AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

EFFECT OF CROSS-LINKING AGENT AND POLYMER ON THE CHARACTERISTICS OF DILTIAZEM HYDROCHLORIDE LOADED MUCOADHESIVE MICROSPHERES

P. Sivanarayana*[1], V. Sai Kishore[2], P.Jithendra Kumar[1]

1. Nalanda Institute of Pharmaceutical Sciences, Kantepudi,- 522438, Andhra Pradesh, India.
2. Bapatla College of Pharmacy, Bapatla-522101, Andhra Pradesh, India.

ABSTRACT

Diltiazem hydrochloride, a calcium channel blocker, is widely used for the treatment of angina pectoris, hypertension and arrhythmias. The usual dose of diltiazem hydrochloride is 180-240 mg/day. The conventional tablet or capsule is administered 3 or 4 times a day due to its low biological half-life of about 3.7 h. The problems of frequent administration and variable low bioavailability (36-50%) after oral administration of conventional tablet or capsules have been attenuated by designing diltiazem hydrochloride in the form of mucoadhesive microspheres. Diltiazem hydrochloride loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum encapsulation efficiency of 99.48± 0.32%.The order of increasing release rate observed with various microspheres was as follows Sodium alginate < Sodium alginate+ NaCMC < Sodium alginate+ HPMC. The order of increasing release rate observed with various cross linking agents was as follows Aluminum chloride < Barium chloride< Calcium chloride. The release behaviour of microspheres, with different cross-linking agents depends upon the valency and size of the cations of the respective cross-linking agent. The dissolution profiles follow zero order kinetics and the mechanism of drug release was governed by peppas model. The *in vitro* wash-off test indicated that the wash-off was faster at simulated intestinal fluid (phosphate buffer, pH 7.4) than that at simulated gastric fluid (0.1 M HCl, pH 1.2). The mucoadhesive microspheres formulated with sodium alginate+HPMC and calcium chloride showed a satisfactory sustained release profile for 12 hours.

Keywords: Mucoadhesive microspheres, Polymer, Cross linking agent, zero order kinetics

*Corresponding Author Email: siva_mpharma@yahoo.com
Received 8 December 2011, Accepted 14 December 2011

Please cite this article in press as: Sivanarayana P *et al.*, Effect of Cross-Linking Agent and Polymer on the Characteristics of Diltiazem Hydrochloride Loaded Mucoadhesive Microspheres. American Journal of PharmTech Research 2012.

INTRODUCTION:

Diltiazem hydrochloride, a calcium channel blocker, is widely used for the treatment of angina pectoris, hypertension and arrhythmias. Due to its low biological half-life (3.7) it requires frequent administration. Sustained therapeutic action is necessary for alleviating patients symptoms and is achieved by mucoadhesive microspheres[1]. The objective of the present study was the development and evaluation of gastroretentive microspheres containing diltiazem hydrochloride using various mucoadhesive polymers for prolonged gastrointestinal absorption. An attempt was also made to develop microspheres with high entrapment efficiency. The method of microencapsulation is based on emulsification-internal gelation technique involving alginate polymers alone and/or in combination with other mucoadhesive polymers. Diltiazem hydrochloride is a water-soluble cardiovascular drug. The use of external gelation process of microencapsulation involving alginate polymers in the aqueous cross-linking agent would minimize the entrapment efficiency due to the diffusion of diltiazem hydrochloride into the aqueous phase during the curing of the gel beads. The other inconveniences include the limitation in reducing microspheres diameter, the teardrop shape of the microparticles produced and difficulty in industrial scale-up. The emulsification-internal gelation technique of microencapsulation use an external oil phase and thereby may reduce the drug diffusion during encapsulation process and improve the drug entrapment efficiency. The gel bead diameter can be easily controlled and that has scale-up potential[2].

MATERIALS AND METHODS

Diltiazem hydrochloride was obtained as a gift sample from Natco Pharma, Hyderabad. Sodium alginate, hydroxyl propyl methyl cellulose (HPMC) and sodium carboxy methyl cellulose (NaCMC) (Loba Chemie Pvt. Ltd., Mumbai), Aluminium chloride,Barium chloride, Calcium chloride and glacial acetic acid (Ranbaxy Fine Chemicals, Chandigarh), light liquid paraffin, Span 80 (Central Dug House, New Delhi). All the solvents and chemicals used were of analytical grade satisfying pharmacopoeial standards.

Preparation of microspheres:

Diltiazem hydrochloride microspheres were prepared by an emulsification internal gelation method3.Microspheres containing diltiazem hydrochloride was prepared employing sodium alginate alone and in combination with HPMC and NaCMC. The homogenous polymer solution was prepared in distilled water (15ml) stirred magnetically with gentle heat. The drug and cross linking agent (Aluminium chloride/Barium chloride/ Calcium chloride) were added to the

polymer(s) solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with a needle of size no.23 into light liquid paraffin (150 ml) containing 1.5% span 80 and 0.2% glacial acetic acid being kept under magnetic stirring at 500rpm in a 250ml glass beaker. The above dispersion was maintained at 100^0C and stirring was continued for 15 minutes. The microspheres were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil. The microspheres were dried at 40 ^{0}C for 12h. The compositions of the microspheres formulations are listed in Table 1.

Table 1: Composition of Diltiazem hydrochloride loaded various mucoadhesive Microspheres formulation

Formulation	Polymer level (% w/v)	Drug level (%w/w)	Cross-linking agent/level (%w/w)
F1	Sodium alginate 4%	5	Aluminum chloride/6%
F2	Sodium alginate, 2% + NaCMC 2%	5	Aluminum chloride/6%
F3	Sodium alginate, 2% + HPMC 2%	5	Aluminum chloride/6%
F4	Sodium alginate, 2% + HPMC 2%	5	Barium chloride/6%
F5	Sodium alginate, 2% + HPMC 2%	5	Calcium chloride/6%

Evaluation of microspheres:

Size Distribution and Size Analysis:

Size and size distribution was determined by sieve analysis was carried out on mechanical sieve shaker. The drug loaded microspheres were separated into different size fractions(% weight fraction) by sieving for 5min using standard sieves having nominal mesh apertures of 1.4mm, 1.2mm, 1.0mm, 0.85mm and 0.71mm (sieve no 12, 14, 16, 18 and 22, respectively). Particles that passed through one sieve but were retained on the other were collected and weighed and the distribution was analyzed based on the weight fraction on each sieve. The particle size distribution and mean particle size of microspheres were calculated using the following formula[4].Mean particle size =Σ (mean particle size of the fraction x weight fraction) /Σ (weight fraction)

Drug content and Microencapsulation efficiency

The amount of diltiazem hydrochloride present in the microspheres was determined by extracting the drug into phosphate buffer of pH 7.4 under magnetic stirring for a period of 2 h. The solution was filtered through whatman filter paper no.5, suitably diluted and estimated for drug content

spectrophotometrically at 237 nm using UV-Visible spectrophotometer. The microencapsulation efficiency was calculated by the following formula.

$$\text{Microencapsulation efficiency} = \frac{\text{Estimated percent drug content}}{\text{Theoretical percent drug content}} \text{X}100$$

Swelling properties

The swelling properties of the drug loaded microspheres were determined in various pH ranges (i.e. 1.2 and 7.4 buffer solutions). Thirty dried microspheres were placed in a small beaker to which 100ml of buffer solutions was added and then allowed to swell at 37^0C. After 2h interval, the equilibrium swollen microspheres were observed and measured by Optical microscopy. The magnitude of swelling was presented by the ratio f the mean diameter of swelling microspheres to the mean diameter of the dried microspheres before the test. Swelling ratio was determined from the following formula[5].

$$\text{Swelling ratio} = \frac{[(\text{Mean diameter at time t-Initial diameter})] \text{X}100}{\text{Initial diameter of microspheres}}$$

In Vitro Release Studies:

The Microspheres (12/16 mesh size (1200 µ) containing equivalent to 90 mg of diltiazem hydrochloride were packed in hard gelatin capsule and subjected to *in vitro* drug release studies. Release of diltiazem hydrochloride from the capsule was studied in phosphate buffer of pH 7.4 (900 mL) using a United States Pharmacopoeia (USP)XXIV 8-station dissolution rate test apparatus (Model TDT - 08L, M/s Electro lab, Mumbai, India) with a rotating paddle stirrer at 100 rpm and 37 ^{O}C ± 1 ^{O}C. 5 ml of samples of dissolution fluid were withdrawn through a filter (0.45 µ) at the regular interval of every one hour and were assayed at 237 nm for diltiazem hydrochloride content using a Shimadzu UV-1700 double beam spectrophotometer (Shimadzu Corporation, Japan). The drug release experiments were conducted in triplicate.

The rate and the mechanism of release of diltiazem hydrochloride from the prepared microspheres were analyzed by fitting the dissolution data into[7], zero-order equation, $Q=Q_0 - k_0t$(1),where Q is the amount of drug released at time t, and k_0 is the release rate. First order equation, $\text{Ln } Q = \text{Ln } Q_0 - k_1t$ (2), where k_1 is the release rate constant and Higuchi's equation, $Q= k_2t^{1/2}$ (3) where Q is the amount of the drug released at time t and k_2 is the diffusion rate constant. The dissolution data was further analyzed to define the mechanism of release by applying the dissolution data following the empirical equation , $M_t/M_\infty = Kt^n$ (4), where Mt/M_α is

the fraction of drug released at time t. K is a constant and n characterizes the mechanism of drug release from the formulations during dissolution process.

In-vitro Wash-off Test:

Freshly excised pieces of intestinal mucosa (2×2cm) from sheep were mounted on to glass slides (3×1inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microspheres were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, 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 of the machine. At the end of 30 minutes, at the end of 1 hour, and at hourly intervals up to 8 hours, the machine was stopped and the number of microspheres still adhering to the tissue was counted[8]. The test was performed at both gastric pH (0.1N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4).

Measurement of the GI Residence Time:

It is a simple procedure involving the use of radio-opaque markers, e.g. barium sulfate, incorporated in mucoadhesive polymers to determine the effects of mucoadhesive polymers on GI transit time. *X-Ray* inspection provides a non-invasive method of monitoring total GI residence time without affecting normal GI motility. Healthy rabbit of 2.8 - 3.1 kg was fasted over night and on the next day morning mucoadhesive microspheres containing barium sulphate (equivalent to diltiazem hydrochloride) was administrated followed by giving 25 ml of water. At different time intervals of 0, 2, 4, 8 and 10 hours rabbit was X-Ray photographed and observed for the nature and position of the mucoadhesive microspheres.

RESULTS AND DISCUSSION

Diltiazem hydrochloride loaded mucoadhesive microspheres were prepared by emulsification-internal gelation technique. Diltiazem hydrochloride, a hydrophilic drug, can partition out into the aqueous processing phase during the preparation of microspheres by external gelation method. Depending on the processing conditions as much as 80 to 90% of the drug can partition out into the external aqueous processing medium. In this study attempt was made to encapsulate diltiazem hydrochloride with sufficiently high encapsulation efficiency. An external oil phase (liquid paraffin) was used as the harvesting medium with the expectation that for diltiazem hydrochloride it would be non favourable to diffuse out of the microspheres before they form as rigid and discrete particles.

Diltiazem hydrochloride loaded mucoadhesive microspheres composed of alginate alone and in combination with HPMC or NaCMC were prepared by the emulsification-internal gelation technique using Aluminium chloride or Barium chloride or Calcium chloride as cross linking agent. The microspheres were found to be discrete, spherical and free flowing. The percent yield was found to be in the range of 90.12-93.36%. The mean particle size of the various formulations was found to be in the range of 1219.8 -1248.4μm with only 10.43-14.84% being of the smaller mean diameter 855μm (16/22mesh). Overall more than 85% of microspheres prepared were of 1200μm mean diameter (12/16mesh).

The particle size ranges are shown in Table 2. It was found that the particle size distribution of each formulation was within a narrow range but the mean particle size was different among the formulations. The micrometric parameters like angle of repose, bulk density and tapped density of all microspheres confirms better flow and packaging properties. All the formulations showed good flow ability represents in terms of angle of repose, Carr's index, and Hausner's ratio (Table 3).

Table 2: Size distribution analysis and mean particle size of diltiazem hydrochloride loaded various mucoadhesive microspheres formulations

S.No	Formulation	% yield	1200μm [12/16]mesh	855μm [16/22]mesh	Average particle size [12/16]
1	F1	92.84	87.28	12.72	1246.4
2	F2	91.86	86.53	13.47	1234.2
3	F3	89.36	87.74	12.26	1232.3
4	F4	90.12	85.16	14.84	1248.4
5	F5	93.36	89.57	10.43	1219.8

Table 3: Micromeritic properties of diltiazem hydrochloride loaded various mucoadhesive microspheres formulations

S.No	Formulation code	Angle of Repose [θ]	Bulk Density [g/ml]	Tapped Density [g/ml]	Carr's Index (CI)%	Hausner's ratio
1	F1	22.24±1.16	0.662±0.14	0.769±0.13	13.91	1.16±0.77
2	F2	23.28±1.22	0.633±0.65	0.743±0.14	14.80	1.17±0.34
3	F3	21.64±0.82	0.723±0.76	0.831±0.12	12.99	1.14±0.18
4	F4	23.22±0.66	0.654±0.73	0.771±0.11	15.17	1.18±0.58
5	F5	22.56±0.80	0.658±0.04	0.773±0.13	14.87	1.22±0.32

The drug content for the prepared microspheres was determined and it ranged from 78.44 to 79.62. The drug entrapment for the prepared microspheres was determined and it ranged from

98.05 to 99.52. The results tabulated in Table 4 indicate that the encapsulation efficiencies were more than 99 % for microspheres cross-linked with Al^{3+} and Ba^{2+}. This may be attributed to the formation of nonporous alginate microspheres due to an increase in the apparent cross-linking density in presence of Al^{3+} and Ba^{2+} which prevent the diffusion of the drug out of the microspheres at the time of curing. The low encapsulation efficiency of alginate microspheres cross-linked with Ca^{2+} could be attributed to the formation of porous microspheres ensuring the diffusion of the drug out of the beads at the time of curing.

Table 4: Drug content, Encapsulation efficiency and Swelling ratio of diltiazem hydrochloride loaded various mucoadhesive microspheres formulations

S.no	Formulation	Drug content	Encapsulation efficiency	Swelling ratio	
				pH 1.2	pH 7.4
1	F1	81.32	99.64	3.1	12.4
2	F2	80.64	99.34	4.2	14.2
3	F3	80.16	99.12	4.8	17.4
4	F4	79.76	98.82	4.6	16.6
5	F5	79.22	98.67	4.2	15.8

The swelling ratio of the microspheres was dependent on the pH of the solution. The swelling ratio of the microspheres was found to be more in alkaline conditions than acidic conditions. The low swelling in acidic media pH1.2 was probably due to insolubility of the polymer in acid pH. The swelling of microspheres were ultimately increases in pH7.4 were due to increased solubility of the polymer in basic pH leading to relaxation of the cross-linked polymeric network.

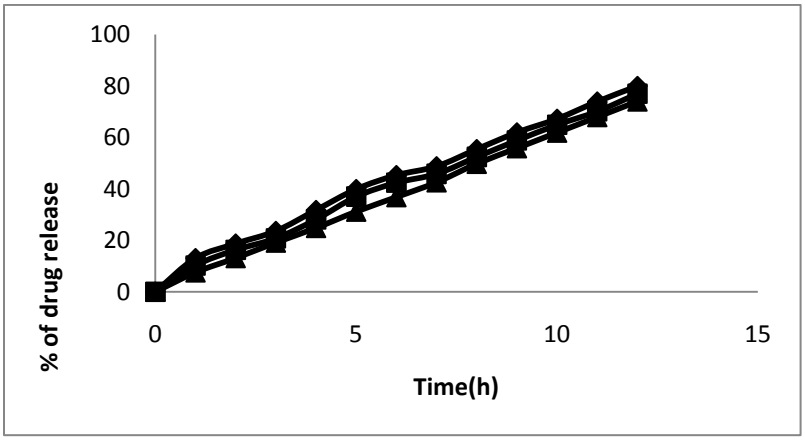


Figure 1: Release Profile Of diltiazem hydrochloride mucoadhesive microspheres Prepared With Various Polymers and Aluminium Chloride as a Internal Cross Linking Agent

F1 - (♦) - Microspheres prepared with sodium alginate polymer
F2 - (■) - Microspheres prepared with Na alginate and NaCMC
F3 - (▲) - Microspheres prepared with Na alginate and HPMC

The *in vitro* drug release studies were carried out in the simulated intestinal fluid (phosphate buffer, pH 7.4). Diltiazem hydrochloride release from the microspheres was slow and spread over an extended period of time. The release increased as the size of microspheres decreased. Microspheres of Sodium alginate in combination with other mucoadhesive polymers gave relatively more release when compared with the microspheres prepared with Sodium alginate alone and are shown are shown in Figure 1.The order of increasing release rate observed with various microspheres was as follows: Sodium alginate < Sodium alginate+ NaCMC < Sodium alginate+ HPMC.

The order of increasing release rate observed with various cross linking agents was as follows: Aluminum chloride < Barium chloride < Calcium chloride

The release behaviour of alginate microspheres, produced by ionotropic internal gelation with different cross-linking agents depend upon the valency and size of the cations of the respective cross-linking agent. Their release profiles in phosphate buffer of pH 7.4 is depicted in Figure 2. The results obtained can be explained on the basis of the extent of cross-linking in the microspheres. Ca^{2+} and Ba^{2+}, being divalent, form two-dimensional bonding structure with sodium alginate inside the alginate matrices. But since Ba^{2+} has the largest size as compared to the other two cations (Ca^{2+} and for $Al\ 3^{+}$), it is expected to form strong alginate microspheres with smaller voids and low water uptake. Therefore, the exchange of larger Ba^{2+} in the microspheres with Na^{+} of dissolution medium (phosphate buffer, pH 7.4) and also their removal in the form of insoluble barium phosphate was hindered, thus resulting in delayed swelling of the microspheres and slow release.

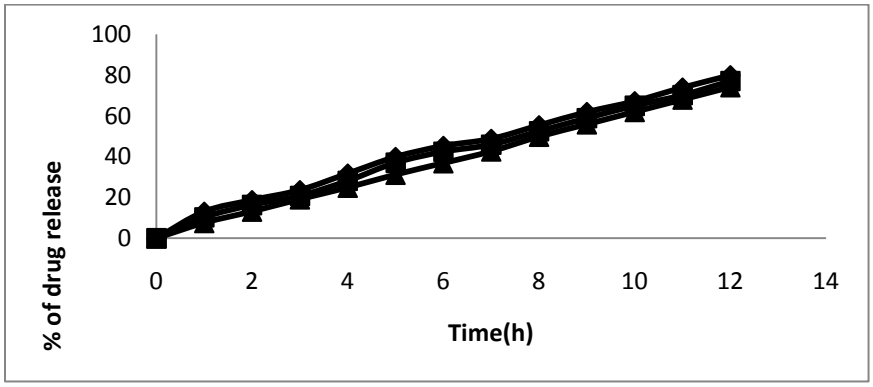


Figure 2: Release profile of diltiazem hydrochloride microspheres prepared with various cross linking agents

F3 -(♦) -Na alginate and HPMC microspheres prepared with aluminum chloride as crosslinking agent.
F4 - (■) -Na alginate and HPMC microspheres prepared with barium chloride as crosslinking agent
F5-(▲)-Na alginate and HPMC microspheres prepared with calcium chloride as crosslinking agent

In case of Ca^{2+} alginate microspheres, the smaller size of Ca^{2+} as compared to Ba^{2+} ensure rapid removal of Ca^{2+} as calcium phosphate from the microspheres due to ion exchange process with Na^{+} of phosphate buffer medium and thus leading to greater water uptake and rapid release. In case of Al^{3+}alginate microspheres, the delay was due to the ability of Al^{3+} to form three-dimensional bonding structure with the sodium alginate inside the microspheres. This three dimensional bonding results in an extended cross linking through the whole microspheres, producing hard alginate microspheres with low water uptake and thus leading to slow removal of Al^{3+}due to ion exchange with Na^{+} in the phosphate buffer. As a result, the swelling of the microspheres are delayed leading to slow disintegration.

To ascertain the mechanism of drug release, the dissolution data was analyzed by zero order, first order, and higuchi and peppas equations. The correlation coefficient values (r) were reported in Table 5. These values revealed that the dissolution profiles followed zero order kinetics and the mechanism of drug release was governed by peppas model. The n values are found to be more than 0.5 (n>0.5) indicted that the drug release was from the microspheres followed the anomalous transport and super case-II transport mechanism controlled by swelling and relaxation of the polymer chains.

Table 5: Correlation coefficient (R) values in various kinetic models and dissolution kinetics of diltiazem hydrochloride loaded various mucoadhesive microspheres formulations

S.no	Formula	Correlation coefficient				Diffusion exponent (n)	T_{50}	Release rate (mg/h)
		Zero order	First order	Higuchi model	Peppas model			
1	F1	0.9986	0.9247	0.9436	0.9989	0.98	8.06	5.58
2	F2	0.9997	0.9276	0.9576	0.9987	0.92	7.78	5.78
3	F3	0.9827	0.9382	0.9585	0.9986	0.91	7.37	6.10
4	F4	0.9957	0.9442	0.9694	0.9975	0.82	6.90	6.52
5	F5	0.9928	0.9347	0.9786	0.9918	0.74	6.73	6.68

The time required to get 50% drug release (T_{50}) and zero order release rate constants were calculated and reported in Table 5. The relevance of difference in the in-vitro dissolution rate profile was evaluated statistically. Statistical analysis by using One-way analysis of variance ($P<0.05$) proves that diltiazem hydrochloride microspheres prepared with various polymers and various cross linking agents have significant difference in dissolution rates of diltiazem hydrochloride.

The microspheres consisting of sodium alginate alone and in combination with HPMC and NaCMC exhibited good mucoadhesive properties as observed in *in vitro* wash-off test when compared to a non-mucoadhesive polymer, ethyl cellulose microspheres. The wash off was slow in the case of microspheres consisting of alginate-mucoadhesive polymers when compared to that of ethyl cellulose microspheres (Table 6). The wash-off was faster at simulated intestinal pH (7.4) than that at simulated gastric pH (1.2). The rapid wash-off observed at simulated intestinal pH may be due to the ionization of carboxyl acid group and other functional groups in the polymers, which increase their solubility and reduce adhesive strength. The results of the wash-off test indicated that the microspheres had fairly good mucoadhesive properties. The developed mucoadhesive microspheres would adhere to the GI walls, thus resisting gastric emptying. It would ensure the prolong residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability.

Table 6: Invitro wash-off test values for the diltiazem hydrochloride loaded various mucoadhesive microsphere formulations.

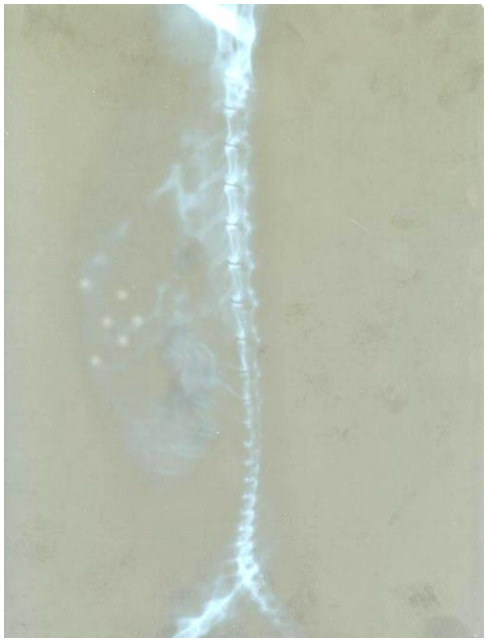
Formulation	Percentage of microspheres adhering to tissue at 4 times intervals (h)							
	0.1 N Hydrochloric acid				7.4 pH Buffer			
	2	4	6	8	2	4	6	8
F1	89±0.43	84±0.53	78±0.32	76±0.44	87±0.46	82±0.44	77±0.64	72±0.66
F2	92±0.32	86±0.42	82±0.44	79±0.56	90±0.68	85±0.72	81±0.72	75±0.44
F3	94±0.62	91±0.74	85±0.14	83±0.22	93±0.12	88±0.12	83±0.55	79±0.52
F4	96±0.52	93±0.26	87±0.32	85±0.14	95±0.42	92±0.68	86±0.74	82±0.24
F5	99±0.52	95±0.36	90±0.52	88±0.52	97±0.25	94±0.56	88±0.66	86±0.46
EC	14	0	0	0	-	-	-	-

GI Residence time associated with the administration of sodium alginate+HPMC mucoadhesive microspheres containing barium sulfate (free from drug) was determined with X-Ray photographs (figure 3). The X-Ray studies showed that the mucoadhesive microspheres formulated with sodium alginate+HPMC and calcium chloride remained in the gastric region even after 10 hours of administration indicating good retention period in the stomach region.

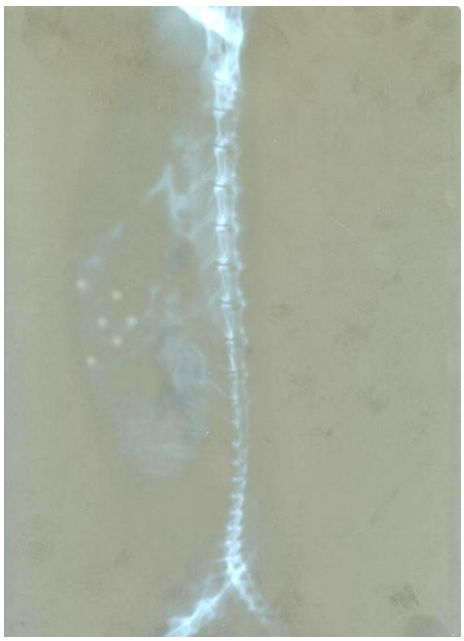
CONCLUSION

Diltiazem-loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique. Various formulation variables such as polymer concentration and cross-linking agents shown significant influence on the drug entrapment efficiency, size distribution, mean particle size, surface morphology, swelling behaviour and *in- vitro* drug

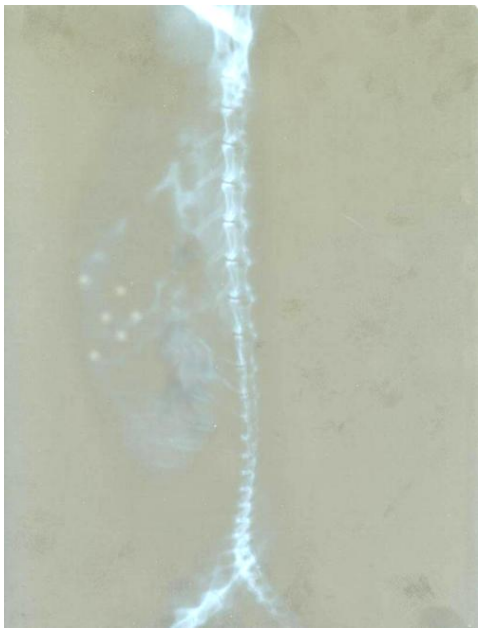
release. The mucoadhesive microspheres formulated with sodium alginate+HPMC and calcium chloride showed a satisfactory controlled release profile for 12 hours.



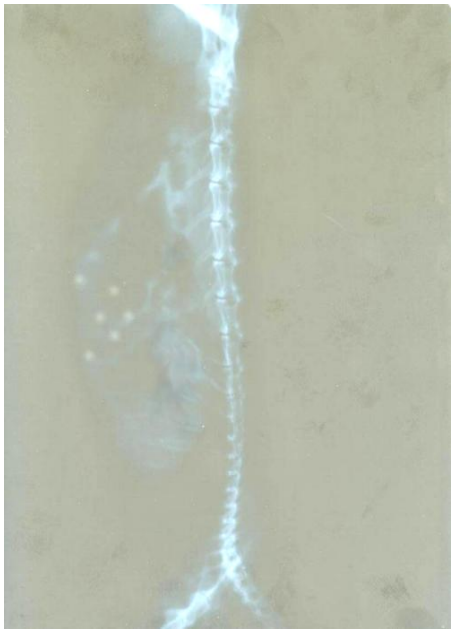
(a) 2nd hr



(b) 4th hr



(c) 8th hr



(d) 10th hr

Figure 3:X-ray photographs of mucoadhesive microspheres formulated with barium sulphate, sodium alginate+HPMC and calcium chloride

REFERENCE

1. Rose MR, Dadiv R. Drugs used for the treatment of myocardial ischemia. In, Joel,G H(ed),Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th edition. New York, McGraw-Hill medical Publishing Division; 2005:767-777.
2. Poncelet D, Lencki R, Beaulieu C, Halle JP, Neufled RJ, Fournier A. Production of alginate beads by emulsification internal gelation. Appl Microbiol Biotech 1992; 38(1): 39-45.
3. Das MK, Divya PM. Evaluation of diltiazem hydrochloride-loaded mucoadhesive microspheres prepared by emulsification-internal gelation technique Acta Poloniae Pharmaceutica - Drug Res 2008; 65(2):249-259.
4. Mann A. Design and evaluation of an oral controlled release microparticulate drug delivery system of Nimesulide treated alginate beads. J Sci Ind Res 1989; 58: 717-720.
5. Pornsak Sriamornsak, Ross A Kennedy. Development of polysaccharide coated pellets for oral administration: Swelling and release behaviour of calcium pectinate gel. AAPS Pharm Sci Tech, 2007; 8 (3):1-8.
6. Government of India. Ministry of health and family welfare. Indian Pharmacopoeia Vol. I & II. The Controller of Publication, New Delhi; 1996: 634.
7. Salomon CJ, Bravo SA, Lamas MA. In-Vitro Studies of Diclofenac Sodium Controlled-release from Biopolymeric Hydrophilic Matrices. J Pharm Pharma Sci 2002; 5(3): 213-218.
8. Chowdary KPR, Rao SY. Mucoadhesive microcapsules of Glipizide: Characterization, *in vitro* and *in vivo* evaluation. Indian J Pharm Sci 2003; 65 (3):279-284.
9. Mathiowitz E, Chickering D, Jacob JS, Santos C. In: Mathiowitz, E, Eds., Encyclopedia of Controlled Drug Delivery, Wiley, New York; 1999; 9-11.