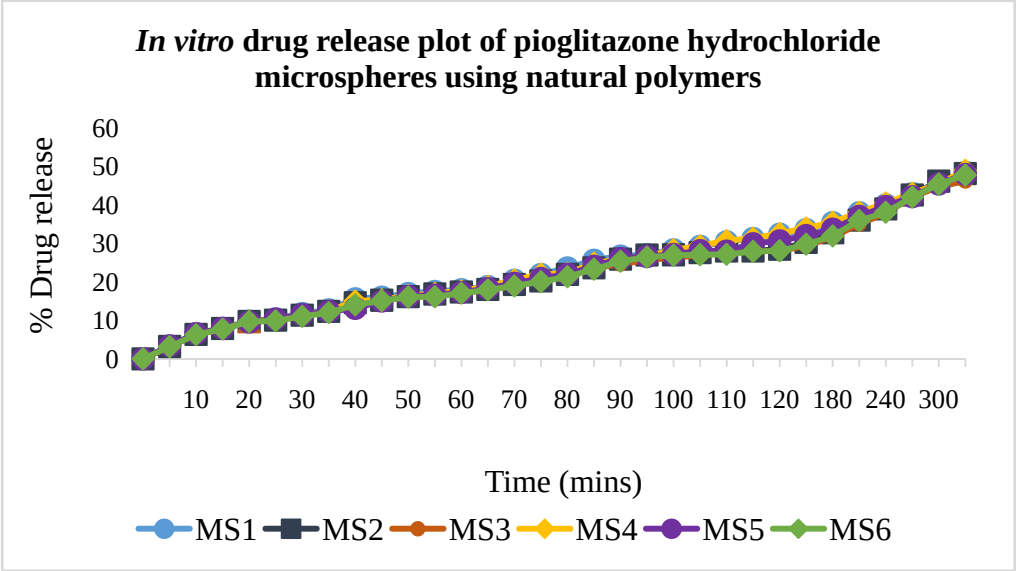

Fig. 9. *In vitro* drug release plot of pioglitazone hydrochloride microspheres using natural polymers

Table 7. *In vitro* drug release kinetic studies for pioglitazone hydrochloride microspheres

Formulation code	*In vitro* drug release kinetic models					
	Zero order	First order	Weibull	Higuchi matrix	Hixson–Crowell	Korsmeyer – Peppas
MS1	0.8896	0.9680	0.9968	0.3699	0.5808	0.9950
MS2	0.9198	0.9760	0.9967	0.3853	0.6000	0.9950
MS3	0.9166	0.9690	0.9965	0.3896	0.5938	0.9948
MS4	0.8961	0.9803	0.9970	0.3687	0.5916	0.9952
MS5	0.9097	0.9770	0.9967	0.3819	0.5983	0.9949
MS6	0.9214	0.9790	0.9967	0.3862	0.6025	0.9950

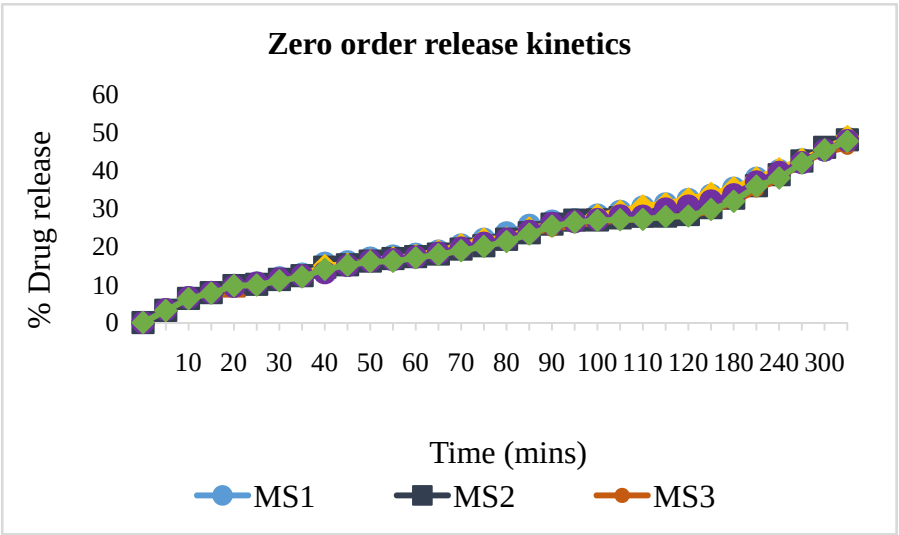

Fig. 10. Zero order release kinetics for the formulations MS1 – MS6

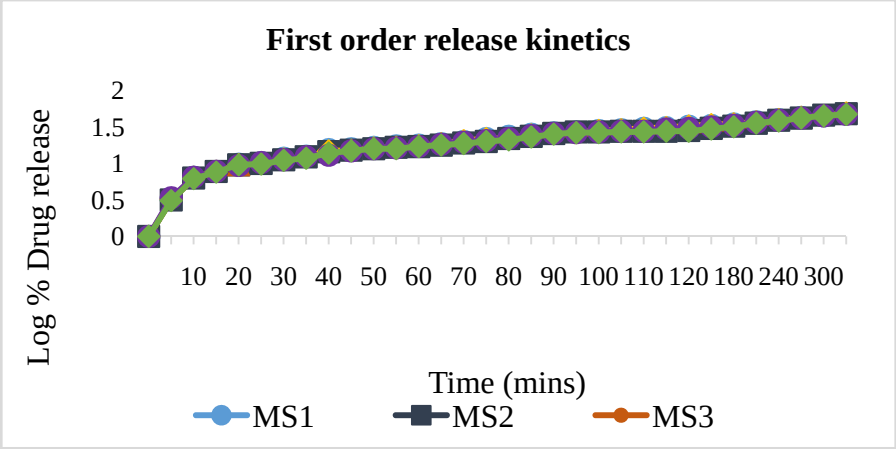

Fig. 11. First order release kinetics for the formulations MS1 – MS6

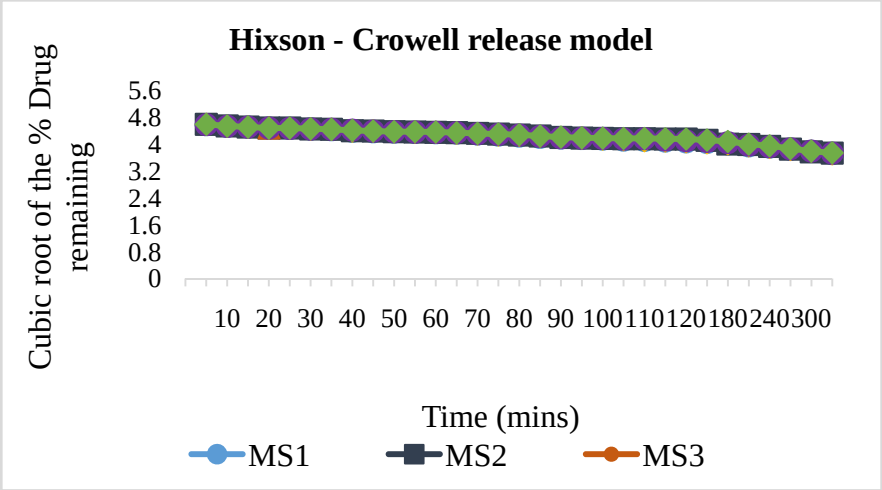

Fig. 12. Hixson – Crowell release model for the formulations MS1 – MS6

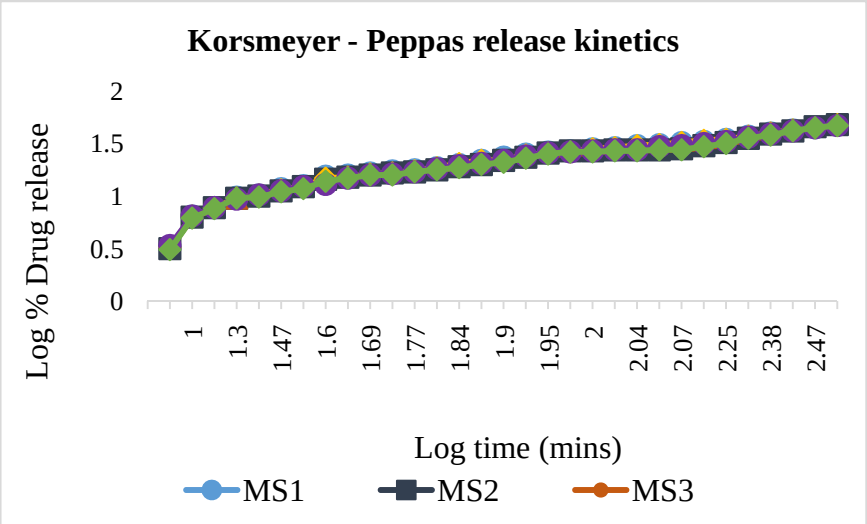

Fig. 13. Korsmeyer – Peppas release kinetics for the formulations MS1 – MS6

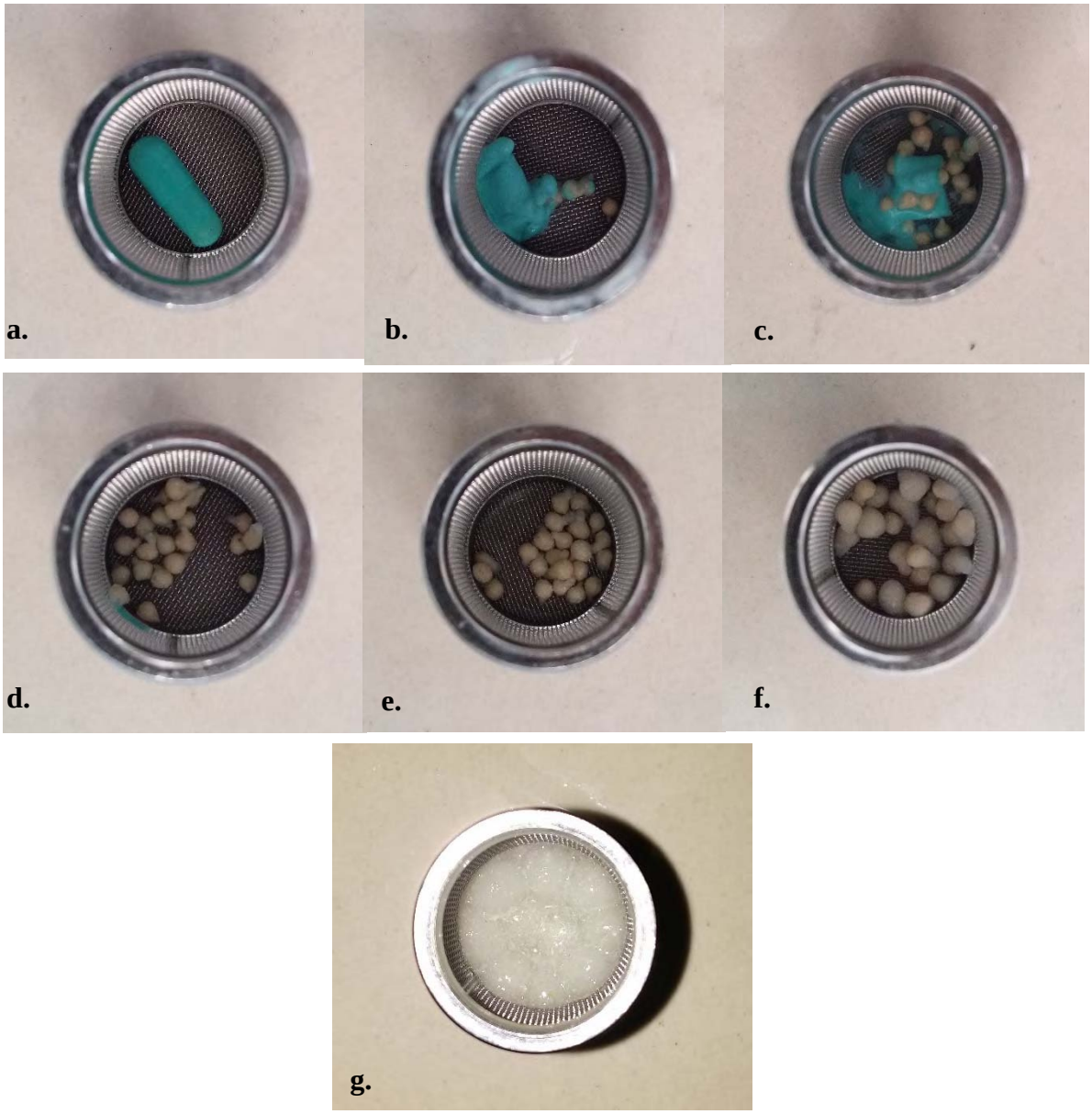

Fig. 14. Formulation(s) at different intervals of time during the *in vitro* dissolution studies;
a. Initially (0 hrs); b. At 5 mins; c. At 10 mins; d. At 15 mins; e. At 1 hr; f. At 4 hrs; g. At 8 hrs.

From the dissolution profiles and release kinetics of all the formulations, it became evident that all the formulations showed a cumulative percentage release of 46.11 – 48.92%, along with the drug release kinetics being in acceptable ranges. Taking the release kinetics and the dissolution profiles into consideration, the formulation MS4 was considered to be the best formulation, which showed the highest cumulative percentage release of 48.92% and showed good release kinetics.

The cumulative percentage drug release for all the formulations was found to range between 46.11 – 48.92% because the natural polymer used, i.e. goru gum exhibited high viscous properties, thus tending to release the drug very slowly in a sustained manner. All the formulations exhibited first order kinetics and the Korsmeyer – Peppas release kinetics were found to be the best fit among the drug release kinetics. It was found that as the concentration of the natural polymer got increased, the rate of drug release from the formulation got retarded and was found to be rather slow. The concentration of calcium chloride solution being used did not seemed to have any effect on the release rate of the formulations. So it was concluded that the concentration of the natural polymer need to be greater, but has to be maintained optimum, as seen in the case of the formulations MS1 and MS4, wherein the drug : polymer ratio was 1:1 and this was found to be optimum.

CONCLUSION

In the present investigation, an attempt was made to formulate mucoadhesive microspheres of pioglitazone hydrochloride using a natural polymer i.e., goru gum. The microspheres were evaluated for various micromeritic properties and it was observed that all the batches exhibited free-flowing properties. Scanning electron microscopy results showed that the microspheres were almost spherical in shape and discrete. The FTIR results showed that there were no interactions between the drug and the excipients and that all the ingredients were compatible. It was found that as the concentration of the mucoadhesive polymer was increased, the mucoadhesive and swelling properties of the microspheres also got increased. From the dissolution profiles and release kinetics of all the formulations, it became evident that all the formulations showed good cumulative percentage release of the drug, along with the drug release kinetics being in acceptable ranges. It may be concluded from the present study that a sustained release of pioglitazone hydrochloride over a long period of time was achieved by the formulation MS4, which showed the highest cumulative percentage release of 48.92% and showed good release kinetics.

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