Evaluation of Aceclofenac Loaded Agarose Beads Prepared by Ionotropic Gelation Method

Farhana Yesmin[1], Md. Mesbah Uddin Talukder[2], Muhammad Shahidul Islam[1], Susmita Laila[3], Tasnuva Haque[1]
Department of Pharmacy, Stamford University Bangladesh[1]
Dhaka-1217, Bangladesh.
Department of Pharmacy,The University of Asia Pacific[2],
Dhaka-1209, Bangladesh.
Department of Pharmaceutical Technology, Faculty of Pharmacy[3]
University of Dhaka, Dhaka-1000, Bangladesh.

Corresponding Author
Tasnuva Haque
Tel: 01712-130454
E-mail: Shoume_du@yahoo.com

ABSTRACT

Aceclofenac loaded agarose beads were prepared by ionotropic gelation method and drug release profile, swelling index (SI %) and entrapping efficiency (EE %) of aceclofenac were investigated. The drug was dissolved in melted 4 % agar solution in different ratios. Beads were prepared by dropping the hot aqueous solution into a beaker of different percentages of chilled $CaCl_2$ solution followed by filtering the solution and drying at room temperature. The entrapment efficiency was 100±5 %. The swelling index was found to be highest (18.22 % in 4 hours) for beads containing aceclofenac-agar (1:2) in 4 % electrolyte solutions and the swelling property was decreased with increasing electrolyte concentration. In vitro dissolution of beads was carried out in USP apparatus-II (paddle method) at 75 rpm. The drug release was measured by using UV-Spectrophotometer at λ_{max} of 268 nm for acid media and 274 nm for buffer media. The dissolution data were treated with zero order, first order and Higuchi model. Half of the formulations were fitted to Higuchi model and rest half to first order model. Finally it can be concluded that with the increasing polymer (agar) concentration, the release rate of aceclofenac was decreased and swelling index was increased and with the increasing electrolyte concentration, the release rate was increased and swelling index was decreased.

Key Words: Aceclofenac, Agarose bead, Electrolyte, Ionotropic Gelation method.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line drugs in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondilytis. Aceclofenac is one of the emerging NSAID molecules for arthritis treatment. It is a newer derivative of diclofenac and has less gastrointestinal complications (Parfitt et al., 1999; Kay et al., 2003 and). The successful treatment of arthritis depends on the maintenance of effective drug concentration level in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow rate over an extended period of time and achieve this object. Short biological half life (about 4 hour) and dosing frequency more than one per day make aceclofenac an ideal candidate for sustain release (Parfitt et al., 1999). Agar is prepared from various species of Gelidium and other red algae. It is an alternating copolymer of 3-linked ß-D galactopyranose and 4-linked 3-6 anhydro-a-L-galactopyranose. It is characterized by its gelling point (35 - 45°C for 1.5% gel). Agar is dissolved as a colloidal solution in water if heated to about 90°C and forms a solid gel upon cooling below its gelling point. Agar gelation is thermo-reversible that is the gel re-melts on heating to about 90°C. In the gel, the agar molecule form double helices which are linked together by hydrogen bonding in bundles to form network. In the bundles 10 to 50 double helical link in parallel aggregates, accounting for the large mesh size typical of agar gels (Waki et al., 1982). In gels of low agar concentration (1% w/v), drug diffusivity can be the same as in water. Oral sustained release agar beads of sulphamethizole were prepared by adding drug to hot gel solution before the beads were formed (Nakano et al., 1979).

There are a number of techniques applied for the formulation as well as in the manufacturing of sustained release dosage form (Nimmi et al., 2005). Purpose of preparation of agarose beads is to control drug delivery system, increasing surface area and to investigate the relationship between the swelling property and sustaining action of agar in the beads cross-linked with the divalent electrolyte ($CaCl_2$).

EXPERIMENTAL

Materials and Methods

Aceclofenac, agar and calcium chloride were from Chaina, Oxoide (Germany) and Merck (India) respectively. Tribasic sodium phosphate, 37% (w/v) HCl and methanol were from Merck (Germany). Among the instruments, electronic balance (sensitivity 0.001) was from Denver Instrument M-310 (Switzerland), pH meter was from Lida (Chaina), Dissolution apparatus was from Pharma Test (Germany) and HACH DR/4000U spectrophotometer was used which was from USA with 1 cm matched quartz cells.

Preparation of aceclofenac loaded Agarose beads

At first agar was weighed out (according to the formulation given in Table 1) and taken in a volumetric flask. 4% of agar solution was prepared by heating with the help of a burner. After complete melting of agar, aceclofenac was added and mixed properly by using a glass rod. When all the ingredients were mixed completely, a gel was formed, then it was taken in a syringe with nozzle of 2.5 mm outer diameter and extruded into a beaker filled with chilled electrolyte solution ($CaCl_2$) of different concentration (4%, 6%, 8%, and 10%) in a drop wise fashion with gentle agitation at room temperature. The distance from the nozzle to the $CaCl_2$ solution was about 5 cm. When gel was poured into the electrolyte solution from syringe as droplet, droplets become spherical as soon as it reaches to different concentration of calcium chloride solution; it became hard since the temperature of $CaCl_2$ solution was below the gelling point of agarose. Thus agarose beads were prepared. The beads formed were allowed to stand in the solution for 15 minutes and then beads were collected removing the electrolyte solution by filtration. Then beads were placed in open space for air-dry for overnight (using mainly fan). Finally aceclofenac loaded agarose beads were prepared. The beads had a diameter of 2 mm (Choudhury et al., 2005).

Table 1: Different formulations of aceclofenac loaded agarose beads.

Formulation	$CaCl_2$ Solution (%)	Aceclofenac (gm)	Agar (gm)	Aceclofenac:Agar
F-1		1	1	1:1
F-2	4	1	1.5	1:1.5
F-3		1	2	1:2
F-4		1	1	1:1
F-5	6	1	1.5	1:1.5
F-6		1	2	1:2
F-7		1	1	1:1
F-8	8	1	1.5	1:1.5
F-9		1	2	1:2
F-10		1	1	1:1
F-11	10	1	1.5	1:1.5
F-12		1	2	1:2

Evaluation of agar based aceclofenac beads

Determination of Entrapment Efficiency (EE %)

The entrapment of drug in the aceclofenac loaded agarose beads was quantitatively determined by immersing the dried beads (equivalent to 100 mg aceclofenac) in 250ml phosphate buffer pH 6.8 in order to dissolve the drug dispersed in the beads. After sonication, the solution was collected and the drug content entrapped inside the beads was determined by UV spectrophotometer at 274nm. The entrapment efficiency (EE) was calculated according to the following equation (Piyakulawat et al., 2007).

$$EE(\%) = \text{Actual drug content} \times 100\% / \text{Theoretical drug content} \dots\dots\dots (1)$$

Preparation of 0.1N HCl and pH 6.8 phosphate buffer

For preparing 0.1 N HCl, 8.3 ml of 37% (w/v) solution of HCl was taken in a volumetric flask and distilled water was added to make the volume up to 1000 ml. Thus, 1000 ml of 0.1N HCl was prepared (pH 1.2). Again for pH 6.8 phosphate buffer, 0.2 molar 114.036 gm tribasic sodium

phosphate was taken in a volumetric flask and distilled water was added to dissolve it and make the volume up to 1500ml. Now, in a beaker 750 ml of 0.1N HCl was taken and 225 ml of 0.2M tribasic sodium phosphate was added. Finally the pH was checked 6.8 using pH meter.

Swelling study

The extent of swelling was measured in terms of percent (%) weight gained by the beads. The swelling behavior of formulations F-1, F-2, F-3, F-4, F-5, F-6, F-7, F-8, F-9, F-10, F-11, F-12 of aceclofenac beads were studied. In this test, 20 mg beads from each formulation were kept in petri dishes containing pH 6.8 phosphate buffers. At the end of 1 hour, the beads were withdrawn, soaked with tissue paper and weighed. Then for every 1 hour, weights of the beads were noted, and the process was continued till the end of 8 hours. Percent weight gained by the beads was calculated by the following formula (Yeole *et al.*, 2006);

$$S.I = \{(M_t - M_o) / M_o\} \times 100 \ldots\ldots\ldots\ldots\ldots (2)$$

Where, S.I = swelling index,

M_t = weight of beads at time 't' and

M_o = weight of beads at time, t = 0.

In vitro release study

The aceclofenac release study of the beads from each formulation was performed in the simulated gastrointestinal condition by the pH chane method at 37°C (Sipahigil *et al.*, 2006). The media of pH 1.2 (0.1N HCl) was chosed to represent the gastric condition; pH 6.8 was a compromise condition between the pH of the gastric and small intestine. The dissolution process was carried out in USP apparatus-II (paddle method) at 75 rpm by taking beads equivalent to 100 mg aceclofenac in 750 ml of 0.1 N HCl media for first 2 hours, followed by 1000 ml pH 6.8 phosphate buffer for 8 hours. The dissolution media was changed to pH 6.8 phosphate buffer adding 225 ml 0.2M tribasic sodium phosphate and 25 ml distilled water in the 750 ml 0.1N HCl media. The total process was continued for 10 hour. The release rate of aceclofenac in acid media was very negligible, and the percent release in acid is not shown here.

Release drug data modeling

The suitability of several equations that are reported in the literature to identify the mechanisms for the release of aceclofenac was tested with respect to the release data. The data were evaluated according to the following equations:

Zero-order model (Donbrow *et al.,* 1980):

$$M_t = M_0 + K_0 t \ldots\ldots\ldots\ldots\ldots (3)$$

Higuchi model (Higuchi *et al.*, 1961):

$$M_t = M_0 + K_H t^{0:5} \ldots\ldots\ldots\ldots\ldots (4)$$

Where M_t is the amount of drug dissolved in time t, M_0 is the initial amount of drug, K_0 is the zero-order release constant and K_H is the Higuchi rate constant,.

First order model (Merchant *et al.*, 2006):

$$LogC = LogCo - kt/2.303 \ldots\ldots\ldots\ldots\ldots (5)$$

Where, C = cumulative percent of drug release, Co = the initial concentration of drug and k = first order rate constant.

RESULTS AND DISCUSSION

Entrapment efficiency of beads

From the entrapment measurement study, it was found that all of the formulations i.e. from F-1 to F-12 had the entrapment efficiency within the range i.e. 95-105% (Table 2).

Swelling behavior

Effect of polymer on Aceclofenac-agarose bead formulations:

The formulations were prepared using three different ratio of agar. F-1, F-4, F-7 and F-10 formulations contained 1gm agarose polymer. F-2, F-5, F-8 and F-11 contained 1.5 gm agarose polymer. F-3, F-6, F-9 and F-12 beads contained 2 gm agarose polymer. Among F-1 to F-3, F-3 showed highest swelling of 18.22% at 4 hours, among F-4 to F-6, F-6 showed 8.42% at 4 hours and F-9 and F-12 showed 5.95% at 5 hours, F-12 3.44% at 3 hours. So, from the result it was evident that with the increasing the polymer content the swelling index was also increased (Table 2 and Figure 1).

Table 2: Entrapment Efficiency (%) and Swelling Index (%) of different formulations of agarose beads.

Formulation	Mean EE (%)	Mean SI (%)
F-1	98.492	9.57% at 4 hours
F-2	102.999	12.2% at 4 hours
F-3	98.332	18.22% at 4 hours
F-4	95.274	9.45% at 3 hours
F-5	97.774	10.2% at 5 hours
F-6	98.734	8.42% at 4 hours
F-7	98.117	3.43% at 5 hours
F-8	99.941	4.99% at 5 hours
F-9	103.321	5.95% at 5 hours
F-10	102.194	3.9% at 4 hours
F-11	101.443	5.81 at 4 hours
F-12	98.364	3.44% at 5 hours

Effect of different concentration of electrolyte ($CaCl_2$) solution on swelling behavior of Aceclofenac-agarose bead formulations:

Swelling study of agarose based aceclofenac beads prepared in different electrolyte solution was also observed. F-1 to F-3 formulations were prepared using 4% $CaCl_2$ solution. F-4 to F-6 formulations were prepared using 6% $CaCl_2$ solution. F-7 to F-9 formulations were prepared using 8% $CaCl_2$ solution. F-10 to F-12 formulations were prepared using 10% solution. F-1, F-4, F-7 and F-10 having same drug-polymer ratio (1:1) but they were prepared in increasing percentage of $CaCl_2$ solution. F-1 swelled 9.57% at 4 hours, F-4 9.45% at 3 hours, F-7 3.43% at 3 hours and F-10 3.9% at 4 hours. It was indicated that with the increasing electrolyte solution concentration, swelling indices were decreasing (from 9.57% to 3.9%) at the same drug-polymer ratio (Figure 1).

Dissolution Study

Dissolution profile

The release rate of aceclofenac in first two hours in acid media was so negligible (less than 4%) that the result was not shown in the release curve (Figure 2). At this pH, aceclofenac exists in its acidic form which is well known to be practically insoluble in the stomach (Sheu et al., 1992 and Kincl et al., 2004). When the dissolution was changed to pH 6.8 phosphate buffer media, the drug release rate was slightly increased, possibly because the aceclofenac was partially converted to aceclofenac salt which is soluble. Among the formulations of F-1 to F-3 (containing aceclofenac-Agar 1:1, 1:1.5 and 1:2 ratios) prepared in 4% electrolyte solution, showed 80.51, 79.37 and 73.53% release of drug respectively at 8 hours. Again among F-4 to F-6, 99.02, 94.45 and 85.89% release were shown by them respectively at 8 hours. F-7, F-8, F-9, F-10, F-11 and F-12 released about 97.74, 96.19, 92.12, 97.49, 96.15 and 95.12% drug respectively at 8 hours. So, among the formulations prepared in same percent of electrolyte solution, the release rate was found to be decreased.

Table 3: Release kinetics of twelve formulations of agarose beads.

Formulation	Zero order		Higuchi		First order	
	K_0	R^2	K_H	R^2	K_1	R^2
F-1	12.428	0.559	31.714	0.925	0.189	0.881
F-2	11.139	0.833	27.002	0.992	0.159	0.742
F-3	12.079	0.434	31.890	0.911	0.184	0.980
F-4	14.522	0.682	36.730	0.963	0.512	0.870
F-5	14.585	0.567	37.193	0.929	0.343	0.974
F-6	12.500	0.750	31.458	0.982	0.231	0.976
F-7	15.380	0.431	39.527	0.867	0.429	0.951
F-8	15.074	0.440	38.717	0.871	0.369	0.937
F-9	14.192	0.553	36.220	0.922	0.296	0.954
F-10	14.423	0.648	36.555	0.949	0.415	0.929
F-11	14.403	0.628	36.574	0.949	0.371	0.960
F-12	14.109	0.651	35.773	0.957	0.342	0.956

Release kinetics

The values of kinetic constant (k) and correlation coefficient (R^2) calculated from equations 3 to 5 are presented in Table 3. As observed from the table, correlation coefficients (R^2) of all formulations were high enough to evaluate the drug dissolution behavior using equation 4 and 5 (R^2: 0.92- 0.99). Depending on the polymer concentration, aceclofenac loaded agarose bead formulations showed best fitting with first order and Higuchi release kinetics and no formulation was fitted with zero order model.

Drug release mechanism

According to Alderman et al., 1984, when the hydrophilic matrix system enters an *in vitro* dissolution medium, drug particles initially pass into solution from the surface (immediate release). The solid matrix also begins to swell (polymer relaxation) as soon as hydration with solvent molecules, diffusion of the dissolved drug and erosion of gelatinous viscous polymer layer into aggregates or granules and these in turn deaggregate into fine particles that also release their drug content by dissolution. Higuchi model describes the release of the drug from the matrix system through diffusion by pore formation. The release mechanism is also influenced by porosity and totuosity of the matrix (Florence *et a*l., 1988). According to the table-3, it is observed that F-1 and F-2 as well as F-5, F-7, F-8, F-9 and F-11 were best fitted with first order model indicating their release kinetics dependent on the concentration of drug in the depot. Again the release kinetics of F-1, F-2, F-4, F-6, F-10 and F-12 were found to be governed by Higuchi model that indicated their release mainly had been occurred by diffusion followed by pore formation.
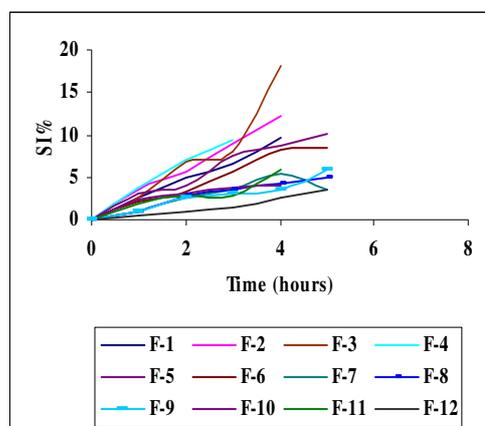

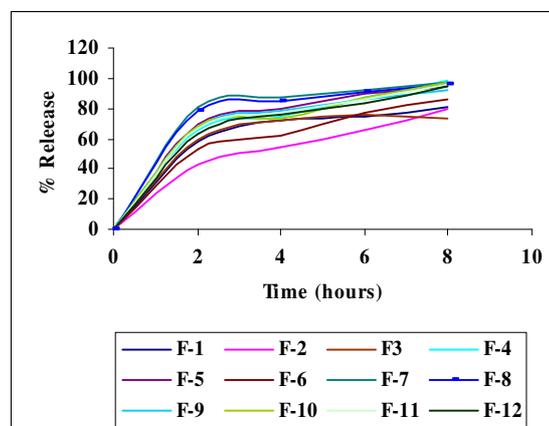**Figure-1: Plot of swelling indices (%) vs. time of twelve formulations of agarose beads.**

Figure-2: Zero order plot of twelve formulations of agarose beads.
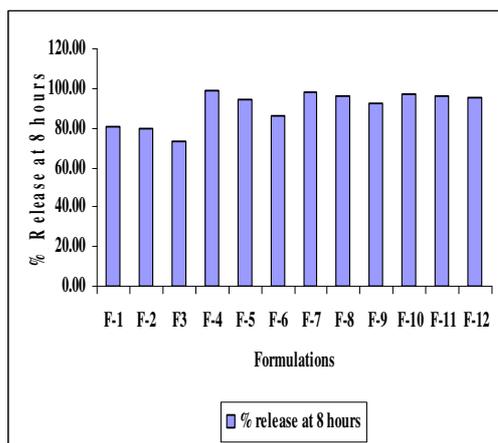
Figure 3: Effect of polymer on release rate of twelve formulations of agarose beads.
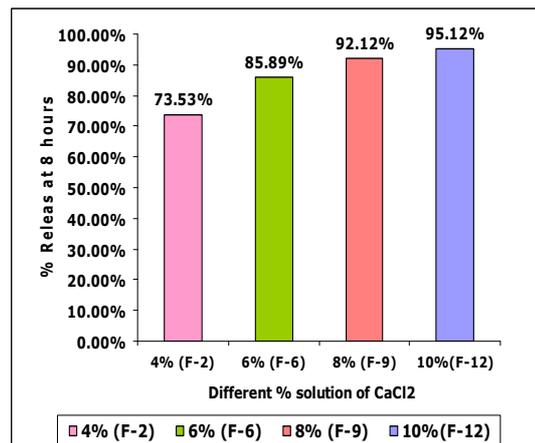
Figure 4: Effect of various percent of electrolyte on release profile of aceclofenac from agarose beads (drug-polymer in 1:2 ratio).

Effect of polymer on release rate of drug from aceclofenac loaded agarose beads

In this study the beads were prepared using different concentrations of polymer. Here agar was commonly used in all the twelve formulations with aceclofenac at ratio of 1:1, 1.5:1 and 2:1 as rate retarding agent. In those formulations the content of agar was increasing proportionally. When the bead surface was wetted by the buffer media the agarose started to partially hydrate, forming a gel layer. Initial burst release of soluble beads from the external surface was found. Buffer penetrated into the beads increasing the thickness of the viscous gel layer and soluble bead was released by diffusion from the gel layer and by exposure through beads erosion. The more concentration of the polymer, the more viscous the gel layer was formed and the less release of drug from the beads took place. At higher concentration (1: 2 drug- polymer ratio) of polymer the formulations F-3, F-6, F-9 and F-12 showed lower release rate than the others. So, it might be said that higher concentration of agarose had a higher retarding capacity (Figure 3).

Effect of Electrolyte (CaCl$_2$) on release rate of aceclofenac from agarose beads

In this study the beads were prepared using different concentrations of electrolyte (CaCl$_2$) solution. Here CaCl$_2$ was commonly used in all the twelve formulations of beads at the concentration of 4%, 6%, 8 % and 10%. Formulations were prepared in different CaCl$_2$ solution i.e. F-1, F-2, F-3 in 4%, F-4, F-5, F-6 in 6%, F-7, F-8, F-9 in 8% and F-10, F- 11, F-12 in 10% CaCl$_2$ solution. Among F-1 to F-3, the highest rate retarding effect was observed from F- 3 (73.53% at 8 hour). Among F-4 to F-6, the highest rate retarding effect was observed from F-6 (85.89% at 8 hour). In case of F-7 to F-9, the highest rate retarding effect was observed from F-9 (92.12% at 8 hour). In the same manner among F-10 to F-12, the highest rate retarding effect was observed from F-12 (95.12% at 8 hour). From the above discussion it was clear that at the same drug-polymer ratio (1:2), the % release from highest rate retarding formulations were increasing with the increase of % CaCl$_2$ solution (Figure 4).

CONCLUSION

Aceclofenac loaded agarose beads were prepared and evaluated to find out the entrapment efficiency, swelling index and release profile. In wet condition, the size of agar beads were measured and found bigger than those in dry condition. The entrapment efficiency was found to be satisfactory. After evaluating the results of swelling index and dissolution profile, both had shown similar result during changing the polymer and electrolyte concentration. The SI (%) was found to be increased and the release rate of aceclofenac was decreased with the increasing concentration of polymer (agar) but the SI (%) was found to be decreased and aceclofenac release rate was increased with the increasing concentration of electrolyte solution. So it can be concluded that it is possible to modify the release rate of aceclofenac from agarose beads by choosing appropriate drug- polymer ratio and suitable electrolyte concentration.

REFERENCES

Alderman DA. (1984) A review of cellulose ethers in hydrophilic matrices for oral controlled release dosage forms. *Int J Pharm Technol Prod Manuf.* 5 : 1-9.

Chudhury PK, Kar M. (2005) Preparation of alginate beads containing Metformin HCl using Emulsion-Gelation method. *Trop. J. Pharma. Res.* 4(2): 489-493.

Donbrow M, Samuelov Y. (1980) Zero order drug delivery from double-layered porous films: release rate profiles from ethylcellulose, hydroxypropylcellulose and polyethylene glycol mixtures. *J Pharm Pharmacol.* 32:463-470.

Florence AT, Attwood D. (1988) Physicochemical Principles of Pharmacy.2nd ed, pp. 323-324 Macmillan Press Ltd., London.

Higuchi T. (1961) Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm. Sci.* 50: 874-875.

Higuchi T. (1963) Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm. Sci.*52:1145 -1149.

Jacques CHM, Hopfenberg HB, Stannett V. (1974) Super case II transport of organic vapors in glassy polymers. In: Hopfenberger HB, ed. Permeability of Plastic Films and Coatings to Gases, Vapors, and Liquids. pp. 73-86 Plenum Press, New York.

Kay AE, Alldred A. ((2003) Rheumatoid Arthritis and Osteoarthritis, In Walker R., Edwards C., (ed.) Clinical Pharmacy and Therapeutics. 3rd ed, pp. 791- 807 Churchill Livingstone, London.

Kincl M, Vrečer F, Veber M. (2004) Characterization of factors affecting the release of low-solubility drug from prolonged release tablets. *Anal Chem Acta.* 502:107-113.

Korsmeyer RW, Gurny R, Doelker E M, Buri P, Peppas NA. (1983) Mechanism of solute release from porous hydrophilic polymers. *Int J Pharm.*15:25-35.

Merchant HA, Shoaib HM, Tazeen J, Yousuf RI. (2006) Once-Daily tablet formulation and in vitro release evaluation of Cefpodoxime using Hydroxypropyl Methylcellulose: A Technical note. *AAPS PharmSci Tech.* 7(3) Article 78.

Nimmi I, Chowdhury JA, Dulal MM, Reza MS. (2005) Effects of electrolytes on Diclofenac sodium from Agarose beads. *Dhaka Univ. J Pharm Sci.* 4 (2):117-120.

Parfitt K. (1999) Analgesics Anti-inflammatory and antipyretics, In: Reynolds JEF. ed. Martindale: The Complete Drug Reference. ed 32nd, pp. 2-12 Massachusetts.

Peppas NA. (1985) Analysis of Fickian and non-Fickian drug release from polymers. *Pharm Acta Helv.* 60:110-111.

Piyakulawat P, Praphairaksit N, Chantarasiri N, Muangsin N. (2007). Preparation and evaluation of Chitosan/Carrageenan beads for controlled release of Diclofenac Na. *AAPS PharmSci Tech.* 8(4) Article 97.

Sheu M, Chou H, Kao C, Liu C, Sokoloski TD. (1992) Dissolution of diclofenac sodium from matrix tablets. *Int J Pharm.* 85: 57-63.

Sipahigil O, Gursoy A, Cakalagaoglu F, Okar I. (2006) Release behavior and biocompatibility of drug-loaded pH sensitive particles. *Int J Pharm.* 311:130-138.

Waki S, Harvey JD, Bellarmy AR. (1982) Study of agarose gels by electron microscopy of freeze fractured surfaces. *Biopolymers.* 21:1909-1926.

Nakano M, Nakamura Y, Takikawa K, Kouketsu M, Arita T. (1979) Sustained release of sulphamethizole from agar beads. *J Pharm Pharmacol.* 31: 869-872.

Yeole PG, Galgatee UC, Babla IB, Nakhat PD. (2006) Design and evaluation of Xanthan gum based sustained release matrix tablets of Diclofenac Na. *Indian J Pharm Sci*. 68 (2): 185-189.