Preparation and evaluation ofhollow calcium pectinate beads for floating-pulsatile drug delivery.

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Abstract: -

The purpose of this work was to develop hollow calcium pectinate beads for floating-pulsatile release of ofloxacin intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. A controlled release system designed to increase its residence time in the stomach without contact with the mucosa was achieved through the preparation of floating drug delivery system by rate controlled drug delivery approach. To overcome limitations of various approaches for imparting buoyancy, hollow/porous beads were prepared by simple process of acid-base reaction during ionotropic crosslinking. The floating beads provided expected two-phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. The floating beads obtained were porous (38% porosity), hollow with bulk density <1 and had $F_{t50\%}$ of 16–22 h. This approach suggested the use of floating pulsatile dosage forms as they have potential for use as controlled-release drug delivery systems for site and time-specific release of drugs acting as per chronotherapy of diseases.

Keywords: Calcium pectinate beads, Chronotherapy, Buoyancy, Floating-pulsatile drug delivery.

1. Introduction:

Pulsatile drug delivery systems are usually of reservoirtype, whereby a drug reservoir is surrounded by a diffusionalbarrier. This barrier erodes, dissolves or ruptures after aspecified lag time, followed by a rapid drug release.Pulsatile drug delivery systems release active ingredientcompletely and rapidly after a defined lag time[1]. Suchsystems are advantageous for (i) drugs with an extensivefirst pass metabolism and developed biological tolerance, (ii) the targeting of locally absorbed or acting drugs to aspecific site in the intestinal tract (e.g. colon), (iii) the adaptationof the therapy to chronopharmacological needs.[1-2]Natural biodegradable polysaccharides like pectin, guar gum, chitosan, carrageenans, sodium alginate and gellan gum have been used in controlled drug delivery.[3-7] Multiparticulate systems obtained by ionotropic crosslinking of these polymers have been used to develop floating drug delivery. Various approaches to induce buoyancy in crosslinked beads, some of which include freeze-drying, entrapment of gas or gas forming agents, use of volatile oils or fixed oils, have been used.[8-10]These approaches are complicated, as they require specific equipment and handling techniques with limited acceptance.The floating dosageforms containing sodium bicarbonate as buoyancy impartingagent are simple to produce which have been alreadyattempted. Comparatively,oil containingbeads have limitations of coalescence of oil droplets yieldingbeads of wider particle size distribution, volatilizationor leaching of oil.[11-13]Floating property of dosageforms containing sodium bicarbonate is based on theevolution of carbon dioxide when in contact with acidicenvironment followed by the ability of polymer gel toentrap it which decreases their density below one. On theother hand, violent gas generation, disintegration of dosageform, burst release, dose dumping and alkaline microenvironment[14] are limitations of these dosage forms.Fatmanuretal[15] developed the alginate based mesalazine tablets for intestinal delivery. Sodium alginate is a biocompatible, natural polymer with pH-sensitive gel-forming ability.Srisagulet al[16]designedmulti-layer coated tabletsbased on gas formationconsists of a drug-containing core tablet coatedwith a protective layer (hydroxypropyl methylcellulose), a gas forming layer (sodium bicarbonate) and a gas-entrapped membrane.Choi et al.[17] have developed porous alginate beads containingriboflavin where the carbon dioxide gas was allowed togenerate during crosslinking only, followed by freeze-dryingto improve porosity.Talukder and Fassihi[18] developeda floatable multiparticulate system by crosslinkinglow methoxylated pectin and

sodium alginate. The beadsoobtained by freeze-drying remained buoyant over 12 h, whereas the air-dried beads remained submerged. Thestudy revealed the presence of air-filled hollow spacesinside the freeze-dried beads, which was responsible for theflotation property of the beads. Sriamornsak et al.[19]developed floating calcium pectinate beads by emulsion-gelationmethod. Such technique can be considered as alternativeto overcome limitations of sodium bicarbonatecontaining floating drug delivery systems.The drug regime based on circadian rhythm,chronopharmacotherapy,isrecently gaining much attention worldwide. Circadian phase dependent patterns have been well documentedin conditions such as asthma, arthritis, epilepsy, migraine, allergic rhinitis, cardiovascular disease (myocardial infarction, angina, stroke) and peptic ulcer disease, with particular times where symptoms are more prominentand/or exacerbated[20].These diseases viz. asthma, hypertension, acidity, and arthritis showcircadian variation that demands time-scheduled drugrelease for effective drug action, e.g., inflammations associatedwith morning body stiffness, asthma, and heart attackin early hours of the day.[21]One must have to design the dosage form which follows above principle such that it can be givenat the convenient time, e.g., bed time for the above-mentioneddiseases with the drug release in the morning.This can be used fordrugs which are easily decomposed in the acidic environmentof the stomach e.g. peptides, or to protect the stomachfrom drug side effects e.g. aspirin. Such systems are alsopromising for a local therapy in the lower parts of theintestine e.g. colon targeting to treat diseases like ulcerative colitis[22]. Pharmacokinetics of some drugs shows circadian variation for anti-inflammatory drugs like ketoprofen, indomethacin, and ofloxacin which have greater absorption inmorning as compared to evening[23], and site-specificabsorption from small intestine[24-25]. To developdosage form for chronopharmacotherapy it is therefore desired thatdrug release should be time-specific as well as site-specific.The drug release from pulsatile release drug delivery systems should exhibit sigmoidalrelease pattern (A) as shown in fig.2 rather than B & C which represents delayed release patterns.

The purpose of the present study was to produce hollow/ porous-floating beads of pectin by a process of evolution of carbon dioxide during cross-linking in acidic environment. Ofloxacin, an acid-insoluble antibacterial drug, was used as model drug. The obtained beads were evaluated for drug content, size analysis, porosity, mechanical strength, *in vitro*floating properties and *in vitro* drug release.

2. Materials and methods:

2.1 Material:

Low methoxy pectin was purchased from Rajesh Chemicals Mumbai. Ofloxacin was obtained as generous gift from Modern Laboretories, Indore. Other materials used in the study calcium chloride dehydrate (Sisco Research Lab. Pvt. Ltd., Mumbai, India), sodium bicarbonate (LobaChemie, Mumbai, India), acetic acid, glacial (100%) (E Merck, Mumbai, India). All chemical reagents used were of analytical grade.

2.2Preparation of beads:

Three hundred milligrams of pectin was dissolved in 10 ml of deionized water, 200 mg ofloxacin and various amounts of sodium bicarbonate were uniformly mixed, as shown in **Table 1.** The dispersion was sonicated for 30 min to remove entrapped air bubbles, if any. The resultant dispersion was dropped via a 23-gauge syringe needle (0.65 mm internal diameter) into 80 ml of 2% w/v calcium chloride ($CaCl_2$) solution containing 10% acetic acid. The content was agitated at 100 rpm using magnetic stirrer for 30 min. The beads were then strained, washed three times with distilled water and followed by oven-dried at 50^0 C for 6 h.

2.3 Drug content:

20 mg beads of each batch were placed in100 ml phosphate buffer having pH 7.4, and mechanically stirred on shaker at 200 rpm for 24 h. The resultant dispersions were filtered and analyzed at 277 nm using UV spectrophotometer (JASCO-V500, Kyoto, Japan). The encapsulation efficiency was determined by the following formula:

Encapsulation efficiency (%) = AQ/TQ x100

where AQ = actual drug content of beads and TQ = theoretical quantity of drug present in beads.

2.4 Bead characterization

2.4.1. Infrared spectroscopy

The infrared spectra of ofloxacin, calcium pectinate beads (without drug, sodium bicarbonate and acetic acid) and drug-loaded porous calcium pectinate beads were recorded on FTIR (JASCO-FTIR 5300).The samples were prepared on KBr press and spectra obtained were used for preformulation studies.**(Fig 1)**

2.4.2. Size analysis

Randomly selected 10 beads were observed under a stereomicroscope(Motic) attached with a digitalcamera (Watec, WAT-202, Japan). Biovis image plussoftware (Expert Tech Vision, India) was used to analyzethe images of beads and were then expressed in terms ofdifferent parameters such as diameter, roundness and circulatoryfactor.

2.4.3. Bead porosity and bulk density

Mercury porosimetry(Autoscan 60 Porosimeter, Quantachrome software, USA)[13]was used to assess bead porosity. The mercury intrusion data were recorded and plottedagainst pressure. The pressure was applied from 0 to 6000 psi.Standard values for the contact angleand surface tension of mercury were used for calculations.The bulk densities of the beads were also measured usingsame mercury porosimeter.

2.4.4.Moisture content

The total moisture content was measured using about50 mg of beads from all the batches by Karl Fisher titration(Veegomatic-D, Mumbai, India).

2.4.5. Buoyancy test

The beads so obtainedwere studied for buoyancy[26] andbuoyancytime using USP XXIII type 2 dissolution test apparatus(Electrolab TDT-06P, Mumbai, India). One hundredbeads of each batch were placed in 900 ml of 0.1 N HCl(pH 1.2) containing 0.02% w/v Tween 80 and stirred at100 rpm, temperature was maintained at 37^0C ± 2. Number of sinking beads wasmonitored visually. Typical profile of drug release profile of pulsatile drug delivery systems is shown in **(Fig.2)**

2.4.6 Molar absorption coefficient and correlation coefficient (r)

Stock solutions (1000 µg/ml) of ofloxacin
was prepared by dissolving separately 100mg of drug in minimum quantity of glacial acetic
acid and finally diluted with PBS (pH 7.4) to make up the volume up to 100 ml. The maximumabsorbance (λmax) of ofloxacinwas
obtained at 277 nm. A series of standard drug solutions in concentration range of 5-30 µg/ml

were prepared by diluting appropriate volumes of the standard stock solutions. The scanning for solution of ofloxacin
was carried out in the range of 200-400 nm against PBS (pH 7.4) solution as blank for obtaining spectra that was used in the analysis. Absorbance and absorptivity of series of standard solutions wererecorded at selected wavelength.The correlation coefficient (r) was found to be 0.9969.

The molar absorption coefficient equation was determined for the ofloxacin using calibration curve equations as shown below in table 0. Further, the molar absorption coefficient was determined by using the equation:

(□) = $E^{1\%}_{1cm}$ × Molecular weight /10

Sandell's Sensitivity = Molar Absorptivity / Molecular weight

Table 0: Absorbance and molar absorption coefficient of Ofloxacin

Sr.No.	Conc(mcg/ml)	Absorbance	$E^{\%}_{1cm}$
1.	5	0.188	361.80
2.	10	0.235	336.00
3.	15	0.328	366.00
4.	20	0.475	308.45
5.	25	0.540	326.70
6.	30	0.622	360.00

2.5. Dissolution studies

The dissolution studies of the prepared beads equivalent to 50 mgof ofloxacin were performedusing USP XXIIItype 1 dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). The drug release study was carried outin 0.1 N HCL for initial 2 or 6 h depending upon floatingcharacteristics of beads, followed by dissolution in phosphatebuffer, pH 7.4, each 900 ml, maintained at37^0C ± 2 and agitated at 100 rpm (n = 3). Samples were withdrawn periodically and filtered and concentration of ofloxacin wasmeasured spectrophotometrically (UV spectrophotometer, JASCO-V500, Kyoto, Japan) at 273 and 277 nm for acidicand basic media, respectively. Analysis of data was doneusing 'PCP Disso v2.08' software.

3. Results and discussion

Polysaccharides have been widely exploited for their use as pharmaceuticalexcipient owing to biocompatible, biodegradable, inexpensiveand non-toxic nature. With the event of ionotropic gelation they form multiparticulatesystem by simple ionotropic gelation, which can be formulatedto provide various desired drug release profiles. Pectin, heterogeneous anionic polysaccharides with an abilityto produce water-insoluble complexes with drug, has beenused in oral novel drug delivery systems. In stomach pectinis not digested by gastric enzymes and has minimumswelling but undergoes rapid gel relaxation/swelling inalkaline environment[27-30].

3.1. Preparation of beads

In our preliminary study, 0.75:1 w/w ratio of sodiumbicarbonate and sodium alginate yielded mechanicallyweak and irregular hollow beads. Compared to calciumalginate beads the calcium pectinate beads of same concentrationshowed greater mechanical strength, therefore pectinwas selected to obtain hollow floating beads. Thehollow/porous beads were produced during ionotropic gellingassisted by in situ reaction between sodium bicarbonatein wet pectin beads with acidified calcium chloridecrosslinking solution. To observe the effect of acid andalkali component Batch A1 and Batch A2 were preparedas shown in Table 1. Batches A3–A6 were, respectively, prepared using increased sodium bicarbonate level to pectin in ratio of 0.25:1, 0.5:1

0.75:1 and 1:1. Batch A6produced beads of poor mechanical strength with no sphericalshape, due to the excessive liberation of gas, whichmade pectin matrix too weak to sustain the shape afterdrying.

3.2. Drug content

Batch A1, prepared in plain crosslinking solution, showed lowest drug encapsulation than other batches; itmay be due to decreased drug solubility in acidic crosslinkingsolution (Table 1). Batch A2 showed high encapsulationthan A3 due to the absence of sodium bicarbonate.In Batches A3–A5, encapsulation efficiency increased withthe increase in amount of sodium bicarbonate (Table 1).Effect of sodium bicarbonate can be attributed to the formationof alkaline microenvironment inside the beadenhancing drug solubility combined with the effervescentaction-giving rise to modifications of bead matrix in situ.In Batch A3, the less amount of sodium bicarbonate actedindividually causing scattered micro channels leading todrug loss. This effect can be supported by the fact thatthe bulk density of this batch is more than 1 (Table 2).For Batches A4 and A5 collective action exerted by theincreased amount of sodium bicarbonate leads to the formationof prominent hollow structures due to entrapmentof generated gas. This entrapment leads to the coalescenceof gas bubbles, which pushed the internal matrix towardsperiphery forming thick boundaries minimizing drugleaching.

3.3. Bead characterization

3.3.1. Infrared spectroscopy

The IR spectra of calcium pectinate beads showed thecharacteristic band C=O vibration of COOH group at1740 cm^{-1} and strong absorption band at 1617 cm^{-1}belonging to the asymmetric stretching of vibration ofCOO. The IR spectra of ofloxacin showed thestrong peak at 1600 cm^{-1} in carbonyl frequency region, peak for − NH stretching of aromatic ring at 1450 $cm^{-1}$and bending at 690 cm^{-1} for meta-di-substituted chlorineon benzene. The IR spectra of drug-loaded calcium pectinatebeads of Batch A5 showed all the above-mentionedpeaks of calcium pectinate beads and the ofloxacin**.**

3.3.2. Size analysis

The drug-loaded calcium pectinate beads without sodiumbicarbonate were comparatively spherical than otherbatches (Table 2). The presence of sodium bicarbonateamounts (at constant pectin concentration) might beresponsible for softening of pectin beads subsequentlydeformed

by the force of agitation. The particle sizeincreases with the increased proportion of sodium bicarbonatein the polymer matrix. This can be attributed tothe presence of entrapped gas bubbles. The increase inporosity was also observed in similar order too(Table 2).

3.3.3. Bead porosity and bulk density

The bulk density of hollow beads (Batches A4 and A5)was less as compared with the beads without sodium bicarbonate(Batch A2). The decrease in bulk density wasobserved with increase in size and porosity (Table 2).

3.3.4. Buoyancy testand dissolution studies

Floating properties of beads were studied by determiningbuoyancy and time required for sinking all the beadsunder study. The surfactant was used in medium to simulatesurface tension of human gastric juice (35–50 mN/m^2).[21] Beads of Batches A1 and A2 were completelynon-floating and sunk immediately, whereas majority ofbeads of Batch A3 were non-floating. Batches A4 and A5produced floating beads without buoyancy lag time**(Fig. 3)** and remained floating for 7 and 12 h, respectively.Ft50, the time required to sink 50% of beads assuming linearapproach of sinking, was presumed to be 14 and 24 h,respectively, for Batches A4 and A5. The floating propertiesof hollow/porous beads may be attributed to the lowbulk density and the porosity of the beads; implying thatthe beads will have the propensity to exhibit an excellent

buoyancy effect *in vivo*.The in-vitrocumulative drug release profile of Batch A5 was studiedby using dissolution apparatus as shown in (**Fig.4)**. It canbe interpreted from the dissolution studied that the A5 beads gave a discretelag of around 4 h, after that mass started to diffuseto some extent in gastric content up to the end of 5 h, untilthe end of study. Hence A5 beads were found to be our best formulation due to their drug release data and other results obtained throughout the study. The beads of Batch A3 were not studied for dissolutionrate. The non-floating beads were assumed to remain instomach for 2 h whereas on the basis of dissolution data floating beads were considered to be gastroretentivefor 6 h, making basis for in vitro dissolution time inacidic medium. All the beads released 3–4% of the drugin acidic medium irrespective of time.

4. Conclusion

Novel hollow calcium pectinate beads containing ofloxacin were prepared by simple technique within situ action of buoyancy imparting agents during formation.Overall, the buoyant beads

provided a lag phase whileshowing gastro retention followed by a pulsatile drugrelease that would be beneficial for chronotherapy of rheumatoid arthritis and osteoarthritis. This work can beextended for time-scheduled drug release of drugs havinglow solubility, poor absorption or degradation in lowergastrointestinal tract.

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Table No. 1Composition, percent yield and encapsulation efficiency profiles of calcium pectinate beads

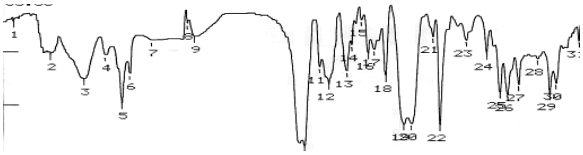
Batch No.	**A1**	**A2**	**A3**	**A4**	**A5**
Amount of pectin (mg)	300	300	300	300	300
Amount of drug (mg)	200	200	200	200	200
SBC (mg)*	-----	-----	0.075	0.1500	0.225
Amount of CaCl2 (g)	1.6	1.6	1.6	1.6	1.6
Acetic acid 10% (v/v) (ml)	--	8	8	8	8
% encapsulation efficiency	63.78 ± 1.92	77.86 ± 2.29	71.48 ± 1.10	76.66 ± 2.66	80.53 ± 1.81
% yield	92.86 ± 1.07	93.55 ± 1.05	90.03 ± 1.03	92.83 ± 1.40	88.55 ± 1.23

- SBC: Sodium bicarbonate.

Table No.2Micromeritic properties of calcium pectinate beads

Batch No.	Diameter (mean) (mm)	Roundness	Bulk density (g/cm3)	Porosity (%)
A1	1.43 ± 0.05	0.77 ± 0.06	1.23 ± 0.01	--
A2	1.47 ± 0.06	0.71 ± 0.08	1.85 ± 0.11	--
A3	1.66 ± 0.06	0.67 ± 0.08	1.28 ± 0.19	20.41
A4	1.82 ± 0.09	0.75 ± 0.06	0.89 ± 0.13	25.61
A5	1.97 ± 0.10	0.75 ± 0.08	0.85 ± 0.07	35.70

Plane calcium pectinate bead



Ofloxacin

Batch A5

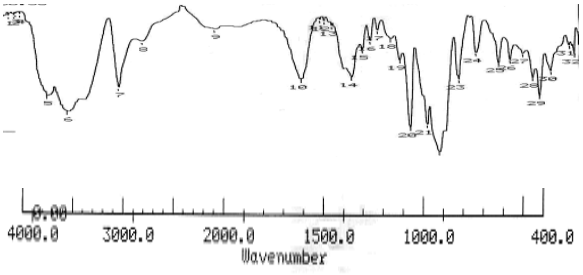


Fig 1.The FTIR spectra. (A) Plane calcium pectinate bead. (B) Ofloxacin. (C) Batch A5.

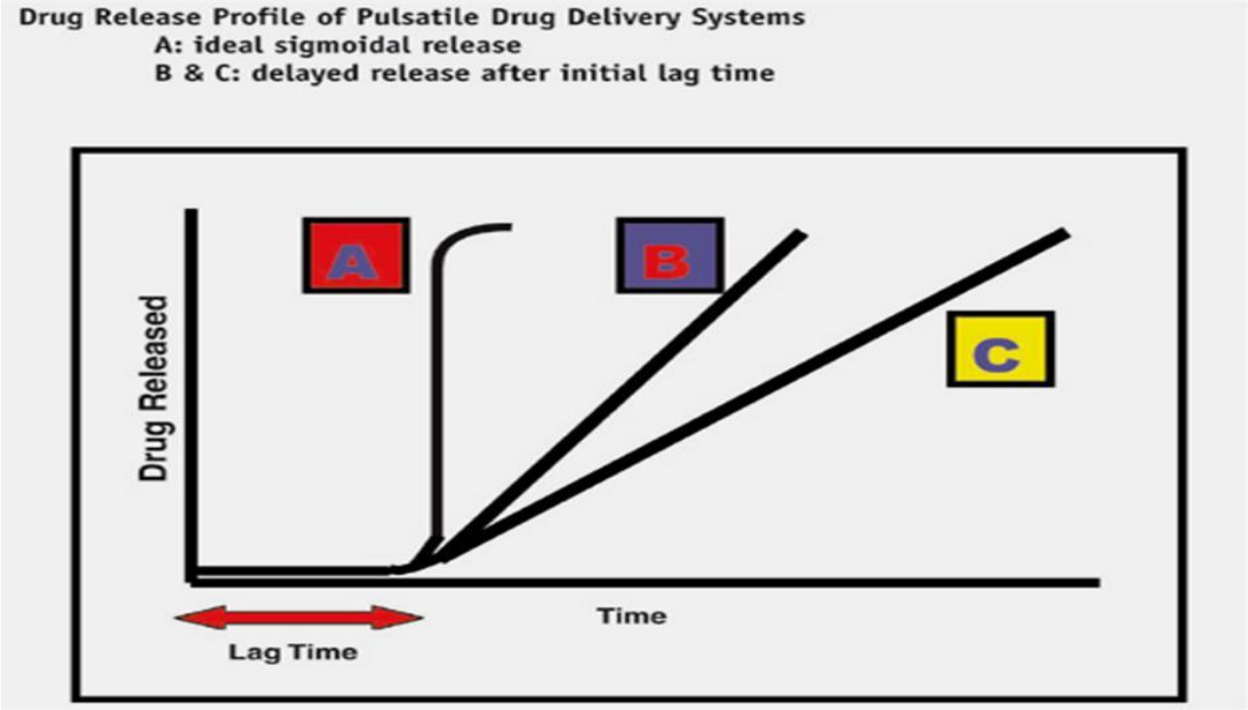


Fig. 2Drug release profile of pulsatile drug delivery systems

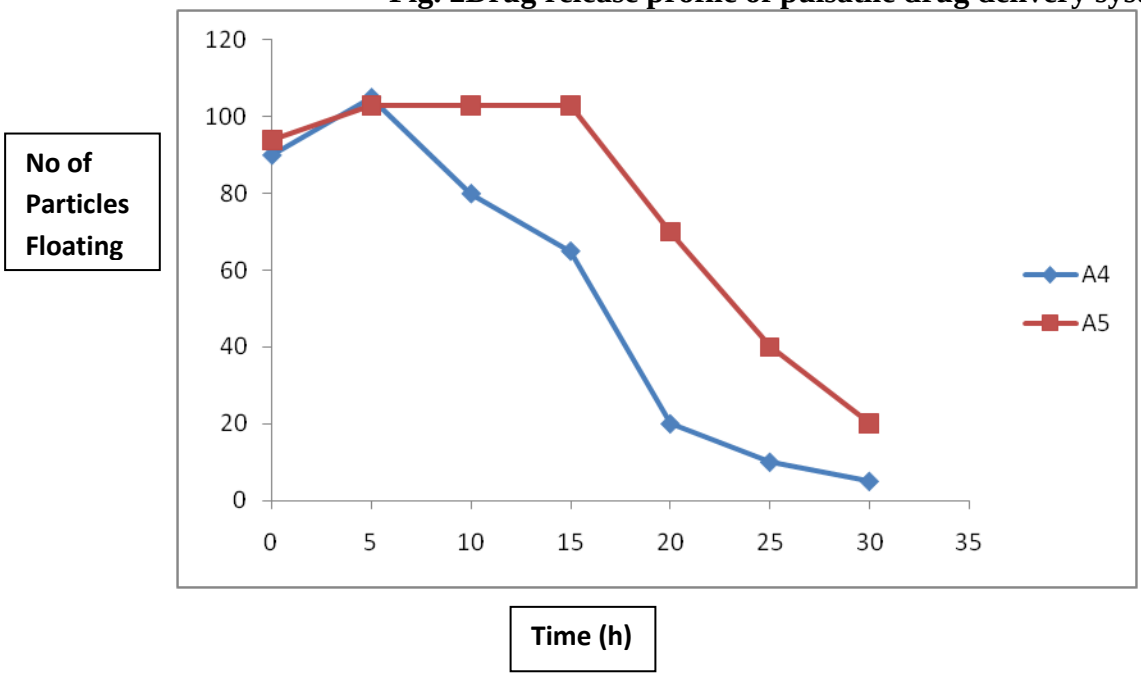


Fig. 3 Floating Profile for Ofloxacin Loaded Calcium Pectinate Beads.

(Time Vs No of Particles Floating)

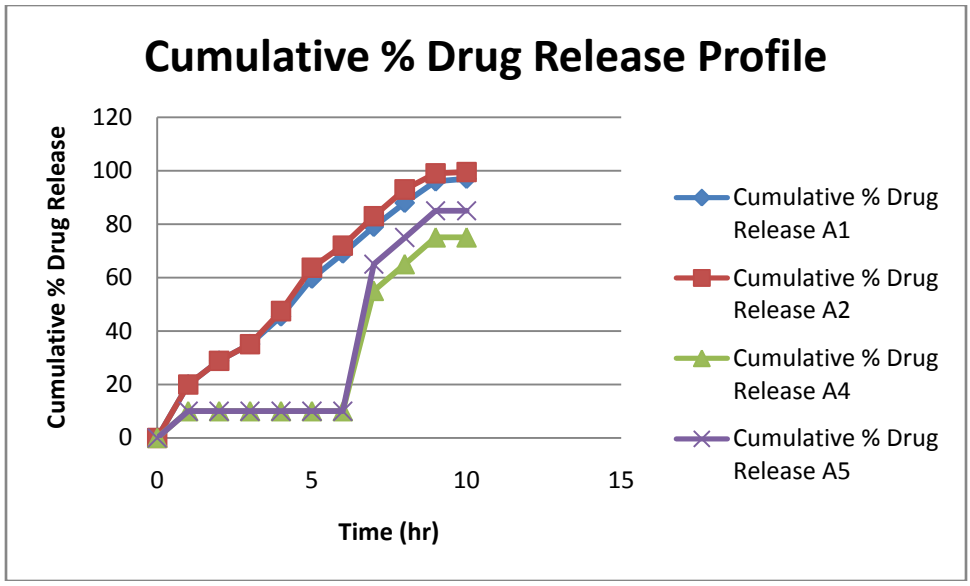


Fig. 4 Cumulative percent drug release profile

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