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Research Article

Design and Characterization of Diclofenac Sodium Microspheres Prepared by Ionotropic Gelation Technique for Oral Controlled Drug Delivery

Zien El-Deen E.E.*, Ghorab M.M, Shadeed Gad and Yassin H.A.

Pharm. Technology Dept., Faculty of Pharmacy, Tanta University*, Tanta, Egypt.

Pharmaceutics Dept., Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.

ABSTRACT

Dicolofenac sodium is a well-known representative of non-steroidal anti-inflammatory drugs (NSAID's) widely used to control pain and inflammation of rheumatic and non-rheumatic origin.Treatment with NSAIDs is observed to have gastrointestinal side effects. The formulation of Diclofenac sodium using biodegradable and biocompatible polymers in the form of microspheres is expected to decrease GI side effects. In the present study, different microsphere formulations of Diclofenac sodium were prepared by ionotropic gelation technique using sodium alginate as carrier and HPMC as release modifying agent. Microspheres of diclofenacsodium prepared in this study were evaluated for flow properties, drug entrapment efficiency as well as drug release from the various formulations proposed. Controlled release microspheres of diclofenac sodium were successfully prepared using Ionotropic Gelation Technique. The prepared formulations were found to control the release of the active substance for 12 hours when examined in phosphate buffer (pH 7.4). The obtained results proved the suitability of the prepared microspheres of Diclofenac sodium as controlled release dosage forms. The flow characteristic showed Hausner's ratios of <1.25 and Carr's index of 5-13 % of the systems prepared while those of the drug alone were >1.25 and > 40% respectively indicating good and excellent flow of the systems and extremely poor flowability of the drug alone. Diclofenac sodium content in different formulations was not affected by neither the polymer type nor drug to polymer ratio which was ranged between 79 -90 %. No significant drug-polymer interactions were observed in FT-IR studies. The surface morphology of drug-loaded microbeads prepared with sodium alginate and HPMC was spherical in shape and has large bridges observed on the outer surface .There was no significant degradation of diclofenac sodium or change in drug release rate in any of the prepared formulations during a six-month period of stability testing. The *in-vitro* release studies showed that the release rate of the drug has been modified. This study presents a new approach for obtaining modified release drug delivery system of diclofenac sodium.

Keywords: Microspheres, Diclofenac sodium, oral controlled drug delivery, design and charecterisation.

1. INTRODUCTION

In recent years a wide variety of newer drug delivery systems like oral controlled release dosage forms are designed and evaluated in order to overcome the limitations of conventional therapy. Diclofenac sodium is a good candidate for the development of oral controlled release formulations. It is used for long treatment of inflammation and pain, with a dose range of 50-75mg as conventional tab/ capsules. Adverse gastrointestinal reactions have been observed, and the short biological half-life (1-2 h) of the drug requires administration 2-3 times a day[1].

Microencapsulation has been employed to control the drug release, reduce or eliminate drug related adverse effects, dose-intake and improve the bioavailability in spite that the drug undergoes extensive first-pass metabolism ultimately improve the compliance in pharmacotherapy of inflammation and pain[2]. Microencapsulation by ionotropic gelation is one of

the widely used methods for preparation of calcium-alginate microspheres/beads which has the ability to form gels due to the reaction with calcium salts. Recently the use of calcium-alginate gel beads as a vehicle for controlled drug delivery system has attracted considerable attention because of their property of reswelling which is susceptible to environmental pH[3].

Consequently, acid sensitive drugs incorporated into beads would be protected from gastric juice[4]. Major characteristics of alginate beads are their fast disintegration in simulated intestinal fluid and high porosity, which result in rapid drug release[5].

Regarding ionotropic gelation technique, the preparation of drug containing microparticles is based on the principle of coalescence of colloidal polymer particles. Ionotropic gelation of the anionic polysaccharide sodium alginate with oppositely charged calcium ions forms microparticles. Subsequent curing step induces the fusion of colloidal polymer particles into the homogenous matrix. During the coating and drying process, the colloidal polymer particles coalesce and fuse into the homogenous film[6]. It is a cost effective technique that establishes intimate contact of the drug with the release retardant.

Alginate is a natural biopolymer which has been used widely due to its non-toxic, biodegradable and biocompatible nature. Alginic acid is a linear copolymer of p-o-mannuronic acid and u-L-guluronic acid linked by (1-4)-glycosidic bonds[7]. Alginate gelation takes place when divalent cations, interact ionically with blocks of guluronic acid residues, resulting in formation of three-dimensional network[8].

Sodium alginate is soluble in water and forms a reticulated structure which is cross-linked with divalent calcium chloride to form insoluble meshwork. Alginate's unique property of forming water insoluble calcium alginate gel through ionotropic gelation with calcium ions is a simple, mild and eco-friendly condition to encapsulate drugs. Another important property of alginate beads is their re-swelling ability. This property is sensitive to the environmental pH[6]. Alginate has a property of coating the drug core and also acts as a release rate retardant[9].

The above mentioned considerations led to the objective of this study, which was to prepare and evaluate oral controlled release product namely microbeads for diclofenac sodium by utilizing the ionotropic gelation method using sodium alginate alone and with HPMC as release rate modifiers to overcome the fast disintegration of alginate beads in simulated intestinal fluid. In the proposed method we dropped the mixture of drug and polymer dispersion into an aqueous calcium chloride, solution gelation occurs instantaneously resulting in the formation of spherical beads, with narrow particle size, high yield and optimum controlled release of the drug in various physiological GI conditions.

2. MATERIALS AND METHODS

2.1. Materials

Diclofenac sodium (Sigma-Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Amriya pharmaceuticals industries, Alexandria, Egypt. Hydroxypropylmethylcellulose (HPMC) and sodium alginate were purchased from RÖhm Pharma GMBH, Darmstadt (Germany). All other reagents were analytical or pharmaceutical grade and used as received.

2.2. Preparation of microbeads:

Microbeads of diclofenac sodium were prepared by ionotropic gelation technique. In this present work four sets of microbeads were prepared by using sodium alginate alone and combination with coating polymers like HPMC and calcium chloride used as counter ion.

The microbeads were prepared in an environment free from organic solvents by dropping a mixture of colloidal copolymer dispersion, the dispersed drug diclofenacsodium, formed mucilage of sodium alginate in calcium chloride solution, which acted as a counter ion. The droplets instantaneously formed gelled spherical beads due to cross- linking of calcium ion with the sodium ion which remain ionized in the solution[10].

Chemical reaction between sodium alginate and calcium chloride to form calcium alginate was utilized for the microencapsulation of diclofenac sodium core material. Preliminary work on the preparation of microbeads revealed that stirring speed and curing time greatly affected the size of microbeads[11]. Smaller particles can be prepared by adjusting stirring rate to 500rpm and curing time for 2h and also depending upon the height of the syringe from the level of counter ion solution, compressed force on the plunger of the syringe. The gelled particles were cured to get sufficiently hardened beads, filtered, washed and dried. The colloidal polymer particles fused into the polymer matrix during drying with the drug being dispersed in the latex. The detailed compositions of the various formulations are mentioned in Table (1).

2.2.1. Preparation of sodium alginate microbeads:

In the first set three batches of drug-loaded microbeads were prepared (F1, F2, and F3). A solution of sodium alginate was prepared in 100ml of

deionized water. In 50ml of sodium alginate solution, weighed quantity of diclofenac sodium was dispersed uniformly. Bubble free dispersion was dropped through a syringe with a needle into 100ml aqueous calcium chloride solution and stirred at 500rpm. After stirring for 30minutes, the gelled beads were separated by filtration, washed with distilled water and finely dried at 70°C for 6h in an oven[12,13].

2.2.2. Preparation of Alginate-HPMC microbeads:
In the second set two batches of drug loaded microbeads (F4 and F5) were prepared using sodium alginate and HPMC as coating polymers. To 50ml of deionized water, HPMC was added and stirred with an electric stirrer to form mucilage. Sodium alginate was added to form uniform dispersion. Weighed quantity of diclofenac sodium was added and homogenized for 5 min. The resulting dispersion was dropped through a syringe with a needle into 100ml of 5%w/v aqueous calcium chloride solution and stirred at 500rpm. After stirring for 30min the formed beads were separated by filtration, washed with distilled water, dried at 70°C for 6h in an oven[14].

3. Characterization and Evaluation of the prepared microbeads:

3.1. Infrared spectral analysis:
The IR spectrum was used to determine the interaction of the drug with the polymers used. The infrared spectra of samples were obtained using a spectrophotometer (FTIR, Jusco, Japan). Samples were mixed with potassium bromide (spectroscopic grade) and compressed into discs using hydraulic press before scanning from 4000 to 400 cm^{-1} [15,16].

3.2. Flowability testing:
The flowability of the prepared powders was tested by measuring their angle of repose. The calculations of Carr's compressibility index as well as Hausner's ratio were also tested. experiments are done in triplicate, the average ±S.D.

3.2.1. Measuring the angle of repose:
The fixed height cone method was adopted[17] where the diameter of the formed cone (d) was determined according to the following equation:

$$\text{Tan}\,\theta = \frac{2h}{d}$$

Where (h) and (d) are the height and the diameter of the cone respectively.

3.2.2. Determination of the initial and tapped bulk densities:
A fixed weight of the powder either drug or the prepared microbeads was poured in a 25 ml graduated cylinder, the powder was allowed to settle with no outer force and the volume occupied was measured as V_I (initial bulk volume).The cylindrical graduate was then tapped on a plan surface at a one inch distance till a constant volume was obtained . The tapped volume of the powder was then recorded as (V_T). The initial and tapped bulk densities were then calculated according to the following equation[18]:

Initial Bulk Density $\rho_I = M / V_I$

Tapped Bulk Density $\rho_I = M / V_T$

Where (M) is the mass of the powder.
The percentage compressibility (Carr's index) was then determined from the following equation[19]:

$$\text{Carr's index} = \frac{\rho_T - \rho_I}{\rho_T}$$

Finally the Hausner's ratio was obtained by dividing V_I by V_T[20].The experiments were carried in triplicate and the average angle of repose; Carr's index and Hausner's ratio of each of the prepared formulae were then calculated.

3.3. Scanning Electron Microscopy Analysis (SEM):
The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique. The particles were dried by vacuum, coated to 200 A^o thicknesses with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications[21].

3.4. Drug content determination:
Percentage yield can be determined by calculating the initial weight of raw materials and the finally obtained weight of granules. Percentage yield can be calculated by using the formula[22]:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoritical yield}} \times 100$$

An accurately weighed amount of the prepared microbeads was taken in a stoppered test tube and extracted with 5×10 ml quantities of phosphate buffer (pH 7.4).The extracts were filtered and collected into 100 ml volumetric flaskand made up to the volume with phosphate buffer (pH 7.4). The solutions were subsequently suitably diluted with pre warmed phosphate buffer (pH 7.4) and spectrophotometric absorbance was taken at 276 nm[23], (UV-Visible recording spectrophotometer,

SHIMADZU (UV-160A) (Japan).Percentage drug entrapment and the percentage entrapment efficiency (PEE) were calculated by the formula given below[24-26].

$$PEE = \frac{\text{Drug loading in the prepared microbeads}}{\text{Theoritical drug loading}} \times 100$$

3.5. *In-vitro* drug release studies:

The USPdissolution test apparatus employing paddle type(Paddle type , Copley , England) was used to measure the dissolution rate of diclofenac sodium from microbeads delivery system at 37°C± 0.2. The dissolution of the drug was conducted in two media pH 1 (0.1 N HCL) as well as pH 7.4 (Phosphate buffer) represent stomach and intestinal conditions respectively.

At predetermined time intervals(up to 12 hours),aliquots (5ml) were withdrawn, filtered through 0.45 membrane filter and replaced with equal volumes of pre warmed fresh medium to maintain constant volume and keep sink condition. After appropriate dilution, the sample solution was analyzed for diclofenac sodium by UV absorbance method at 276 nm[27].

3.6. Stability study:

A stability test was conducted by storing the prepared formulation in hard gelatin capsules at ambient temperature, 31, 37, 43°C (the relative humidity was controlled at 75%, except at ambient temperature). The content of diclofenac sodium and the dissolution of drug from the prepared formulations were tested monthly for six months. The dissolution study of the tested formulations followed the same procedures as previously described.

4. RESULTS AND DISCUSSION

4.1. Infrared spectral analysis:

The IR spectra are shown in Figure 1. The IR spectrum of pure drug (diclofenac sodium) (Fig.1a) shows a characteristic peaks at 3386 cm^{-1}due to N-H stretching frequency of secondary amine. The absorption bands at 1305 and 1282 cm^{-1}resulted from C-N stretching and the peaks at 1556 and 1574 cm^{-1}due to C=C stretching and C=O stretching of carboxylate group, respectively. The C-Cl stretching characteristic peak was observed at 746 cm^{-1}. The IR spectrum of diclofenac sodium with sodium alginate microbeads (Fig.1b) and diclofenac sodium sodiumalginate-HPMC microbeads (Fig.1c) shows all the principal characteristic peaks related to diclofenac sodium without any change in their position, indicating no possibility of chemical interaction between the drug and formulation ingredients**.**

4.2. Flowablity testing:

Table (2) shows the results of the flowablity testing of diclofenac sodiumas well as the prepared formulations, it is clear that, the rheological parameters like angle of repose and bulk density of all the proposed microbeads confirms better flow and packing properties. All the prepared formulations showed excellent flowability, as represented in terms of angle of repose (<40°)[17]. The batches prepared with the coating polymers show good flowability due to formation of smooth layer on the surface of the microbeads. Bulk and tapped density of the microbeads showed good and acceptable ranges and found to have higher packability. The improvements of micromeritic properties suggest that the prepared micobeads can be easily handled.

Hausner's ratio of the drug alone was found to be 1.86which indicates a poor flow property of the free drug. The F1 formula showed 1.14, while F2 formula revealed 1.08. Also,F3 formula showed 1.12.The F4 formula revealed 1.09,while F5 formula revealed 1.04indicating a good flow[20].

Carr's index for the drug alone was found to be 50.21% indicating extremely poor flow of the free drug. The F1 formula showed an index of12.23%, whileF2 formula showed an index of 11.34%. On the other hand, F3 formula showed an index of12.76%.F4 formula showed an index of 13.11%,while F5 formula showed an index of 13.56%indicating excellent and good flow[19].

4.3. Scanning Electron Microscopy Analysis (SEM):

The physical parameters like shape, surface morphology and also particle size were analyzed by scanning electron microscopy (SEM).Scanning electron micrographs of drug loaded microbeads prepared by sodium alginate (F2) and with the coating polymer (F5) are shown in Figure 2(a and b). The surface of the sodium alginate microbeads were discrete and spherical in shape with a rough outer surface and have a sandy appearance because of the surface-associated crystals of drug. Moreover, higher concentration of drug uniformly dispersed at the molecular level in the alginate matrices (Figure 2a). The surface morphology of drug-loaded microbeads prepared with sodium alginate and HPMC was spherical in shape and has large bridges observed on the outer surface (Fig 2b).**(a)**

4.4. Drug Entrapment Efficiency (DEE):

The drug entrapment efficiency increased progressively with increasing sodium alginate concentration (Table 3), This will result in the formation of larger microbeads entrapping greater

amounts of the drug. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and, consequently, the greater degree of cross-linking as the amount of sodium alginate increased. Since the solubility of diclofenac sodium was slightly higher in calcium chloride than in distilled water, prolonged exposure caused greater loss of drug in the curing medium. Increase in alginate concentration reduced loss of drug in the curing medium due to the formation of dense matrix structure. On other hand drug entrapment efficiency was significantly increased with the increase in the concentration of coating polymers (F4 and F5).The adhering property of the coating polymers played an important role in reducing the loss of drug in the curing medium.

4.5. *In-vitro* drug release studies:

Since Diclofenac sodium has a pka value of 4, it is practically insoluble in acidic medium; there was no observable release of Diclofenac sodium in 0.1 N HCl.

Diclofenac sodium release from the prepared microbeads was studied in acidic buffer (pH 1.0) for initial 2h and phosphate buffer (pH 7.4) for a period of 12 h. The release of diclofenac sodium from microbeads showed more controlled behavior with the increase in sodium alginate concentration.

At acidic pH the alginate beads shrink due to tightening of the gel network, resulting in decreasing drug release from microbeads. The polymer is eroded at alkaline pH and the contents of microbeads are released in a controlled manner by both diffusion and slow erosion of polymer matrix. Based on these suggestions in-vitro release kinetic data proved that the drug release was very slow in acidic medium (pH 1.0) and subsequently increases at higher pH levels. The maximum drug release of about 94.12%- 97.9 %w/w (F1- F3) was observed (Figure 3), due to the fact that alginate beads showfast disintegration in simulated intestinal fluid(pH7.4) and the observed high porosity results in rapid drug release. Whereas more controlled drug release polymers were observed 85.22-89.30 %w/w (F4, F5).The release profile of diclofenac sodium from the proposed formulations is shown in Figure(3)

Table 1
Composition of the prepared formulations

Formulation code	Diclofenac sodium (mg)	Sodium alginate % (w/v)	Calcium Chloride% (w/v)	HPMC% (w/v)
F1	50	2	5	-
F2	50	3	5	-
F3	50	4	5	-
F4	50	3	5	0.5
F5	50	3	5	1

Table 2
Flowability test of diclofenac sodium and its modified release formulations

Parameter	Pure drug	F1	F2	F3	F4	F5
V_I (ml)	3.4	1.77	1.56	1.08	0.73	0.52
V_T (ml)	1.53	1.51	1.42	0.96	0.53	0.41
P_I(gm.cm^{-3})	0.22±0.46	0.61±0.22	0.70±0.64	0.80± 0.78	0.75±0.13	0.77±0.56
P_T(gm.cm^{-3})	0.41±0.81	0.70±0.55	0.76±0. 32	0.90±0. 32	0.82±0.98	0.84±0.32
P_T / P_I	1.86	1.14	1.08	1.12	1.09	1.04
Carr's index (%)	50.21	12.23	11.34	12.76	13.11	13.56
Angle of repose	55±0.43	30.25±0.46	29.67±0.34	27.75±0.87	25.04±0.11	22.45±0.76

Where,V_I: Initial bulk volume, V_T: Tapped bulk volume, P_I:Initial bulk density, P_T: Tapped bulk density, P_T / P_I:Hausner ratio, each formulation containing 50mg of diclofenac sodium. Results show are the± SD, n=3 for Angle of repose, Bulk density and Tapped density.

Table 3
Drug Entrapment Efficiency in different formulations in phosphate buffer (pH7.4)

Formula	DEE
F1	79.30±1.20
F2	82.13±1.25
F3	84.22±0.88
F4	86.91±0.96
F5	90.22±0.43

Table 4
Stability of diclofenac sodium in different prepared formulations

Time (months)	% Drug Remaining				
	F1	F2	F3	F4	F5
1	98.18±1.78	99.44±1.29	98..33±1.39	99.45±1.43	99.18±0.34
3	97.88±0.43	98.32±1.03	97.23±0.67	98.12±0.33	97.98±0.23
6	9698±1.34	97.76±0.11	97.11±0.17	96.89±0.77	96.65±0.23

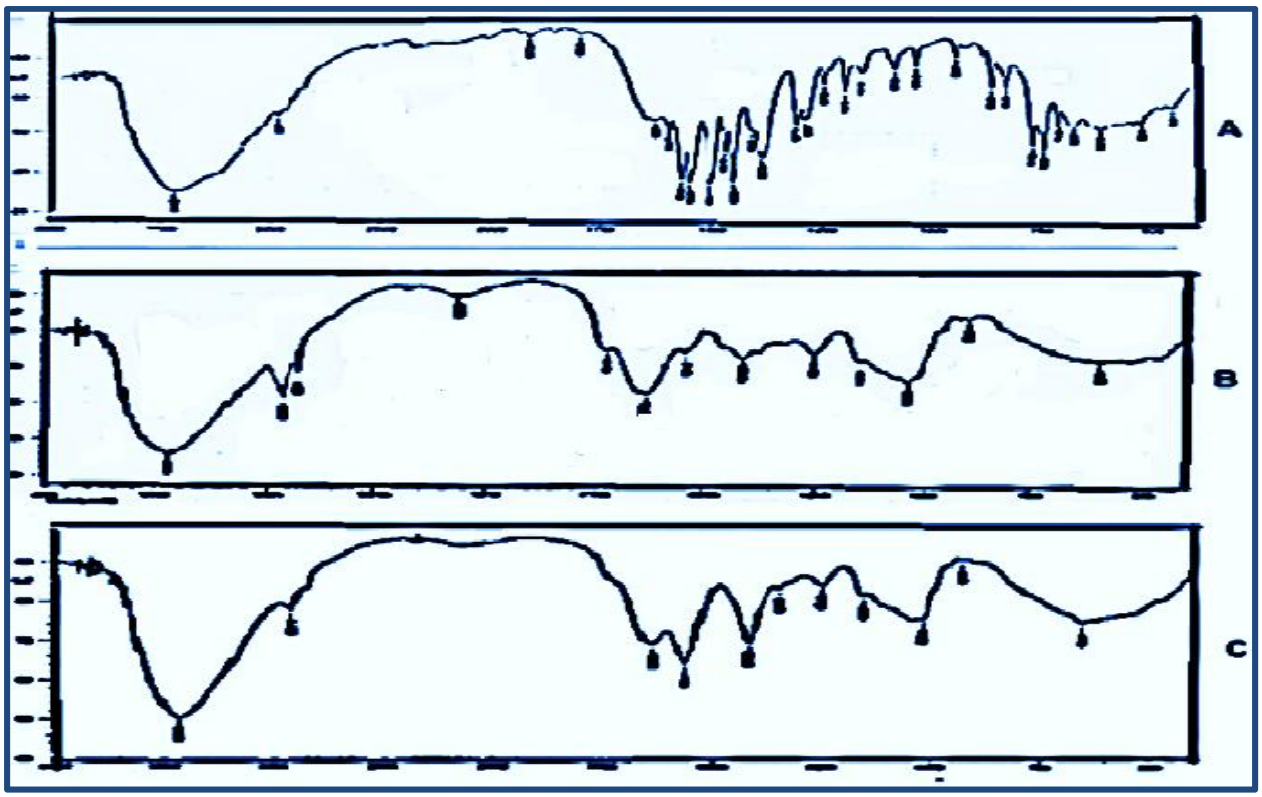
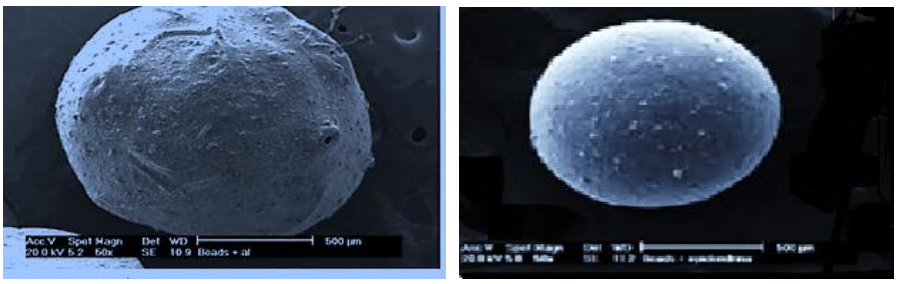


Figure 1
IR spectra of pure drug diclofenac sodium (a), diclofenac sodium with sodium alginate microbeads (b) and diclofenac sodium sodium alginate-HPMC microbeads (c).



(a) (b)

Figure2
Scanning Electron Micrographs of diclofenac sodium microbeads 2(a), 2(b) for formulations F2 and F5

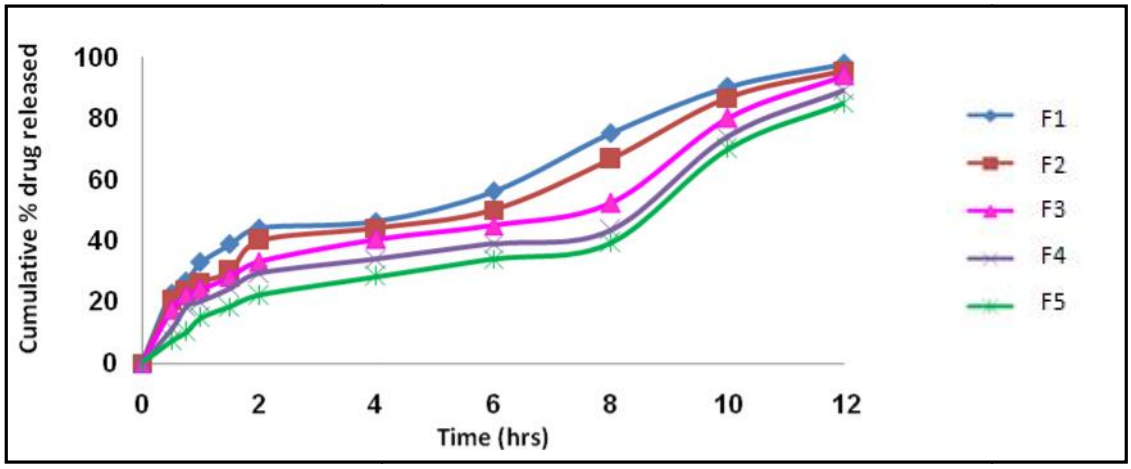


Figure 3
***In vitro* drug release profile of the prepared formulations**

4.6. Stability test:
Three batches of each formulation were prepared and the dissolution rate of the drug was evaluated under the same conditions. The release profiles of Diclofenac sodium from three different batches were constructed. The tested batches showed no significant difference regarding the release profile for each set of the three batches, indicating that the proposed manufacturing process is reliable and reproducible. Table (4) shows stability results of Diclofenac sodium in the different prepared formulations showing the percentage remaining in each formulation. Overall, results from the stability studies indicated that the proposed formulations were physically and chemically stable for more than 6 months.

5. CONCLUSION
Controlled release formulations of Diclofenac sodium were successfully prepared using sodium alginate in combination with HPMC by ionotropic gelation technique. The *in vitro* release data showed controlled release of the formulation up to 12 hours. The microspheres were prepared without the use of organic solvents. FT-IR studies did not reveal any significant drug interactions. Diclofenac sodium release from microbeads formulated with sodium alginate with HPMC as coating polymers showed a satisfactory controlled release profile. There was no significant degradation of diclofenac sodium or change in drug release rate in any of the proposed formulations during a six-month period of stability testing. Diclofenac sodium content in different formulations wasn't affected by neither the polymer type nor drug to polymer ratio. Therefore, one can assume that the diclofenac sodium microbeads are promising pharmaceutical dosage forms by providing controlled release drug delivery systems and avoiding the dose related side effects in the entire physiological region. The entire process is feasible in an industrial scale and demands pilot study.

6. REFERENCES

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