Research Article

FORMULATION DEVELOPMENT OF SUSTAINED RELEASE ASPIRIN BEADS FOR INTESTINAL DELIVERY

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ABSTRACT

The purpose of this research work was to prepare beads of Aspirin (NSAIDs) by ionotropic gelation technique. Sodium alginate was used as a biopolymer. The beads were coated using cellulose acetate phthalate enteric polymer to avoid the drug release in acidic pH and provide sustained release in basic pH. The processing parameters like drug: polymer ratio, concentration of sodium alginate, concentration of calcium chloride and stirring time were studied and the beads were evaluated for flow properties, particle size, % drug entrapment and *In vitro* drug release. FT-IR and DSC confirmed the compatibility between drug and polymer. Surface morphology was studied by SEM analysis of coated and uncoated beads. 6% Cellulose acetate phthalate solution was optimized for enteric coating and In-vitro dissolution study showed negligible release in acidic pH and sustained release of drug for 8 hr in basic pH.

Keywords: Sustained release, beads, Sodium alginate, Ionotropic gelation, Cellulose acetate phthalate, Aspirin.

INTRODUCTION

NSAIDs are amongst the most commonly prescribed medications in the world. However, numerous spontaneously Oral drug delivery is the most desirable and preferred method of administering therapeutic agents for their systemic effects. For many drug substances, conventional immediate release formulations provide clinically effective therapy while maintaining the required balance of pharmacokinetic and pharmacodynamic profiles with acceptable level of safety to the patient.[1] In recent years, a wide variety of newer oral drug delivery systems like sustained/controlled release dosage forms are designed and evaluated in order to overcome the limitations of conventional therapy.[2] Reported adverse drug reactions, case control, cohort, and post marketing surveillance studies have revealed that NSAIDs are associated with extensive side effects. A trend of dosage form development for NSAIDs development has been an attempt to improve therapeutic efficacy and reduce severity of side effects through modified release such as enteric-coating (EC) or sustained release (SR) formulations.[3] Aspirin is a non-steroidal anti-inflammatory drug used extensively in the treatment of minor aches and pains (e.g. caused by headache, muscle aches, backache, arthritis, the common cold, toothache, and menstrual cramps). It works by inhibiting several different chemical processes within the body that cause pain, inflammation, and fever. The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding, and tinnitus due to higher doses. It is rapidly and completely absorbed after oral administration, having plasma half-life is approximately 2-3 hours which requires frequent dosing to maintain plasma drug concentration.[4] To reduce the frequency of administrations and improve patient compliances, Aspirin is suitable candidate for making sustain release and enteric coated dosage form. Sodium alginate was used as a biopolymer for preparation of beads and Cellulose acetate phthalate as enteric polymer. Aspirin beads spread out more uniformly in the GI tract, thus avoiding exposure of high concentration of drug to the mucosa at once and ensuring more reproducible drug absorption. The risk of dose dumping and side effect due to NSAID also seems to be lower than with a single unit dosage form.[5] Thus, the purpose of study by taking Aspirin as a suitable drug candidate was to understand the usefulness of sustained release dosage form for intestinal targeting in order to reduce the dosing frequency as well as side effects associated with conventional dosage forms.

Sodium alginate is a salt of alginic acid, a natural polysaccharide found in all species of brown algae and certain species of bacteria. It is a linear polymer of β (1-4) mannuronic acid (M) and α (14) guluronic acid (G) residues in varying proportions and arrangements. It has been shown that the G and M units are joined together in blocks, and as such, the following 3 types of blocks may be found: homo-polymeric G blocks (GG), homopolymeric M blocks (MM), and heteropolymeric sequentially alternating blocks (MG). The reactivity with calcium and the subsequent gel formation capacity is a direct function of the average chain length of the G blocks. Hence, alginates containing the highest GG fractions possess the strongest ability to form gels. This initially arises from the ability of the divalent calcium cation to fit into the guluronate structures like eggs in an "egg box junction". Consequently, this binds the alginate chains together by forming junction zones, sequentially leading to gelling of the solution mixture and bead formation. When aqueous solution of sodium alginate is added to drop wise to an aqueous solution of calcium chloride, it forms a spherical

gel with regular shape and size, also known as an "alginate bead". Enteric coated sustained release alginate beads have the advantages of being nontoxic orally, high biocompatibility, and inability to swell and release the drug in acidic environment, whereas they easily reswell in an alkaline environment and show sustained drug release. So stomach irritant drugs (like aspirin) incorporated into the beads would protect the stomach from gastric ulcer or irritation.[6]

MATERIALS AND METHODS

Aspirin was gifted by Mepro pharmaceuticals Pvt ltd., Wadhwan city, Gujarat, India. Calcium Chloride dihydrate (98.0%) was procured from Sisco research lab. Pvt ltd, Mumbai. Sodium alginate, sodium hydroxide was obtained from Loba chemie Pvt ltd, Mumbai. Cellulose acetate phthalate was purchased from GM chemicals, Mumbai. Dibutyl phthalate and isopropyl alcohol were purchased from Oswal chemicals, Ahmedabad. Water used was double distilled.

Preparation and optimization of aspirin – loaded alginate beads

Beads were prepared by ionotropic external gelation technique. Sodium alginate (1-10% w/v) was dissolved in distilled water using magnetic stirring. Accurately weighed quantity of Aspirin (after passing it through 100 mess sieve) was added and dispersed uniformly. The dispersion was sonicated for 30 min to remove any air bubbles that might have been formed during stirring process. The bubble free sodium alginate-drug dispersion was added drop wise via a 22-guage hypodermic needle fitted with a glass- syringe into a beaker containing calcium chloride solution (4-15%w/v) and stirred at 200rpm for 30min. The droplets from the dispersion instantaneously gelled into discrete matrices upon contact with the solution of gelling agent. The beads were allowed to harden for 30 min in same condition. The beads were collected by decanting calcium chloride solution, washed with deionized water and dried in hot air oven overnight at 40°C.[5, 7]

Several batches of drug loaded beads were prepared to investigate the effect of process variables, such as drug to polymer ratio, concentration of cross-linking agent, cross-linking time and stirring time on mean particle size, drug entrapment efficiency and In-vitro drug release. To study the effect of these variables, each time one variable was varied, keeping the others constant and optimized to get small, discrete, uniform, smooth surfaced and spherical beads. The detailed composition of the various formulations is mentioned in table 1.

Characterization of aspirin loaded beads

Determination of Drug-entrapment Efficiency [8]

Aspirin content in the beads was estimated by UV-spectrophotometric method. Accurately weighed 100mg of beads were crushed and suspended in 100 ml 0.1 N NaOH solution. The resulting solution was agitated using a mechanical stirrer for a period of 24 h to determine the amount of aspirin. After 24 h the samples were filtered, suitably diluted and spectrophotometrically measured at 296nm using Shimadzu UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug.

The drug entrapment efficiency was determined using following relationship;

Entrapment efficiency = (Drug loaded / Theoretical Drug loading) ×100

Table 1: Preparation and Optimization of Alginate Beads

Batch code	D:P ratio	Sodium alginate (%w/v)	Calcium chloride (%w/v)	Stirring time (h)	Stirring rate (rpm)
F1	1:1	1	4	0.5	200
F2	1:1	3	4	0.5	200
F3	1:1	5	4	0.5	200
F4	1:1	7	4	0.5	200
F5	1:1	9	4	0.5	200
F6	2:1	10	4	0.5	200
F7	2:1	5	6	0.5	200
F8	2:1	7	6	0.5	200
F9	2:1	9	6	0.5	200
F10	2:1	5	4	0.5	200
F11	2:1	5	6	0.5	200
F12	2:1	5	10	0.5	200
F13	2:1	5	15	0.5	200
F14	2:1	7	4	0.5	200
F15	2:1	7	6	0.5	200
F16	2:1	7	10	0.5	200
F17	2:1	7	15	0.5	200
F18	2:1	9	4	0.5	200
F19	2:1	9	6	0.5	200
F20	2:1	9	10	0.5	200
F21	2:1	9	15	0.5	200
F22	2:1	9	6	Overnight	200

Table 2: Preparation and Optimization of Coating solution

Batch code	Ingredients					
	(%w/w) of beads				(ml)	
	Cellulose acetate phthalate	Dibutyl phthalate	Titanium dioxide	Tartrazine yellow (lake)	Isopropyl alcohol	Methylene chloride
CS1	2	1	0.2	0.15	40	60
CS2	4	1	0.2	0.15	40	60
CS3	6	1	0.2	0.15	40	60
CS4	2	2	0.2	0.15	40	60
CS5	4	2	0.2	0.15	40	60
CS6	6	2	0.2	0.15	40	60

Measurement of micromeritic properties[9]

The flow properties were investigated by measuring the angle of repose of drug loaded beads using fixed base cone method. Beads were allowed to fall freely through a funnel fixed at 1cm above the horizontal flat surface until the apex of the conical pile just touched to the tip of the funnel. The height and diameter of the cone was measured and angle of repose was calculated by using the following formula. Each experiment was carried out in triplicate [n=3].

$\theta = \tan^{-1}(h/r)$

h=cone height (cm), r= radius of circular base formed by the beads on the ground (cm).

Compressibility index or Carr's index value of beads was also measured using equation:

Carr's index (%) = [(Tapped density-Bulk density)] x100/Tapped density

Hausner's ratio of beads was determined by comparing the tapped density to the bulk density by using the equation:

Hausner's ratio= Tapped density / Bulk density

Particle size analysis[9]

Particle Size was measured using Micrometer (Mitutoyo, Japan). Around 50 particles were measured from each batch for the particle size measurement before and after coating.

In-vitro drug release studies

In vitro release studies of prepared beads were carried out using USP XXI dissolution apparatus Type –II (paddle Type), Electrolab ED 2 AL. In-vitro drug released was determined using phosphate buffer (pH 6.8) maintained at a temperature of 37±0.5°C. The speed of the paddle was set at 50 rpm. At scheduled time intervals, the sample (5ml) was withdrawn and replaced with same volume of fresh medium. The withdraw sample was filtered through whatman filter paper of 0.45µ pore size and after appropriate dilution with 0.1 N NaOH, estimated for aspirin concentration at 296nm spectrophotometrically (Shimadzu, Japan).[10]

Criteria for Optimized Batch[11]

Three limits were arbitrarily selected;

i. Percentage drug release in first hr. (20-22%)

ii. t_{90}: time for 90 % drug release (6-8 hr.)

iii. % Drug entrapment efficiency (more than 80%)

Fourier Transformed Infrared (FT-IR) Spectroscopy

Drug polymer interactions were studied by FT-IR spectroscopy using KBr pellet. FT-IR spectra were obtained by powder diffuse reflectance on a FT-Infrared spectrophotometer type Shimadzu 8400S, Tokyo, Japan.[5]

Differential Scanning calorimetry (DSC)

DSC curves were recorded on a scanning calorimeter equipped with a thermal analysis data system (Shimadzu, Differential Scanning Calorimeter (Tokyo, Japan)). Samples weighing 3-5 mg were heated in sealed aluminium pans from 50-300°C at a scanning rate of 10°C/min under nitrogen purge, with an empty aluminium pan as reference. DSC was conducted first with Aspirin pure drug, beads, Sodium alginate and compared for possible drug-polymer interactions.[11]

Preparation of enteric coating solution

The coating solution for selected batch of beads was prepared by dispersing Cellulose Acetate Phthalate in methylene chloride (60 ml) with continuous stirring for 30 minutes. Titanium dioxide and color was dissolved in iso propyl alcohol (10 ml) and mixed with polymer solution with continuous stirring. Remaining iso propyl alcohol (30 ml) and Dibutyl phthalate was then added as plasticizer and allowed to stir for 1 hour. The resulting solution was filtered through muslin cloth.

Different concentrations of Cellulose Acetate Phthalate (enteric polymer) and Dibutyl phthalate (plasticizer) were taken and optimized to get effective protection in stomach and free flowing beads respectively.

Enteric coating of beads by spray coating

Aspirin loaded beads were coated by spray coating method. Accurately weighed aspirin beads were taken in coating pan and it was allowed to rotate at 50 rpm. Coating solution was sprayed using fine spray nozzle. Inlet temperature was maintained with the help of blower which allows rapid evaporation of solvent and drying of the beads. It was continues to rotate and hot air sprayed until all the beads were completely dried. Then beads

were collected with helped of scraper and kept for air drying for some time.

Characterization and evaluation of enteric coated beads

***Measurement of Particle size*[9]**

Particle Size was measured using Micrometer (Mitutoyo, Japan). Around 50 particles were measured from each batch for the particle size measurement before and after coating.

***Measurement of flow property*[9]**

The flow properties were investigated by measuring the angle of repose of drug loaded beads using fixed base cone method. Beads were allowed to fall freely through a funnel fixed at 1cm above the horizontal flat surface until the apex of the conical pile just touched to the tip of the funnel. The height and diameter of the cone was measured and angle of repose was calculated by using the following formula. Each experiment was carried out in triplicate [n=3].

$\theta = \tan^{-1}(h/r)$

h=cone height (cm), r= radius of circular base formed by the beads on the ground (cm).

In-vitro drug release study

In vitro release studies of enteric coated beads were carried out using USP XXI dissolution apparatus Type –II (paddle Type), Electrolab ED 2 AL. Dissolution was carried out using 0.1 N HCl (pH 1.2) for first 2 h and phosphate buffer saline (pH 6.8) for the rest of the period maintained at a temperature of 37±0.5°C. The speed of the paddle was set at 50 rpm. At scheduled time intervals, the sample (5ml) was withdrawn and replaced with same volume of fresh medium The withdraw sample were filtered through whatman filter paper of 0.45µ pore size and after appropriate dilution with 0.1 N NaOH, estimated for aspirin concentration at 296nm spectrophotometrically (Shimadzu, Japan).[10]

Criteria for Optimized Batch[11]

Two limits were arbitrarily selected;

i. Percentage drug release in 0.1 N HCl for 2 hr. (there should be no or minimum release)

ii. Flow property of enteric coated beads (should be at least good)

Kinetics of drug release

In order to understand the mechanism and kinetics of drug release, the drug release data of the *in-vitro* dissolution study was analyzed with various kinetic equations like zero-order (% release v/s time), first- order (Log % retained v/s time), Korsmeyer-Peppas (log fraction dissolved v/s log time), Higuchi (% release v/s Sq.rt time), Weibull (log-log fraction undissolved v/s log time), and Hixon-Crowel (cube.rt of % unreleased v/s time) equation. Coefficient of correlation (r) values were calculated for the linear curves obtained by regression analysis of the above plots.[5,12]

Sphericity and Surface morphology

The sphericity of uncoated and coated beads were studied by microscopy using Charge coupled device (CCD camera).

The surface characteristics of the coated beads and uncoated beads were studied by Scanning electron microscopy (JEOL, JSM 50A, Tokyo, Japan). The accelerating voltage was 30 kV.[11,13]

RESULTS AND DISCUSSION

Side effects, mainly at the gastric level are well known, following oral administration of an NSAID. Therefore the efforts of many researchers have been concerned to solve these problems, through a variety of techniques of protection of the gastric mucosa or alternatively to prevent the NSAID release in this gastric region. In this paper we evaluate the potential utility of the enteric polymer such as cellulose acetate phthalate to inhibiting the release of Aspirin in the gastric environment.[14] Since among the micro particulate systems, beads have a special interest as carriers for NSAID, mainly to extend the duration period of the dosage form, we aimed to investigate possible applicability of sodium alginate in various proportions as drug release modifier for the preparation of beads of aspirin as a sustained release manner. We prepared beads containing aspirin by ionotropic gelation method and examined the effects of various processing and formulation factors like concentration of sodium alginate, concentration of calcium chloride, stirring time and nature of beads, these may be affects the physical characteristics and drug release potential.

Preparation and optimization of alginate beads

The beads were prepared in an environment free from organic solvents by dropping a mixture of aspirin and sodium alginate polymer dispersion in calcium chloride solution, which acted as a counter ion. The droplets instantaneously formed gelled spherical beads due to cross- linking of calcium ions with the sodium ions of alginate which remain ionized in the solution.

Aspirin loaded beads formulated with 1.0 % of sodium alginate in 4.0 percent calcium chloride solution were not spherical and had a flattened base at the points of contact with the drying vessel. However, increase in the concentration of sodium alginate made the particles more spherical. This indicates that at low alginate concentration the particles were composed of loose networks structure which collapsed during drying. On the other hand higher sodium alginate concentration formed dense matrix structure which prevented collapse of beads. But forming high viscous polymer dispersion (with 10% of sodium alginate) did not pass easily through the needle during the manufacturing process moreover found a small tail at one end of beads which significantly affects

the flow properties and particle size distribution. It was found that optimum concentration of sodium alginate could influence the beads size, encapsulation efficiency and the release characteristics.[5]

Characterization of Aspirin loaded Beads

Beads prepared in different batches were evaluated with regards to various parameters and the results are given in the Table 3. The final composition of the formulation was decided on the basis of % Drug entrapment efficiency, Percentage drug release in first hr and t90: time for 90 % drug release. Accordingly, the final batch was prepared and coated for enteric effect.

The effects of various processes and formulation parameters on the physicochemical properties of drug loaded beads were evaluated. The rheological parameters like angle of repose, bulk density and tapped density of all beads confirmed better flow and packaging properties. All the formulations showed excellent flowability represented in terms of angle of repose (<30), Carr's index, and Hausner's ratio (table 3). Here, sodium alginate concentration had a significant positive effect on the angle of repose. Particle size increased with increase in the concentration of sodium alginate and resulted in a decreased angle of repose. However, higher calcium chloride concentration, crosslinking time and high stirring speed influenced the formation of smaller beads because of shrinkage and showed an increased angle of repose.[5] Bulk and tapped density of beads showed good acceptable range indicated a good packability. The density of beads increased as the concentration of the polymer increased suggested that the beads formed at high polymer concentration are more compact and less porous than those prepared at low polymer content.[5] Carr's index and Hausner's ratio of all the formulations were estimated and found to be in the range of 11.42 to 18.32 and 1.13 to 1.22 respectively (table 3), and explained the formulated beads had excellent compressibility and good flow properties. The improvement of flow properties suggest that the beads can easily handled during processing.

The mean particle sizes of drug loaded beads were measured by micrometer. The mean particle size of the various formulations (F1-F22) of beads was obtained in the range between 1.24 to 1.59 mm (table 3). It was found that the mean particle size was different among the formulations. The results indicated that the proportional increase in the mean particle size of beads increased with the amount of sodium alginate in the formulations. This could be attributed to an increase in relative viscosity at higher concentration of sodium alginate and formation of large droplets during addition of polymer solution to the gelling agent.[5]

The drug entrapment efficiency of drug-loaded beads obtained was in the range between 3.45±0.56 to 83.00±0.86 (table 4). It was observed that changing the drug: polymer ratio from 1:1 to 2:1 in the formulation significantly increased the drug entrapment efficiency, due to the more amount of drug dispersed in the polymeric dispersion.

Table 3: Micromeritic properties of aspirin-loaded beads

Formulation Code	Mean Particle size* [mm]	Angle of Repose* [θ]	Bulk Density [g/ml]	Tapped Density [g/ml]	% Carr's Index	Hausner's ratio
F1	1.24±1.04	29.20±0.62	0.477	0.584	18.32	1.22
F2	1.32±0.98	26.18±0.55	0.515	0.615	16.26	1.19
F3	1.41±0.75	24.56±1.07	0.555	0.659	14.96	1.18
F4	1.49±1.10	21.15±0.54	0.588	0.689	14.65	1.17
F5	1.59±1.23	19.11±0.55	0.595	0.690	13.77	1.16
F6	-	-	-	-	-	-
F7	1.39±1.08	20.40±0.64	0.585	0.678	13.71	1.16
F8	1.46±1.07	19.10±0.87	0.653	0.750	12.93	1.15
F9	1.56±0.96	18.85±0.55	0.712	0.810	12.09	1.14
F10	1.41±1.44	24.56±0.43	0.555	0.659	15.78	1.18
F11	1.39±1.08	20.40±0.64	0.585	0.678	13.71	1.16
F12	1.38±0.86	20.15±0.85	0.635	0.724	12.29	1.14
F13	1.36±1.15	19.95±0.45	0.648	0.740	12.43	1.14
F14	1.49±0.87	21.15±0.64	0.588	0.689	14.65	1.17
F15	1.46±1.07	19.10±0.87	0.653	0.750	12.93	1.15
F16	1.44±1.43	18.85±0.46	0.697	0.794	12.22	1.14
F17	1.43±1.05	18.85±0.68	0.709	0.805	12.17	1.14
F18	1.59±0.88	19.11±0.56	0.595	0.690	13.77	1.16
F19	1.56±0.96	18.85±0.55	0.712	0.815	12.63	1.14
F20	1.53±1.06	18.66±0.82	0.745	0.855	12.86	1.14
F21	1.51±0.75	18.45±0.38	0.768	0.867	11.42	1.13
F22	1.51±0.81	18.45±0.76	0.745	0.842	11.52	1.13

*Values are mean of 3 observations ± S.D

Table 4: Optimization of Aspirin-loaded beads

Batch code	Drug entrapment efficiency (%)	% Drug release in first hour	Time for 90 % drug release (t 90) (hr)
F1	3.45±0.25	59.33±0.45	1.0-1.5
F2	7.00±0.32	53.26±0.12	2.0
F3	18.30±0.55	48.53±0.32	2-2.5
F4	27.54±0.50	42.40±0.46	4.5-5.0
F5	38.41±0.75	38.54±0.37	5.0-5.5
F6	-	-	-
F7	58.30±0.60	40.16±0.32	2.5
F8	72.69±0.42	38.91±0.67	5.5
F9	83.00±0.86	22.04±0.44	6.5-7.0
F10	54.53±0.77	44.53±0.87	2-2.5
F11	58.30±0.60	40.16±0.32	2.5
F12	56.40±0.75	41.6±0.78	2-2.5
F13	54.80±0.84	40.05±0.88	2.5
F14	66.38±0.96	40.42±0.65	5.0-5.5
F15	72.69±0.42	38.91±0.67	5.5
F16	65.90±0.85	31.85±0.77	5.5-6.0
F17	58.80±0.58	30.5±0.53	5.5-6.0
F18	74.42±0.75	28.54±0.78	6.0-6.5
F19	83.00±0.86	22.04±0.44	6.5-7.0
F20	78.59±0.90	26.0±0.55	6.0-6.5
F21	69.50±0.97	22.82±0.46	6.5-7.0
F22	69.83±0.86	22.00±0.54	6.5-7.0

*Values are mean of 3 observations ± S.D

Table 5: Optimization of coating solution

Batch code	Angle of Repose [θ]	% Drug Release in two hour (acidic buffer pH 1.2)	% Drug release in first hour in pH 6.8
CS1	24.40±0.55	2.46±0.07	28.88±0.43
CS2	24.56±0.93	1.09±0.12	22.54±0.32
CS3	26.18±0.67	0.48±0.08	19.86±0.28
CS4	19.15±0.75	2.02±0.14	28.34±0.21
CS5	18.85±0.86	0.98±0.16	21.96±0.42
CS6	18.11±0.64	0.42±0.09	19.70±0.22

Values are mean of 3 observations ± S.D.

By increasing the concentration of sodium alginate from 5 to 9 %w/v, the drug entrapment efficiencies were found to in the range of 58.3±0.66 to 83.00±0.86 (table 4). It was observed that the drug entrapment efficiencies increased progressively with increasing the concentration of sodium alginate resulting in the formation of larger beads entrapping the greater amount of the drug. This might be attributed to a 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. Increases the concentration of alginate may also reduced loss of drug in the curing medium due to the formation of dense matrix structure. The sodium alginate concentration in the formulation greatly influenced the sustained release of drug from the beads. As the sodium alginate increased, the release rate of aspirin from the beads decreased. The slower in the release rate could be explained by the increase in the extent for swelling and the gel layer thickness which acted as a barrier for the penetration medium thereby retarding the diffusion of drug from the swollen alginate beads.[5]

Keeping the other parameters constant, Calcium Chloride concentration increased from 4-15 %w/v with different three concentration of sodium alginate (5, 7, 9 %w/v), the drug entrapment efficiencies were found to be in the range 54.53±0.75 to 58.30±0.23, 58.8±0.34 to 72.69±0.52 and 69.5±0.35 to 83.0±0.5 for 5, 7 and 9 %w/v of sodium alginate respectively (Table 4). From the results, it is obvious that increasing calcium chloride concentration produced beads with higher levels of Ca^{2+} ions. Consequently, the cross linking of the polymer and compactness of the formed insoluble dense matrices also increased, resulting in more drug entrapment in the beads. On the other hand, further increase in the concentration of calcium chloride above (6%w/v) did not enhance the drug entrapment efficiencies. This could be due to possible saturation of calcium binding sites in the guluronic acid chain, preventing further Ca^{2+}ions entrapment and, hence, cross-linking was not altered with higher concentration of calcium chloride solution. Also increasing the concentration of calcium chloride above 6%w/v (10 %w/v and 15%w/v) resulted decrease in the drug entrapment efficiencies, since the solubility of aspirin was slightly higher in calcium chloride solution than in distilled water.[5] The effect of cross-linking agent

on aspirin release from different batches of beads was also studied. The results indicate that rate and extent of drug release decreased significantly with increase of concentration of calcium chloride, because sodium alginate as a linear copolymer consisting of β (1→4) mannuronic acid and α (1→4) L-guluronic acid residues; a tight junction is formed between the residues of alginate with calcium ions. However, in case of higher calcium chloride concentration due to increased surface roughness and porosity and also poor entry of dissolution medium into the polymer matrix might have delayed drug release.[5]

It was observed that the drug entrapment efficiencies decreased on keeping the beads in curing medium for overnight period. Increasing the cross-linking time resulted decrease in the drug entrapment efficiencies, this was might be due to hydrolysis or degradation of aspirin. Prolonged exposure in the curing medium caused greater loss of drug through the alginate beads.

Perusal to table no 4 and above discussion, it was concluded that beads formulated in batch F19 showed a desired drug entrapment (83%) and satisfactory sustained release profile (22.04% drug release in 1st hr and t_{90} was 7 hr.) and so it was further coated with enteric polymer to retard the drug release in acidic pH.

Coating of Aspirin loaded Beads

The effects of different concentration of cellulose acetate phthalate and Dibutyl phthalate on the drug release of enteric coated beads in acidic media pH 1.2 were evaluated. It was observed that coating solution with 6%w/w concentration of Cellulose acetate phthalate and 2%w/w concentration of Dibutyl phthalate gave negligible release in gastric pH 1.2 after two hour (table 5). Moreover it showed desired percentage release in phosphate buffer pH 6.8 and has excellent floe property.

Particle Size Measurements

Particle Size of coated and uncoated beads of finalized batch was measured using Micrometer (Mitutoyo, Japan).

Type of Formulation	Average particle size in mm ± SD*
Uncoated beads	1.56 ± 0.25
Coated beads	1.61 ± 0.30

*values are mean of 50 observations.

***In-vitro* drug release study**

Aspirin release from optimized batch of beads was performed in different media, in 0.1 N HCL (pH1.2) for initial 2h and phosphate buffer pH6.8 for the period up to 8h and the results are given in the Table 6. it was observed that the drug release from enteric coated alginate beads was pH dependent, showed negligible drug release in acidic pH 1.2 due to the stability of cellulose acetate phthalate at lower pH. In other hand, the enteric polymer became soluble at pH 6.8 and the alginate got swelled at alkaline pH and the contents were released in a sustained manner by both diffusion and slow erosion of polymer matrix.[12] However, the swelling behaviors of drug-loaded Ca-alginate beads at higher pH could be explained by the ionotropy that occurs between Ca^{2+} ion of alginate and Na^{+} ions present in phosphate buffer and consequently, capturing of the Ca^{2+} by phosphate ions. The ion exchange with phosphate buffer which resulted in swelling of the beads and formation of the solute Ca-phosphate all have influence on the drug release rate at higher pH levels.[5] When the pH changed from 1.2 to 6.8 phosphate buffer, the drug released in a sustained manner (table 6). Based on these results it was conclude that the release rate of aspirin from alginate matrices is modulated by a swelling-dissolution and diffusion process.[12]

Table 6: *In-vitro* drug release profile

Time (min.)	Cumulative % Drug release	
	Uncoated beads	Enteric coated beads
0	0	0
30	16.58±1.2	2.37±1.06
60	22.04±0.84	2.38±0.91
90	49.04±0.87	3.34±1.07
120	62.57±0.97	3.83±0.76
150	64.34±0.87	19.70±0.83
180	71.32±0.95	21.94±0.92
210	75.03±0.86	47.64±0.96
240	80.64±0.66	61.64±1.08
270	82.03±0.94	65.30±0.95
300	84.37±0.85	69.44±0.72
330	87.66±0.94	73.61±0.98
360	90.97±1.05	79.70±1.16
390	93.35±0.48	81.08±0.96
420	94.79±0.90	84.83±0.89
450	97.66±1.07	88.13±0.92
480	101.02±0.91	92.86±0.84

Values are mean of 3 observations ± S.D.

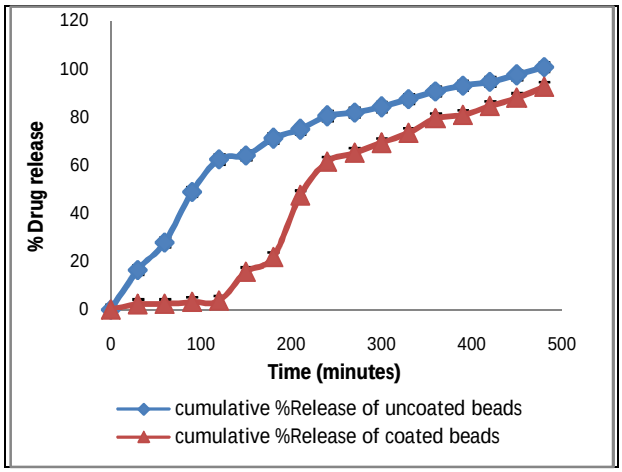


Figure 1: *In vitro* drug release from coated and uncoated beads

Kinetics of drug release

The best fitted kinetic model for drug release was determined by studying the parameters like SSR (Sum of square root of error), R values (Regression analysis i.e multiple R & R square) and F- value (Fischer ratio) for given models. Higher the value of multiple R & F-value and Lower the value of SSR is the best fitted model for

drug release.[12] Accordingly, the kinetic data for drug release was best fitted to HIGUCHI's model and good regression coefficient was observed. Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent and thus, it was concluded that coated as well as uncoated beads were following Higuchi's model for drug release kinetics, i.e. drug release through diffusion mechanism.

Also, the n values (release coefficient or x-variable) of uncoated and coated beads in korsmeyer-peppas model were 0.602 (follows Anomalous transport) and 1.71 (super case II transport) respectively, indicating the drug release from beads was modulated by swelling and relaxation of polymer chains.[12]

Table 7: Kinetics of drug release from uncoated and coated beads

Uncoated beads						
Model	**MULTIPLER**	**R SQUARE**	**Intercept**	**X-VARIABLE (SLOP)**	**SSR**	**FISCHER RATIO**
Zero order	0.921	0.849	25.957	0.181	2145.6	84.0
First order	0.677	0.459	2.473	-0.006	56500.7	12.7
Higuchi	0.965	0.931	-17.602	10.961	966.9	203.7
Korsemeyer peppas	0.963	0.927	-1.562	0.602	967.5	179.0
Weibull	0.935	0.875	-2.54	1.17	248.53	98.45
Hixon Crowel	0.958	0.927	0.10	0.007	103078	225.86
Coated beads						
Model	**MULTIPLE R**	**R SQUARE**	**Intercept**	**X VARIABLE**	**SSR**	**FISCHER RATIO**
Zero order	0.967	0.935	-8.531	0.23	1343.2	217.1
First order	0.971	0.943	2.173	0.00	4618.7	249.5
Higuchi	0.983	0.965	-14.839	10.31	719.2	418.5
Korsemeyer peppas	0.944	0.891	-4.508	1.71	3036.5	114.5
Weibull	0.950	0.903	-5.222	2.098	845.35	131.60
Hixon Crowel	0.979	0.958	-0.368	0.0061	58065.3	349.98

Fourier Transformed Infrared (FT-IR) Spectroscopy

IR spectra of Aspirin, Sodium alginate, physical mixture of Aspirin and sodium alginate and prepared beads are shown in Figure 2. The study of IR spectra of Aspirin (Figure 2) demonstrated that the characteristic absorption bands for aromatic rings (C=C stretching), C=O stretching, C-O stretching and C-H stretching vibration appeared at 1516, 1755, 1095 and 2941 cm^{-1}, respectively. The almost identical absorption bands were obtained in prepared beads, but with lower intensity as shown in Figure 2. The above observed absorption bands were similar to the reported value. Thus, the IR study indicated a stable nature of Aspirin in prepared beads.

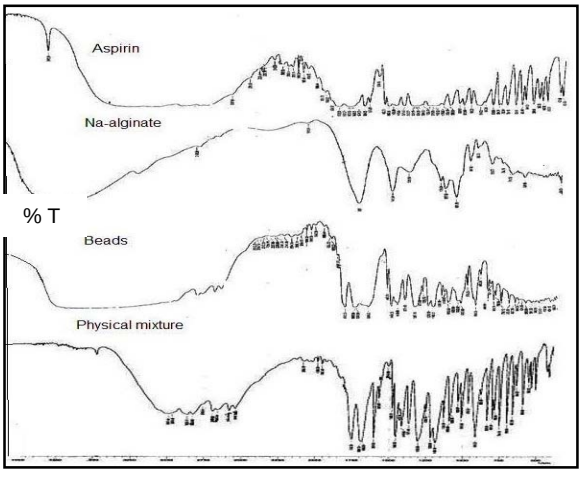


Figure 2: IR Spectra of Drug, Polymer, Physical mixture and beads

Differential Scanning Calorimetry (DSC)

The DSC thermogram of pure drug and beads (figure 3) showed almost similar melting endotherms at 143.07°C and 137°C, respectively. However, the intensity of the endotherm in beads was comparatively less than that of the pure drug. DSC of polymer Sodium alginate did not show any peak in this range. These results were an indication of absence of any chemical interaction between drug and excipient.[11]

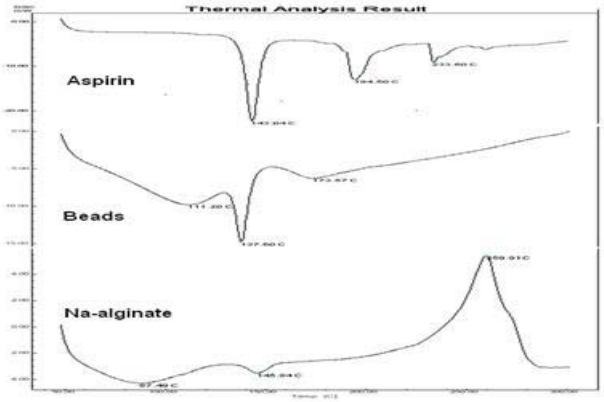
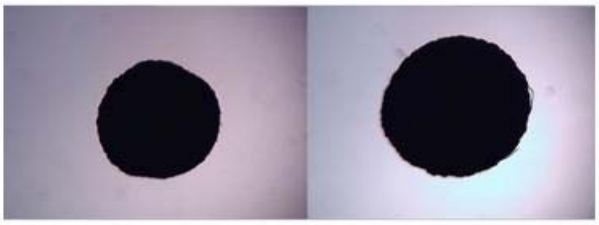


Figure 3: DSC Profile of drug, polymer and beads

Sphericity and Surface Characterization

Sphericity of beads: The sphericity of uncoated and coated beads was studied by microscopy using Charge coupled device (CCD camera). Fairly spherical shape of uncoated as well as coated beads was observed (Figure 4-a and 4-b). This sphericity was responsible for excellent flow property of beads. The size of bead was increased

after coating with enteric polymer which was an indication of uniform coating.



(a) (b)

Figure 4: Sphericity of (a) uncoated bead and (b) coated bead

Surface morphology of beads: Surface morphology of uncoated and coated beads was studied by Scanning electron microscopy (figure 5 and 6). It shows a smooth surface of uncoated beads with very rare findings of dents. The beads with CAP coat showed some dents on its surface. These dents were because of the faster evaporation (due to supply of hot air) of organic solvents used in the formulation of coating liquid. This evaporation might have left a little extent of uneven surface as well as dent formation superficially so did not release the drug in acidic pH.

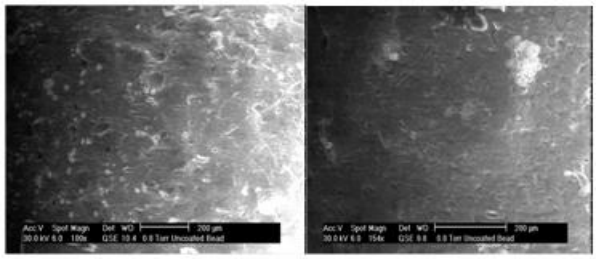


Figure 5: Surface morphology of uncoated beads

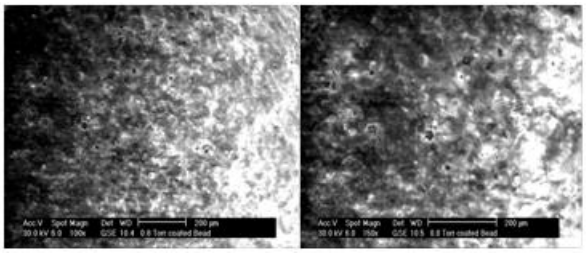


Figure 6: Surface morphology of coated beads

CONCLUSION

From the present study, it was concluded that ionotropic gelation technique can be successfully used for preparation of aspirin beads using sodium alginate as drug release modifier. Various formulation variables such as polymer concentration, calcium chloride concentration and cross-linking time which can influence the drug entrapment efficiency, mean particle size, surface morphology and *in-vitro* drug release. Out of all other parameters, concentration of polymer has profound effect to control and sustain the release of Aspirin. Cellulose acetate phthalate was used to prepare enteric coated beads which was affected by the pH of the dissolution medium resulted a negligible release in 0.1 N HCL and sustained effect in phosphate buffer (pH 6.8) up to 8 hr. It was concluded that coated as well as uncoated beads were following Higuchi's model for drug release kinetics, i. e. drug release through diffusion mechanism. FT-IR and DSC studies did not reveal any significant drug interactions. Therefore, one can assume that the aspirin beads are promising pharmaceutical dosage forms by providing sustained release drug delivery systems and avoiding the dose related side effects in the entire physiological region.

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