Formulation And Evaluation of Diclophenac HPMC Gelatin Microspheres

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Abstract— Diclofenac sodium is a well-known representative of non-steroidal anti-inflammatory drugs (NSAIDs), widely used to suppress pain and inflammation of rheumatic and non-rheumatic origin. NSAID treatment has been observed to have gastrointestinal side effects. Formulation of diclofenac sodium using biodegradable and biocompatible microsphere polymers is expected to reduce GI side effects. In this study, various microsphere formulations of diclofenac sodium were prepared by ionotropic gelation technique using sodium alginate as a carrier and HPMC as a release modifying agent. The diclofenac sodium microspheres prepared in this study were evaluated for flow properties, drug entrapment efficiency and also drug release from various designed formulations. Controlled release diclofenac sodium microspheres were successfully prepared using the ionotropic gelation technique. The prepared formulations were found to control the release of the active substance for 12 hours when tested in phosphate buffer (pH 7.4). The obtained results proved the suitability of the prepared diclofenac sodium microspheres as controlled-release dosage forms. Flow characterization showed Hausner ratio < 1.25 and Carr index 5-13% of the prepared systems, while drug alone ratios were > 1.25 and > 40%, respectively, indicating good and excellent flow of the systems and extremely poor fluidity of the drug. alone. The content of diclofenac sodium in the different formulations was not affected by the type of polymer, nor by the ratio of drug to polymer, which ranged between 79-90%. The surface morphology of the drug-loaded microspheres prepared with sodium alginate and HPMC was spherical in shape and large bridges were observed on the outer surface. During the six-month stability testing period, there was no significant degradation of diclofenac sodium or change in drug release rate in any of the prepared formulations. In vitro release studies showed that the rate of drug release was modified. This study presents a novel approach to obtain a modified release drug delivery system for diclofenac sodium.

***Index Terms*—HPMC, Controlled Release, Biodegradable, Biocompatible, Microsphere.**

1. Introduction

The correct delivery of the drug in the human body has a significant effect on the overall bioavailability of the formulation [1, 2]. In this direction, the multi-articular drug delivery system plays an important role to improve target specificity, stability and predetermined controlled drug release [3]. Carrier technology has recently been actively used in pharmaceutical sciences, viz. microspheres, nanoparticles, liposomes. Among all carrier microspheres, they are more stable and versatile to carry many hydrophobic drugs [4]. The unique characteristic of microspheres is essentially a fluidity in the range of 1-1000 µm containing synthetic polymers and proteins [5]. These types of unique dosage forms have an advantage in patient compliance with reduced toxicity. In microspheres, drug molecules are trapped in an encapsulated matrix of hydrophilic substances [6]. Materials such as polymer wax, protective materials are used. The drug release mechanism for microspheres is dissolution diffusion [7]. At the maximum time, the formulations are in matrix or encapsulated form. There are many methods by which microspheres can be prepared, i.e. coacervation, phase separation, interfacial polymerization [8]. Coating materials are very indispensable when adjusting the thickness of microspheres or microcapsules (1-20 µm) [9]. In this experiment we considered gelation; natural polymer, such as our polymerization agent for the preparation of microcapsules or microspheres by the coacervation method [10]. In preparation, we consider diclofenac sodium as a model drug. Diclofenac is a non-steroidal anti-inflammatory drug that is used to treat inflammatory disease See. gout, joint stiffness, arthritis and swelling. The greatest advantage of diclofenac microspheres is that it can reduce cytotoxicity and organ toxicity due to the lower encapsulation of the drug (1 mg/10 mL) in controlled release microspheres.

2. Methods

A. Preparation of Diclofenac Gelatin Microspheres

Accurately weighed gelatin was dissolved in 60 ml of water to form 0.25%, 0.50% and 0.75% w/v. solution. Next, an appropriate amount of the drug was incorporated into three different gelatin solutions. The drug solution was stirred for 10 minutes at 300 rpm. For a better emulsification process, 2 drops of castor oil were added to three different gelatin solutions during mixing, unless and until castor oil was added, the leachability would not be good for the suspension, so possible phase separation could occur. Stirring was continued for 45 minutes for proper infusibility to produce a clear solution.

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B. Evaluation of Mucoadhesive Microspheres

1) Percentage yield:

The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components that were used to prepare the microspheres.

% Yield = (Actual weight of microspheres / Total weight of drug and polymer) x 100

2) Particle size determination:

An optical microscope was used to determine the size of the microspheres. This method involves calibrating the eyepiece micrometer for which the bench micrometer is used. In the micrometer scale, one mm is divided into 100 equal divisions, and therefore each division is equal to 10 mm, and the particles are measured by a chosen solid line through the center of the particle. The average mean was calculated using the following formula.

Average diameter = Σnd/n×C.F. Where n = number of microspheres, d = diameter of microspheres, C.F = calibration factor

3) Shape and surface morphology:

The morphology of the microspheres was examined using optical Leica microscopy. Photographs of optimized formulation taken by Leica microscope. Leica microscope results showed that the microspheres of diclofenac sodium using HPMC15000cps combination with carbopol934p as polymer (F5) were spherical and their surface was smooth and without cracks which gave them a good appearance.

4) Drug Entrapment efficiency (DEE %):

The drug entrapment efficiency of diclofenac sodium microspheres was determined by taking accurately weighed 100 mg microspheres into a glass trivet and crushed with a glass pestle and treated with 100 mL phosphate buffer pH 7.4 in a sealed volumetric flask and left overnight. It was then transferred to a 250 mL beaker and stirred with a magnetic stirrer using Teflon-coated magnetic beads, the temperature being maintained at 37 °C ±0.5 °C. At the end of 1 hour, it was centrifuged and the supernatant was filtered, the filtrate was analyzed spectrophotometrically at 284 nm (Jasco-V-530). Dilutions were made whenever needed using phosphate buffer pH 7.4. The corresponding drug concentrations in the samples were calculated from the calibration graph. The entrapment efficiency of the microspheres was calculated using the formula.

DEE % = (Practical Drug Loading / Theoretical Drug Loading) X 100

5) Bulk Density:

An accurately weighed sample of microspheres was carefully placed into a 10 ml graduated cylinder using a funnel. Usually, the initial volume was recorded. Carefully align the microspheres without compaction if necessary and read the unsettled apparent volume V0 to the nearest divided unit. Calculate the density vg/cm3 according to the formula.

Df = M / V0

Where Df is bulk density, M is weight of samples in grams and V0 is volumes of sample in cm3.

6) Tapped Density:

The compaction density was obtained by dividing the weight of the powder by the compaction volume in cm3. A sample of microspheres is carefully placed in a 10 ml graduated cylinder. The cylinder was dropped at 2-second intervals onto a hardwood surface 100 times from a height of 1 inch. The shaken density of each preparation was then obtained by dividing the weight of the sample in grams by the final shaken volume of the sample contained in the cylinder in cm3. It was calculated using the equation below

$$Df = \frac{M}{V0}$$

Where Df is bulk density, M is weight of samples in grams and V0 is volumes of sample in cm3.

7) Carr's Index:

The compressibility index and hausner ratio are determined by measuring both the bulk volume and the shock volume of the microspheres. The compressibility percentage of the microspheres was calculated according to the equation below.

% Compressibility Index=(Vr-Vo)/Vf x100

Where V0is bulk density and Vfis Tapped density.

8) Hausner ratio:

The Hausner microsphere ratio was calculated according to the equation below (USP NF 2007).

Hausner ratio: =Vf/Vo

Where V0 is bulk density and Vf is Tapped density.

9) Swelling index:

Pre-weighed diclofenac sodium microspheres (W0) formulated with HPMC and Carbopol using different shell: core ratios were placed in phosphate buffer pH 7.4 maintained at 37 °C ± 0.5 °C. After 6 hours, the microcapsules were collected and blotted to remove excess water and weighed (weight). The swelling index was calculated according to the following formula.

Swelling index =(Wt-Wo)/Wo x100

Where Wt = weight of microspheres observed at 6th hour; W0 = initial weight of microspheres.

10) Mucoadhesion test:

The mucoadhesive properties of the microspheres were evaluated by an in vitro adhesion testing method known as the washout method. Freshly excised pieces of goat intestinal mucosa (2 x 2 cm2) were attached to the glass slide with cotton thread. About 50 microspheres were spread on each prepared glass slide, and the slides were immediately suspended on a USP tablet disintegration tester. When the test apparatus has been operating, the sample is subjected to deceleration up and down in the test fluid at 370 °C ± 0.5 0C contained in the 1 liter vessel of the apparatus. At an interval of 1 hour to 6 hours, the machine is stopped and the number of microspheres still adhering to the mucosal surface is counted. The test was performed in intestinal conditions (Phosphate buffer pH 7.4).

$$\% \text{ Mucoadhesion} = \frac{Total\ no.of microspheres remains}{Total\ no.of applied microspheres} X\ 100$$

Table 1
Formulation of the microspheres prepared

Formulation Code	Diclofenac sodium (mg)	HPMC15cps +Carbopol934p +Gelatin(ratio)	HPMC15000cps +Carbopol934p +Gelatin(ratio)	Liquid paraffin(ml)	Span-80 (0.5%)
F1	50	1 :1:1	-	100	0.25
F2	50	1:2:1	-	100	0.25
F3	50	1:3:1	-	100	0.25
F4	50	1:4:1	-	100	0.25
F5	50	-	1:1:1	100	0.25
F6	50	-	1:2:1	100	0.25
F7	50	-	1:3:1	100	0.25
F8	50	-	1:4:1	100	0.25

Table 2
Percentage yield, Mean particle Size, Shape and drug entrapment efficiency of Different Batches of Mucoadhesive microspheres of Diclofenac sodium.

S.No.	**Formulation code**	**Yield (%)**	**Mean particle size (µm)**	**Shape**	**%Drug entrapment efficiency**
1	F1	86.16	321.12	Oval	70.14
2	F2	81.29	340.10	Oval	63.12
3	F3	80.18	424.14	Irregular	61.28
5	F5	88.83	251.26	Round	76.07
4	F4	78.37	450.46	Irregular	54.14
6	F6	86.82	362.86	Oval, round	62.21
7	F7	87.63	434.78	Oval	63.42
8	F8	84.59	525.56	Irregular	60.28

Table: 3
Bulk density of Different Batches of Mucoadhesive microspheres

S.No.	**Formulation Code**	**Bulk density (gm/cm^3)**	**Tapped density (gm/cm^3)**
1	F1	0.265	0.382
2	F2	0.244	0.363
3	F3	0.309	0.323
4	F4	0.311	0.372
5	F5	0.245	0.326
6	F6	0.367	0.385
7	F7	0.347	0.412
8	F8	0.324	0.434

Table 4
Percent compressibility, Hausner ration, Sweeling index and percent mucoadhesion of different Batches of Mucoadhesive microspheres

S. No.	Formulation code	Compressibility index (%)	Hausner ratio	Swelling Index after 6th hours (%)	Mucoadhesion after 6th hours (%)
1	F1	12.97	1.15	68%	49
2	F2	13.53	1.14	84%	52
3	F3	14.67	1.17	88%	54
4	F4	15.14	1.16	92%	60
5	F5	10.49	1.11	70%	71
6	F6	14.12	1.16	83%	73
7	F7	16.30	1.18	89%	72
8	F8	16.74	1.20	94%	76

11) In-vitro drug release study:

Microspheres equivalent to 20 mg of the drug Diclofenac sodium were filled into hard gelatin capsules (No. 4) and coated with 1% w/v. with a solution of cellulose acetate phthalate (CAP) by the dip coating method. Coating was performed by immersing the filled capsules three times in the CAP solution and air drying after each coating step sequentially. In vitro drug release on size 4 capsules was performed using a USP 2 apparatus at 50 rpm, in 900 mL medium at 37°C ± 0.5°C with a wire dipper. For enteric capsules, 2 hours exposure in 0.1 N hydrochloric acid (pH 1.2) followed by testing in phosphate buffer pH 7.4 for 10 hours. After an appropriate time, interval, an appropriate volume of sample was withdrawn and an equal volume of fresh medium was replaced to maintain a constant total volume. Samples were filtered using a 0.45 µm filter. Diclofenac sodium concentrations were determined by UV spectrophotometry (Jasco-V-530) at a wavelength of 284 nm.

3. Result and Discussion

A. Percentage yield:

The percentage yield of the different formulations was determined by weighing the microspheres after drying. It was observed that as the proportion of polymer in the formulation increases, the yield of the product decreases slightly. The probable reasons may be the high viscosity of the solution, the adhesion of the polymer solution to the beaker wall, and the magnetic beads, which ultimately reduced the microsphere production yields.

The maximum practical yield of around 92.83% was obtained with F5 batches. Very less practical yield was obtained from other batches such as Fl, F2, F3, F4, F6, F7 and F8.

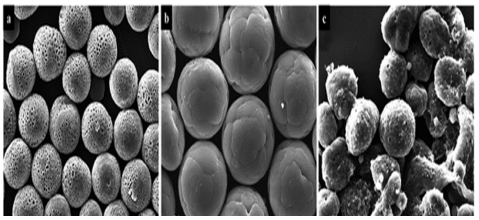
B. Particle size analysis:

Particle size was determined by optical microscopy. It plays an important role in mucoadhesive ability and drug release from microspheres. The mean size increased with increasing polymer concentration, which is due to a significant increase in viscosity, leading to an increase in droplet size and finally a large size of microspheres.

A uniform average particle size was obtained for formulation F5. Microspheres obtained using batch F5 showed a uniform average particle size of 253.26 µm. The average particle size of the mucoadhesive microspheres was in the range of 253.26 – 518.56 µm.

C. Shape and surface morphology:

The morphology of the microspheres was examined using Leica optical microscopy. Leica microscope photographs of the optimized formulations are shown in Figure 6 and Figure 7. The Leica microscope results revealed that the diclofenac sodium microspheres using the combination of HPMC15000cps with carbopol934p as polymer (F5) were spherical and their surface was smooth and crack-free, giving them a good appearance.



D. Drug Entrapment efficiency (DEE %):

The drug entrapment efficiency decreases slightly with increasing Carbopol content and decreasing the ratio of HPMC in the microspheres. This is due to the permeation characteristics of Carbopol, which could facilitate the diffusion of a part of the entrapped drug into the surrounding medium during the preparation of the microspheres. The % drug entrapment efficiency of various formulations was found to be in the range of 58.14 - 80.07% w/w.

E. Bulk Density:

The bulk density of each formulation was determined by dividing the sample weight in grams by the final volume of the sample in cm3 contained in a 10 mL graduated cylinder. The bulk density value of mucoadhesive microspheres ranges from 0.277 to 0.357 gm/cm3. Usually, bulk density is of great importance when considering the size of a high-dose capsule product or the homogeneity of a low-dose formulation in which there are large differences in the density of drug and excipient. Knowing the expected dose and density of the shaken

formulation, it is possible to determine the appropriate size of the capsule formulation. For free-flowing microspheres, such an interaction is generally less significant and the bulk and shock densities will be closer in value. For poorly flowing materials, there are greater interparticle interactions and greater bulk and shock density will be observed. These differences are reflected in the compressibility index and Hausner ratio.

F. Tapped density:

The shaking density was determined by the shaking method. The value of the shaken density of various mucoadhesive microspheres ranges from 0.322 to 0.434 gm/cm 3.

G. Percentage Compressibility index and hausner ratio:

The compressibility index and Hausner ratio are a measure of the porosity of the microspheres to be compressed as such are a measure of the relative interparticle interaction. For free-flowing microspheres, such an interaction is generally less significant and the bulk and shock densities will be closer in value. For poorly flowing materials, there are greater interparticle interactions and greater bulk and shock density will be observed. These differences are reflected in the incompressibility index and the Hausner ratio.

The compressibility index and the closely related hausner ratio have become simple, fast, and popular methods of predicting powder flow characteristics. The compressibility index was proposed as an indirect method of bulk density. Size shape, surface area, moisture content and cohesion of the materials as these can all affect the observed compressibility index. It is determined using the value of the wiped weight and the volumetric weight. The percentage compressibility index was found to be in the range of 11.49 to 17.74% as shown in Table 4. The percentage compressibility value is less than 20 for all formulations. The hausner ratio was found to be in the range of 1.12 to 1.21

H. Swelling index:

The degree of swelling is expressed as the percentage of water in the hydrogel at any time during swelling. Swellability is an important property because it affects mucoadhesion as well as drug release profiles in polymeric drug delivery systems.

The in vitro swelling ability of the microspheres was studied in phosphate buffer pH 7.4. From the data, it can be concluded that as the polymer concentration increases, so does the degree of swelling. So, we can say that the amount of polymer directly affects the degree of swelling. As the polymer to drug ratio increased, the degree of swelling increased from 69% to 97% for diclofenac sodium microspheres using HPMC and carbopol as copolymer. The swelling index of various formulations of diclofenac sodium mucoadhesive microspheres was determined in phosphate buffer pH 7.4 and ranged from 69% to 97%.

I. Mucoadhesion test:

The mucoadhesive properties of the microspheres were determined by an in vitro adhesion testing method known as the washout method. As the polymer concentration increases, mucoadhesion increases. High viscosity grade HPMC15000cps with carbopol934p combination, formulation (F5 to F8) showed the highest mucoadhesiveness compared to formulation (F1 to F4) showed lower mucoadhesiveness. The percentage of mucoadhesion of the different formulations was in the range of 50-79%.

J. In-vitro Drug release study:

In-vitro drug release test was to assure that the micspheres of diclofenac sodium are delivered to the target area,

and to elucidate the release kinetic for the developed formulation. diclofenac sodium is unstable at gastric pH and therefore, the microspheres of the drug were capsulated in hard gelatin capsules, coated with 1% w/v CAP and studied for in-vitro release of drug using USP dissolution apparatus 2 in pH 1.2 for 2 h followed by release in pH 7.4. In the initial 2 h,diclofenac sodium was not released more than 10% in the gastric fluid, thereafter the release was initiated when the pH was changed to intestinal fluid (phosphate buffer, pH 7.4) for the next 10 h.The drug release pattern from microspheres of diclofenac sodium was in a sustained manner, in contrast to the Dissolution of pure drug.

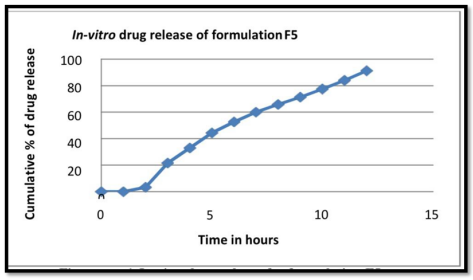


Fig.1. In-vitro drug release for formulation F5

4. Conclusion

Sustained release formulations of diclofenac sodium have been successfully prepared using sodium alginate in combination with HPMC using the ionotropic gelation technique. In vitro release data showed controlled release of the formulation for up to 12 hours. The microspheres were prepared without the use of organic solvents. FT-IR studies revealed no significant drug interactions. The release of diclofenac sodium from microspheres formulated with sodium alginate with HPMC as coating polymers showed a satisfactory controlled release profile. During the six-month stability testing period, there was no significant degradation of diclofenac sodium or change in drug release rate in any of the proposed formulations. The content of diclofenac sodium in the different formulations was not affected by the type of polymer or the ratio of drug to polymer. Therefore, it can be assumed that diclofenac sodium microspheres are promising pharmaceutical dosage forms by providing controlled release drug delivery systems and avoiding dose-related side effects across the physiological range. The entire process is feasible on an industrial scale and requires a pilot study.

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