



Design and *in vitro* Evaluation of Mucoadhesive Microcapsules of Pioglitazone

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ABSTRACT

Mucoadhesive microcapsules of pioglitazone were prepared using sodium alginate as a shell forming polymer and Carbapol 974, HPMC, Sodium CMC as a mucoadhesive polymer for the potential use of treating acute and chronic diabetes mellitus. Large spherical microcapsules with a coat consisting of sodium alginate and a mucoadhesive polymer could be prepared by orifice-ionic gelation process. The microcapsules exhibited good mucoadhesive properties and drug release from these mucoadhesive microcapsules was slow and extended over longer periods of time, depending on the composition of the coat. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of pioglitazone.

Key words: Controlled release, microcapsules, mucoadhesive, orifice-ionic gelation method, pioglitazone, sodium alginate

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INTRODUCTION

Pioglitazone is an oral antidiabetic agent that acts primarily by decreasing insulin resistance. Pioglitazone is used in the management of type-2 diabetes mellitus also known as noninsulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes.^[1]

It decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Pioglitazone is a potent and highly selective agonist for peroxisome proliferator-activated receptor-gamma (PPAR).^[2] Activation of PPAR nuclear receptor modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism.^[3]

Pharmacological studies indicate that pioglitazone improves glycemic control while reducing circulating insulin level.^[1] Pioglitazone has short biological half-life of 3–5 hours and is eliminated rapidly.^[4]

Therefore controlled release (CR) products are needed for pioglitazone to prolong its duration of action and to improve patient compliance; there are few reports^[5] 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. 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 of dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs.^[6–8]

MATERIALS AND METHODS

Materials

Pioglitazone was a gift sample from Sun Pharmaceutical Industries (Dadra, India). Sodium carboxymethylcellulose (sodium CMC), Methyl cellulose (Mc) was kindly provided by Stadmed Private LTD. (Kolkata, India), Hydroxypropylmethylcellulose (HPMC) was a gift sample from Colorcon Asia PVT, LTD. (Verna, India).

Methods

Preparation of mucoadhesive microcapsule

Microcapsules were prepared by orifice-ionic gelation method. Sodium alginate and mucoadhesive polymer were dissolved in purified water to form a homogenous polymer solution. The active substance, pioglitazone is 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

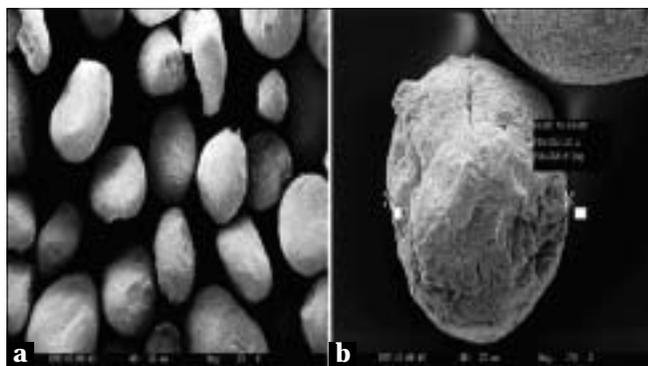


Figure 1: Scanning electron microscopy of Pioglitazone microcapsules, (a) spherical microcapsules with size ranging approximately from 300 to 600 μm (x25); (b) size of individual microcapsules is 353.33 μm (178 xs)

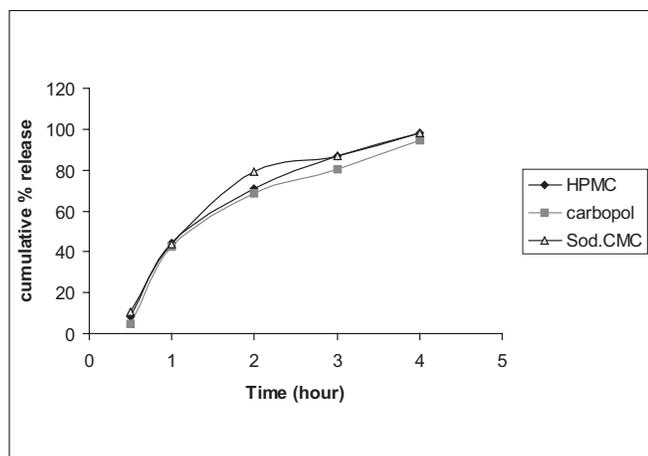


Figure 3: Release profile of Pioglitazone microcapsules (1:1); PC1 (\blacktriangle), PC2 (\diamond), PC3 (\square); in 0.1 N HCL of pH 1.2 (n = 3)

through a syringe with a needle of size no. 18. The added droplets are mixed into the 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, washed repeatedly with water and dried at 45°C for 12 hours.^[9-11]

Estimation of pioglitazone

Pioglitazone was estimated spectrophotometrically at 269 nm using Shimadzu 1701 (Japan) spectrophotometer standard curves for the estimation was prepared in phosphate buffer of pH-7.4 and pH-1.2 in concentration range between 1 $\mu\text{g}/\text{ml}$ –10 $\mu\text{g}/\text{ml}$. In this concentration range good linearity was observed with correlation coefficient (R^2) – 0.9998 in pH 7.4 phosphate-buffer and (R^2) – 0.9991 in pH 1.2 HCL.

Drug loading

About 500 mg of microcapsule was accurately weighed and transferred into 1,000 ml beaker, which contained

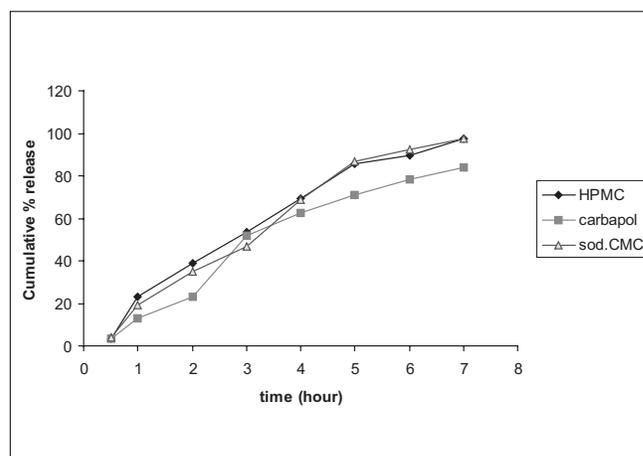


Figure 2: Release profile of pioglitazone microcapsules (1:1); PC1 (\blacktriangle), PC2 (\diamond), PC3 (\square); in Phosphate buffer, pH 7.4 (n = 3).

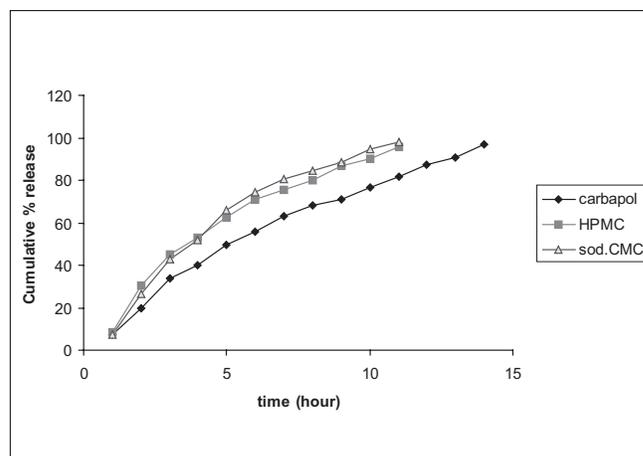


Figure 4: Release profile of Pioglitazone microcapsules (9:1); PC1-b (\blacktriangle), PC2-b (\diamond), PC3-b (\square); in phosphate buffer, pH 7.4 (n = 3)

Evaluation of Mucoadhesive Microcapsules of Pioglitazone

900 ml of phosphate buffer of pH 7.4 at 37°C. The phosphate solution was stirred continuously until all the microcapsules were dissolved. 1% SLS solution was used to enhance the solubility of pioglitazone in 7.4 phosphate buffer. Drug loading was determined at 269 nm spectrophotometrically. Drug loading (Microencapsulation efficiency) was calculated by the following formula:

$$\% \text{ Drug loading} = (\text{Estimated drug content} / \text{theoretical drug content}) \times 100$$

Morphological characterization of microcapsules

The surface and inner part of the microcapsules was observed through the scanning electron microscopy (SEM), SEM was performed for morphological characterization of microcapsules using the electron microscope (SEM-LEICA, S430, London, UK).

Drug release study

In vitro drug release (in pH 7.4-phosphate buffer medium)

In vitro pioglitazone release rate was tested using dissolution test apparatus with a rotating paddle stirrer at 50 rpm. 500 mg of pioglitazone microcapsules, which contained 100 mg pioglitazone, were suspended in 900 ml of phosphate buffer of pH 7.4 with a temperature of 37°C, 9 gm of SLS mixed in the buffer to enhance the solubility of pioglitazone in the phosphate buffer. The rotating rate of basket was adjusted to 50 rpm and 5 ml of samples were withdrawn at different intervals. Sample was then filtered through Whatman filter paper. The same volume of dissolution medium was replaced after each withdrawal. The

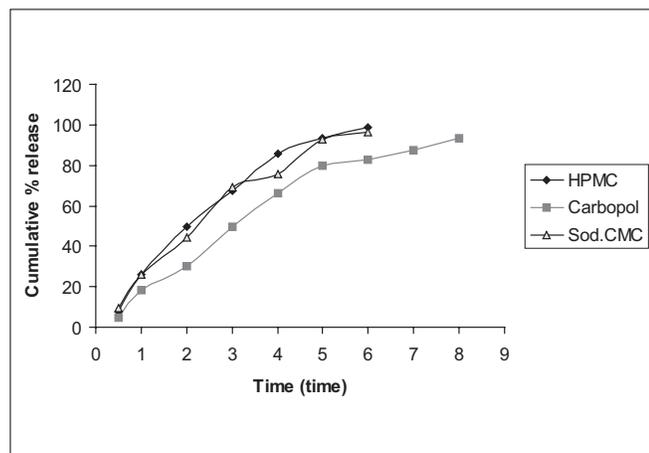


Figure 5: Release profile of Pioglitazone microcapsules (9:1); PC1-b (▲), PC2-PC3-b (□); in 0.1 N HCL of pH 1.2 (n = 3)

Table 1: Coating composition, drug content, and microencapsulation efficiency of microcapsules

Microcapsules	Polymer-polymer ratio	Drug-polymer ratio	(%) Drug loading
PC1-a	Sodium Alginate: Sodium CMC (1:1)	1:4	80.67
PC2-a	Sodium Alginate: HPMC (1:1)	1:4	82.77
PC3-a	Sodium Alginate: Carbapol (1:1)	1:4	90.11
PC1-b	Sodium Alginate: Sodium CMC (9:1)	1:4	92.12
PC2-b	Sodium Alginate: HPMC (9:1)	1:4	81.27
PC3-b	Sodium Alginate: Carbapol (9:1)	1:4	86.25

Table 2: Result of *in vitro* wash-off test to show mucoadhesive properties of microcapsules in 0.1 N HCl

Time	Percentage of microcapsule adhering to tissue at 0.1N Hcl					
	PC1-a	PC2-a	PC3-a	PC1-b	PC2-b	PC2-b
1	71.66 ± 1.52	83.33 ± 1.52	80.00 ± 1.58	74.33 ± 2.08	77.66 ± 1.52	82.33 ± 2.05
2	68.66 ± 3.21	79.23 ± 1.52	76.66 ± 1.15	70.66 ± 2.03	67.33 ± 1.50	69.66 ± 2.08
4	64.00 ± 1.00	74.32 ± 0.57	74.00 ± 1.00	56.83 ± 1.52	59.10 ± 1.01	60.00 ± 1.00
6	54.33 ± 2.08	68.00 ± 2.00	71.32 ± 1.54	36.00 ± 2.46	36.33 ± 1.52	33.00 ± 2.00
8	48.66 ± 1.54	62.00 ± 2.64	66.66 ± 1.35	18.32 ± 1.15	22.56 ± 1.52	18.33 ± 0.57

Table 3: Result of *in vitro* wash-off test to show mucoadhesive properties of microcapsules in 0.1 N HCl

Time	Percentage of Microcapsules adhering to tissue at 7.4 pH					
	PC1-a	PC2-a	PC3-a	PC1-b	PC2-b	PC-b
1	62.66 ± 2.08	66.66 ± 1.52	69.00 ± 1.73	71.00 ± 1.00	68.33 ± 3.05	70.00 ± 2.00
2	54.66 ± 1.527	63.00 ± 1.00	61.66 ± 3.51	55.33 ± 1.52	61.00 ± 3.00	54.33 ± 1.527
4	22.33 ± 1.52	19.33 ± 1.15	25.33 ± 3.05	21.66 ± 1.527	35.00 ± 1.00	30.33 ± 1.52
6	12.33 ± 2.08	11.66 ± 2.52	19.66 ± 1.27	1.66 ± 2.08	17.33 ± 1.527	8.33 ± 3.51
8	3.66 ± 3.214	6.66 ± 0.577	10.00 ± 1.00	-	6.00 ± 3.00	2.66 ± 3.055

samples were assayed at 269 nm for pioglitazone content spectrophotometrically. The drug release experiments were conducted in triplicate (n = 3).

In vitro drug release (in pH 1.2 HCl medium)

Pioglitazone release in the pH 1.2 HCl mediums was carried out using same procedure as in phosphate buffer of pH 7.4. Only dissolution medium was replaced with 0.1N HCl of pH 1.2.

Mucoadhesive testing by in vitro wash-off test

The mucoadhesive property of the microcapsules was evaluated by wash-off *in vitro* method. Freshly excised pieces of intestinal mucosa ($2 \times 2 \text{ cm}^2$) from sheep were mounted onto glass slides (3×1 inches) with cyanoacrylate glue. Approximately 50 microcapsules were spread onto each wet rinsed tissue specimen, and immediately the slide was hung onto the arm of a 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 one liter vessel. After the definite time interval, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. (Gastric pH) 0.1N HCL, pH 1.2 and (intestinal pH) phosphate buffer of pH 7.4 were used as test fluid.^[9]

RESULTS AND DISCUSSION

Morphological characteristics of the microcapsules

The mucoadhesive microcapsules of pioglitazone prepared by the orifice-ionic gelatin method were found to be discrete, spherical, free flowing. The microcapsules were uniform in size, with size range of 300 to 600 μm . The SEM photographs indicated that microcapsules were spherical and completely covered the coat polymer [Figure 1] and the microencapsulation efficiency was in the range of 80–93% [Table 1].

***In vitro* release of Pioglitazone**

Pioglitazone release from the microcapsules was studied in phosphate buffer of pH 7.4 and 0.1 N HCl of pH 1.2 for 24 hours. Pioglitazone release from the microcapsules was slow and followed zero-order kinetics. Microcapsules of alginate-carbapol in phosphate buffer of pH 7.4 gave slow release compared to others. The slower increasing release rate observed in order Sodium alginate: Carbapol < Sodium alginate: Sodium CMC < Sodium alginate: HPMC. Pioglitazone release from microcapsules SP4 was slow and extended over a period of 14 hours, So MS4 was found to

be suitable for oral sustained release [Figures 2-5].

***In vitro* evaluation of adhesiveness**

Microcapsules with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in vitro* wash-off test. The wash-off was faster at intestinal pH than at gastric pH [Tables 2, 3].

CONCLUSION

The method employed gave spherical, discrete, and free flowing (sodium alginate and mucoadhesive polymers) microcapsules of pioglitazone. The size could be readily separated; drug content was found to be uniform in a batch of microcapsules. Pioglitazone released from the microcapsules was found to be slow and spread over extended period of time, drug release depends on the percent of coat material, wall thickness, and size of the microcapsules. Microcapsules with a coat comprised sodium alginate and mucoadhesive polymer exhibited good mucoadhesive properties. In the *in vitro* wash-off test, the wash-off was faster at intestinal pH than at gastric pH. The mucoadhesive microcapsules are thus suitable for oral controlled release of pioglitazone.

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