RESEARCH ARTICLE

Development and Optimization of Gellan Gum-Based Gastro-Retentive Microbeads for Enhanced Controlled Release of Acyclovir

Ranjan Kumar Sethi[1], Sunit Kumar Sahoo[1*], Pravat Ranjan Guru[2]

[1]*University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.*
[2]*Dadhichi College of Pharmacy, Vidya-Vihar, Sundargram, Cuttack, Odisha, India.*

Received: 15th March, 2024; Revised: 23rd May, 2024; Accepted: 13th August, 2024; Available Online: 25th September, 2024

ABSTRACT

Modified release systems offer enhanced spatial and temporal control over drug delivery, leading to better blood plasma profiles, targeted therapy, reduced side effects, and improved patient compliance. Recent advancements focus on using biocompatible polymers to achieve sustained or controlled drug release, particularly for gastroretentive systems that enhance oral bioavailability and minimize dose-dependent toxicity. This study explores the formulation of floating microbeads of acyclovir (ACV) using various hydrophilic polymers. The prototype oral formulations demonstrated superior micrometric properties, including bulk density, tapped density, Carr's index, Hausner's ratio, angle of repose, average particle size, yield, and drug content. *In-vitro* dissolution studies revealed controlled release characteristics, with optimal floating time and entrapment efficiency achieved using specific concentrations of olive oil and polymers. Drug release trailed the Korsmeyer-Peppas model, indicating a diffusion-controlled mechanism. The formulations exhibited good storage stability, *in-vivo* floating ability, and higher bioavailability in rabbits compared to ACV solutions. The results suggest that hydrophilic polymers are effective for formulating floating microbeads with controlled release and reproducible profiles.

Keywords: Acyclovir, Microbeads, Polymer, Drug delivery.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.21

How to cite this article: Sethi RK, Sahoo SK, Guru PR. Development and Optimization of Gellan Gum-Based Gastro-Retentive Microbeads for Enhanced Controlled Release of Acyclovir. International Journal of Drug Delivery Technology. 2024;14(3):1396-1403.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Microspheres are a class of multiarticulate drug delivery systems designed to deliver medications with enhanced specificity, bioavailability, and stability. These spherical particles, ranging from 1 to 1000 μm in diameter, contain pharmaceuticals either dissolved or in microcrystalline form.[1,-5] They offer a promising alternative to traditional dosage forms due to their ability to provide sustained or controlled drug release, thereby improving therapeutic efficacy and patient compliance. Microspheres can be prepared using various techniques, including Spray drying, emulsion solvent evaporation, phase separation or coacervation and emulsification diffusion. Among all methods, the emulsion solvent evaporation technique is particularly popular due to its simplicity and effectiveness in preserving the potency of the active ingredient while producing stable microspheres.

The preparation of microspheres typically involves the creation of a stable emulsion where drug and polymer-containing dispersed oil droplets are evaporated to form microspheres. These microspheres can be made from a variety of materials, including natural and synthetic polymers, glass, and ceramics. They can be categorized into solid or hollow types, each serving different purposes. Solid microspheres are versatile and can be used in various applications depending on their size and material composition.[6] On the other hand, resonating microspheres are often used to reduce the bulk of materials, making them useful in applications like lightweight fillers. Glass microspheres, though less versatile, have specialized uses in adhesives, cosmetics, weight-reduction fillers, and retro-reflectors for road safety.

Recent research has shed light on the potential of gum Konda gogu, a natural gum belonging to the substituted rhamnogalacturonan group. This gum exhibits unique morphological, structural, and physicochemical properties that make it suitable for various applications, including as an emulsifier, stabilizer, and thickener in food products. Understanding the rheological properties of gum Konda gogu is crucial for its effective use in these roles. The background of drug delivery system (DDS),[7-10] the development of floating microbeads offers a significant advancement. These systems are designed to enhance gastric retention time and achieve controlled or sustained release of drugs, thereby improving

**Author for Correspondence: ranjan_pharma@yahoo.com*

oral bioavailability and reducing dose-dependent toxicity. The formulation of such floating microbeads can be optimized using various polymers and techniques. This study focuses on the formulation of oil-entrapped floating microbeads containing acyclovir, a model drug, using gellan gum and sodium alginate. The IEG technique, guided by a 3 factorial design, is engaged to develop these microspheres.

The study aims to explore the properties of different polymers and additives on the characteristics of the microbeads. Specifically, it investigates the use of low-density oils, such as olive oil, to improve the floating drug and a sustained release profile of the acyclovir microspheres. The optimization process involves evaluating various formulation parameters, including the concentration of release retardants based on viscosity and compatibility, as well as process variables such as the height of dropping, needle size, magnetic stirrer speed, concentration of curing agent, and curing time. Additionally, response surface methodology is utilized to refine the formulation and achieve the desired product characteristics.

By examining these factors, the study seeks to develop a robust and effective gastro-retentive delivery system for acyclovir that offers controlled release and enhanced bioavailability. The development of drug delivery technologies provides a more efficient means of administering medications with improved therapeutic outcomes.

MATERIAL AND METHOD

Material

Acyclovir - IPCA Laboratories Ltd., Mumbai, metronidazole - IPC, Ghaziabad, New Delhi gellan gum - Cipla Ltd., Mumbai, karaya gum - Nutriroma, Hyderabad, India, sodium alginate - Loba Cheme. Pvt. Ltd., calcium chloride - E-Merck (India) Limited, Mumbai, methanol - E-Merck (India) Limited, Mumbai, sodium hydroxide - Fisher Chemicals, Mumbai, hydrochloric., Mumbai, potassium dihydrogen phosphate-Fisher Chemicals, Mumbai, Barium sulfate and perchloric acid - SD Fine-Chem Ltd., Mumbai. White rabbits (*Oryctolagus cuniculus)* were bred in Animal House, Dadhichi College of Pharmacy (DCP), Odisha, India

Formulation of floating micro beads Acyclovir (ACV) by using Gellan gum

A significant challenge in designing a sustained-release DDS is to ensure that the dosage form remains in the stomach or upper small intestine long enough for the complete release of the drug within the desired timeframe.[11] This is crucial because most drugs are preferentially captivated in these areas of the gastrointestinal tract.

Process development

The fundamental principle of ionotropic gelation lies in the ability of polyelectrolytes to form hydrogel beads, or gelispheres, through cross-linking in the presence of counter ions. This mechanism has led to increased interest in using natural polymers as drug carriers. In gelispheres, polymer relaxation dictates the drug release rate. These spherical, cross-linked, hydrophilic polymer entities can significantly swell and gel in biological fluid simulations. This process allows for the encapsulation of biomolecules within the gelispheres under mild conditions, preserving their structural integrity.[12-14] Natural polymers, valued for their and biodegradability and biocompatibility, are increasingly used in drug delivery systems employing ionotropic gelation. Commonly used natural or semi-synthetic polymers include alginates, gellan gum, chitosan, pectin, and CMC. These polymers possess specific anions or cations in their chemical structures that interact with counter ions to form a cross-linked meshwork, facilitating gelation. Besides serving as a protective cover for the drug core, these naturally occurring polymers also contribute to a controlled release rate.

Retardant selection

Alginates are naturally occurring polysaccharides sourced from specific types of brown algae and certain bacteria, known for their non-toxic and biodegradable properties. Sodium alginate, a form of alginic acid, is a natural polysaccharide composed of 1,4-linked β-D-mannuronic acid (M) and α-D-guluronic acid (G) residues arranged in various configurations. When sodium alginate, which is soluble in water, interacts with divalent or polyvalent cations, it forms a cross-linked, insoluble network. The acid groups of alginate cross-link with calcium and zinc cations, creating this mesh-like structure. Commercially, gellan gum is produced through the aerobic submerged fermentation of *Sphingomonas elodea* bacteria.[15] To initiate gelation, a concentrated solution of gellan gum in water is first heated. Upon cooling, the chains undergo a conformational shift from random coils to double helices, resulting in a thermo-reversible hydrogel. This transition creates organized junction zones, leading to the sol-gel transition and forming a structured gel network.

Buoyancy-generating material (Inherent low-density agents) selection

Among the agents used to impart low bulk density to a dosage form are substances characterized by an inherently low-density property.[16] The concentration of oil as a low-density agent has been shown to influence the time to float. Oil-free beads sank in water, physiological saline, or HCl solution mimicking gastric juice, thereby increasing gastric residence time (GRT). The buoyancy of these dosage forms is achieved by including substances or agents that generate low density in the system.[17-19] Olive oil, commonly used in cooking, cosmetics, and pharmaceuticals, is generally non-toxic and non-irritant, making it a suitable low-density agent in FDDS.

Table 1: Composition of trial batch

Ingredients	*T1*	*T2*	*T3*	*T4*	*T5*
Acyclovir (mg)	250	250	250	250	250
Gellan Gum (mg)	125	125	125	125	125
Sodium Alginate (mg)	125	125	125	125	125
Olive oil (mL)	2	4	6	8	10

Table 2: Result of trial batch

Ingredients	*T1*	*T2*	*T3*	*T4*	*T5*
Yield (%)	78.54	79.32	82.56	64.45	58.34
Drug entrapment efficiency (%)	36.45	58.43	39.42	31.98	28.81
Mean microsphere size (µm)	321.08	325.87	420.56	534.67	585.12
Cumulative drug release (2 h)	34.56	29.87	36.34	40.46	41.51
Cumulative drug release (8 h)	58.76	52.56	55.51	67.45	69.89
Sphericity	Spherical	Spherical	Spherical	Irregular	Irregular

Table 3: Composition of factorial design batch

Formulation code	*Drug (mg)*	*Polymer*		*Olive oil (mL)*	*Water (mL)*	*$CaCl_2$ (%)*
		Gellan gum (mg)	*Sodium alginate (mg)*			
FG1	250	125	125	2	20	10
FG2	250	125	125	4	20	10
FG3	250	125	125	6	20	10
FG4	250	250	250	2	20	10
FG5	250	250	250	4	20	10
FG6	250	250	250	6	20	10
FG7	250	375	375	2	20	10
FG8	250	375	375	4	20	10
FG9	250	375	375	6	20	10

Floating microbeads manufacture

Microbeads were prepared by ionotropic emulsion–gelation technique according to the process flow presented below and then stored in a desiccator until use. Tables 1, 2 and 3 represent different batches of microbeads formulation.

Ionotropic emulsion–gelation technique-Process flow

- The corresponding quantities of drug and polymers were accurately weighted
- The powders were screened using ASTM #100.
- The corresponding amount of gellan gum was dissolved in 20 mL de-mineralized water and was heated to 80˚C to dissolve using a magnetic stirrer.
- After cooling corresponding amount of sodium alginate, acyclovir and olive oil were added to the above solution with continuous stirring for 30 minutes.

Determination of drug content and % entrapment efficiency of floating beads

Table 4 shows %yield, drug content, and % entrapment efficiency of floating beads. A mortar was used to crush a certain quantity of microbeads into a powder. The powder was dissolved in 0.1 N HCl to a weight of around 100 mg.[20-24] After two hours of ultrasonication, the solution was diluted to 100 mL. About 1 mL of the filtrate was further diluted to 100 mL following filtration using a 0.45 µm membrane filter using High-performance liquid chromatography analysis. The process used was a somewhat altered version of a previously published technique, utilizing an LC-20AD (Shimadzu). The following features of an Enable C18 column were used for chromatographic separations: Particle size: 5 µm; interval: 250 x 4.6 mm. About 50 aliquots at a flow rate of 1.5 mL/min.[25,26]

$$\%\text{Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoritical drug content}} \times 100$$

Determination of micromeritic properties of microbeads

The prepared microbeads were subjected to different studies as shown in Table 5.

Floating lag time and floating duration of floating beads

Floating lag time

Floating lag time is the time taken for all the beads to come over the surface of the solution from the time of dropping. The results are presented in Table 6.

Bouncy time

The test for bouncy is usually determined in 900 mL 0.1 N HCl solution maintained at Temperature 37 ± 0.5°C using dissolution apparatus II.[27, 28] The amount of time the dosage form needs to float is termed as the floating time. The results are presented in Table 6.

Swelling studies of floating beads

When determining the swelling capacity of monolithic systems (such as tablets or capsules) made of swellable hydrophilic polymers, the swelling index is frequently computed.[29] As these polymers swell, air is trapped inside of them, reducing the dosage form density. Drug-loaded microbeads that had

Table 4: %yield, drug content and %encapsulation efficiency of various formulations

Formulation	*Yield* * *(%)*	*Drug content* * *(mg)*	*Entrapment efficiency (%)*
FG1	80.12 ± 1.12	4.14 ± 1.17	37.73
FG2	81.80 ± 1.15	4.08 ± 2.01	66.12
FG3	83.89 ± 1.34	1.76 ± 1.67	41.12
FG4	82.78 ± 1.06	4.41 ± 1.82	44.59
FG5	85.37 ± 1.56	3.47 ± 1.34	59.82
FG6	87.77 ± 1.24	1.91 ± 1.29	46.47
FG7	89.50 ± 0.98	4.12 ± 1.78	45.67
FG8	90.54 ± 1.48	4.81 ± 1.30	87.61
FG9	94.39 ± 1.56	2.66 ± 1.53	67.34

*Values are represented as mean ± s.d.(triplicate)

been weighed were inserted into the USP dissolving equipment II's wire basket. Using 0.1 N HCL. The basket was taken out at various intervals and the weight was then measured again.[30] One way to quantify the swelling index is to look at its dimensions changes, weight gain, or water uptake16. Thus, the following formula is used to determine the swelling index.

$$\%\,\text{Swelling index}\ \% = \frac{(W_t - W_0)}{W_0} \times 100 \text{ -----------(Eq. 1)}$$

Where W_t is the final weight of dosage forms at a time "t" and W_o is the initial weight of dosage forms. The results are presented in Table 6 & Figure 1.

Scanning electron microscopy studies

SEM was utilized to analyze the particle size distribution, surface topology, texture, and fracture surfaces of the microbeads. SEM provides detailed three-dimensional images of the sample's surface relief by detecting secondary electrons emitted from the surface.[30] For SEM analysis, the sample was prepared by lightly sprinkling the powder onto a double adhesive tape affixed to an aluminum stub. The stubs were subsequently coated with a thin gold layer, approximately 300 Å thick, using a gold sputter module within a high-vacuum evaporator. This coating process was conducted under an argon atmosphere to ensure optimal conductivity of the samples.

Following the coating, the samples were examined using a SEM. The SEM was set to various magnifications to capture detailed photomicrographs of both the intact and cross-sectioned microbeads. The resulting images provided insights into the surface characteristics and structural integrity of the microbeads, as illustrated in Figure 2. These studies are essential for understanding the morphology and quality of the microbeads and for ensuring that they meet the required specifications for their intended application.

In-vitro dissolution study

Studies on *in-vitro* dissolution were planned to approximate *in-vivo* circumstances as much as feasible.[31] These investigations aimed to offer a quick, simple, and economical strategy that is consistent with the dosage form's performance in human participants. The conditions for the *in-vitro* dissolution test were standardized and clearly defined, facilitating the comparison of results across different samples. About 1-mL of the sample was pipetted directly for the dissolution study, filtered, and then diluted to a final volume of 25 mL with the appropriate dissolution media. The samples were then analyzed using HPLC, following the same setup and procedures as described earlier, to determine the drug content and entrapment efficiency.

The n (release exponent) values and rate constant (K_m) are given in Figures 3, 4 & 5.

In-vivo Floating Ability (Buoyancy) of ACV Microbeads Using X-ray Photography

To evaluate the spatial and temporal gastric retention of floating drug delivery systems, several techniques such as string tests, endoscopy, and gamma scintigraphy have been used. For this study, X-ray photography was chosen to assess the gastric residence time of floating microbeads. The experimental protocol for these in-vivo radiographic studies was reviewed and approved by the Institutional Animal Ethics Committee at Dadhichi College of Pharmacy, Cuttack, Odisha, India (Registration No. IAEC/DCP/029).

Table 5: Micromeritic properties of microbeads

Formulation Code	*Bulk density (gm/cc)* *	*Tapped density (gm/cc)* *	*Carr's index*	*Hausner's ratio*	*Angle of repose* *	*Avg. particle size (mm) (mean ± S.D)* *
FG1	0.355 ± 0.001	0.383 ± 0.001	7.31	1.07	21.88 ± 0.16	324.58 ± 7.3
FG2	0.334 ± 0.011	0.357 ± 0.008	6.44	1.06	23.09 ± 0.21	326.61 ± 7.8
FG3	0.335 ± 0.002	0.362 ± 0.001	7.45	1.08	23.09 ± 0.15	419.44 ± 8.21
FG4	0.304 ± 0.001	0.335 ± 0.016	9.25	1.10	21.45 ± 0.22	350.13 ± 8.4
FG5	0.297 ± 0.001	0.310 ± 0.001	4.19	1.04	20.95 ± 0.26	424.42 ± 5.8
FG6	0.361 ± 0.001	0.385 ± 0.006	6.23	1.06	20.29 ± 0.15	487.46 ± 6.4
FG7	0.363 ± 0.002	0.383 ± 0.002	5.22	1.05	24.02 ± 0.13	442.69 ± 5.4
FG8	0.359 ± 0.002	0.405 ± 0.002	11.35	1.12	21.65 ± 0.29	452.47 ± 6.8
FG9	0.486 ± 0.011	0.539 ± 0.007	9.67	1.107	22.26 ± 0.009	492.26 ± 8.4

*Values are represented as mean ± s.d.(triplicate)

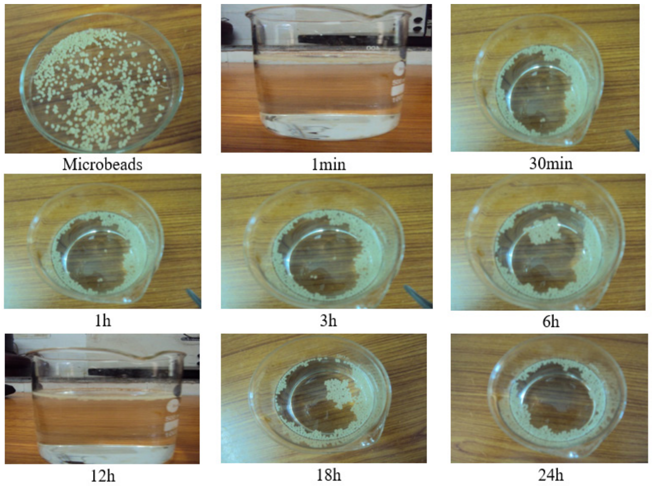


Figure 1: Photographs of capsule containing floating microbeads formulation of ACV

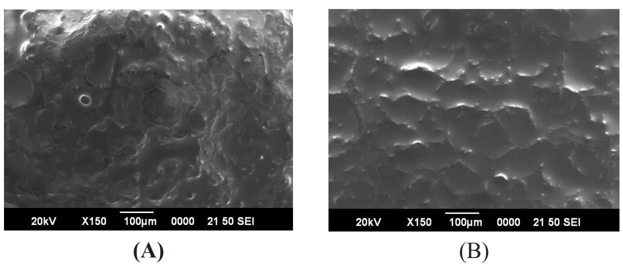


Figure 2: A. 22. SEM of intact (A) and cross-section (B) of microbead formulation (FG$_o$)

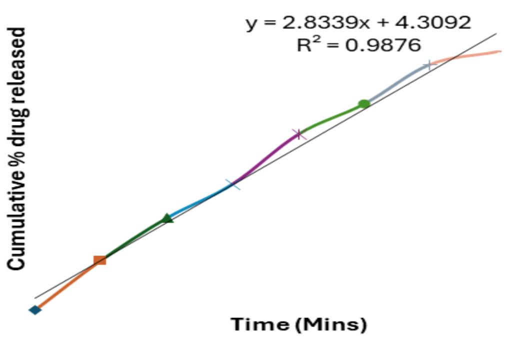


Figure 3: Dissolution profile of pristine drug (ACV)

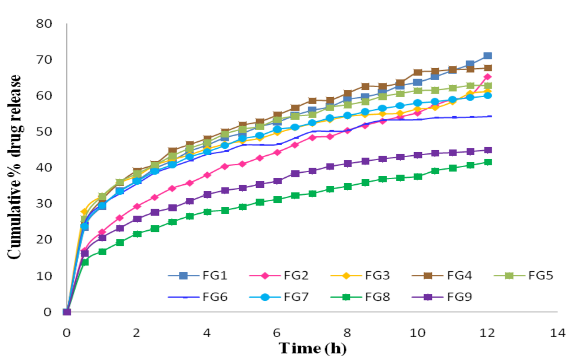


Figure 4: Cumulative %drug released of different formulations

Table 6: Floating properties of microbeads

Formulation code	*Floating lag Time(sec)*	*Bouncy time (h)*	*%Swelling index*
FG1	0	>24	1.4
FG2	0	>24	1.74
FG3	0	>24	2.0
FG4	0	>24	1.67
FG5	0	>24	1.90
FG6	0	>24	2.14
FG7	0	>24	1.84
FG8	0	>24	2.21
FG9	0	>24	2.34

X-ray imaging was observed to examine the intra-gastric floating behavior of optimized ACV microbeads in fasted rabbits (12 hours).[32-37] The rabbits were maintained standard laboratory at a temperature of 25 ± 2°C, acclimated for one week, and provided a fixed standard diet. The study involved healthy white rabbits weighing approximately 2.2 kg.

Barium sulfate, was used as a radiopaque marker, was incorporated into the microbead formulation. Before administration, a baseline radiographic image was captured to confirm the absence of radiopaque material in the gastrointestinal tract. The microbead-containing capsule was administered orally by placing it at the back of the rabbit's tongue, accompanied by a sufficient amount of water.[38-42]

The placement of the formulation in the stomach was monitored using X-ray imaging at 0.5, 1, 6, and 12-hour intervals. The distance between the X-ray source and the subject was kept constant throughout the imaging process. Between imaging sessions, the rabbits were allowed to move freely and engage in normal activities but were not permitted to eat.

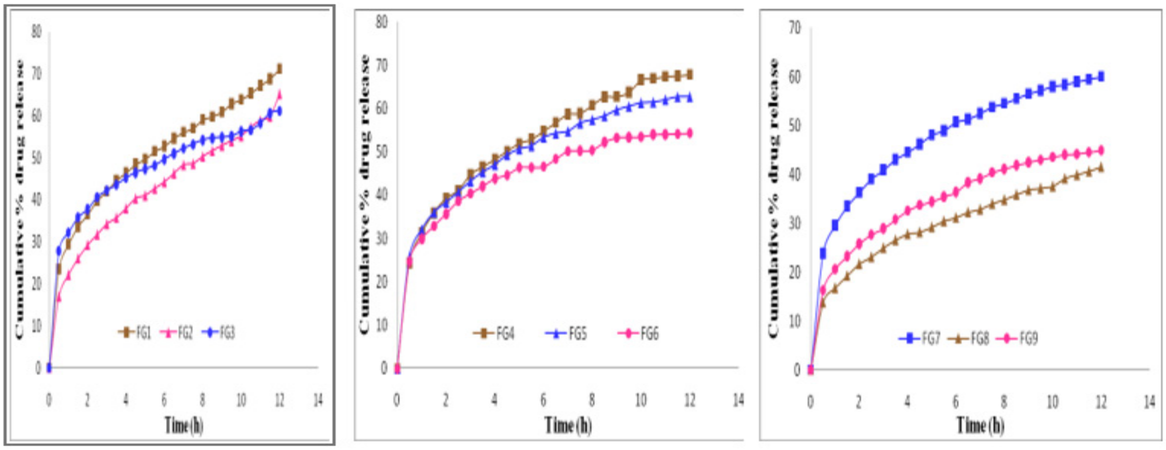
An *in-vivo* buoyancy study was performed to assess the gastric retention of the floating microbeads. Hard gelatin capsules containing $BaSO_4$-loaded floating microbeads were administered orally, and radiographic imaging was used to track their distribution and retention in the stomach.[43-45]

One hour after administration, the radiographic images clearly showed the presence of microbeads in the stomach. Initially, the microbeads appeared as dense, prominent images. Over time, the images became lighter, likely due to the distribution and scattering of the microbeads throughout the gastrointestinal tract. Despite this change, the radiographic results are indicated as shown in Figure 6.

This study demonstrates that the floating microbeads effectively maintained buoyancy and gastric retention, which is essential for their controlled-release function.

RESULTS AND DISCUSSIONS

The mean plasma concentration-time profiles for acyclovir resulting in the oral administration of both acyclovir-loaded floating microbeads and acyclovir solution are depicted.



(A) **(B)** **(C)**

Figure 5: (A) Cumulative% of drug released from the formulations FG1 to FG3 (B) Cumulative% of drug released from the formulations FG4 to FG6 C) Cumulative% of drug released from the formulations FG7 to FG9

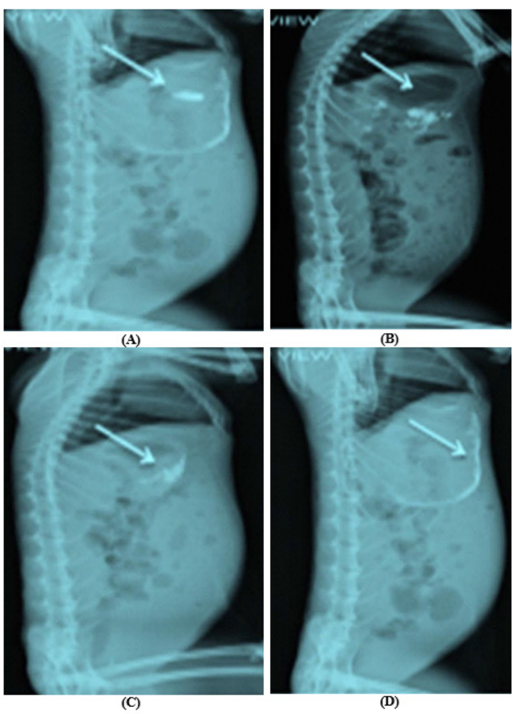


Figure 6: X-ray photographs of gastric FM of ACV FG_O & containing barium under fasted state (A) 0.5 hour, (B) 1 hour, (C) 6 hours (D) 12 hours

Following administration of the drug solution, acyclovir concentrations quickly peaked at 1.16 hours and then decreased rapidly. In contrast, the profiles for acyclovir-loaded floating microbeads showed a smoother pattern, with high plasma concentrations observed at 2.83 hours. These concentrations were maintained relatively steadily for up to 10 hours and slowly declined, with detectable plasma concentrations still present after 24 hours. In contrast, the plasma concentration of acyclovir solution fell below the limit of quantitation after 12 hours.

The elimination rate constant (Kel) was K_{el} (0.080h), $T_{1/2}$ (8.742 hours) and $AUC_{0-\infty}$ (34618.99 ng h/mL) for ACV solution, indicating a slower elimination for the formulation GO. The half-life (T1/2) was 8.742 hours for formulation GO and 3.801 hours for the solution. The area under the concentration-time curve from 0 to infinity (AUC0-∞) was significantly higher for ACV formulation GO (34618.99 ng·h/mL) compared to ACV solution (10446.1 ng·h/mL).

The improved oral bioavailability of acyclovir observed with the ACV formulation GO can be attributed to the extended residence time of the floating microbeads in the GIT. This prolonged retention allows the microbeads to maintain close contact with the absorption site, thereby enhancing drug absorption and overall bioavailability.

CONCLUSION

In this study, buoyant beads containing ACV were successfully developed using a 3^2-factorial design with both gellan gum–alginate and karaya gum–alginate formulations. These oil-entrapped microbeads demonstrated a sustained drug release profile, high drug entrapment efficiency, and effective buoyancy with minimal lag time. Such properties significantly enhance the bioavailability of ACV.

The extended gastric retention of the floating microbeads enabled enhanced absorption of ACV in the upper GIT. Primary pharmacokinetic studies in rabbits demonstrated a significant increase in the oral bioavailability of ACV, attributed to the prolonged residence time of the floating microbeads in the stomach.

The ionotropic emulsion–gelation technique employed for producing these buoyant beads proved to be straightforward, reproducible, cost-effective, and easily controllable. This method can be adapted for various other medications to develop similar gastro-retentive systems, potentially improving their bioavailability and therapeutic efficacy for gastrointestinal conditions.

REFERENCES

1. Singh BN, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. Journal of Controlled Release. 2000;63(3):235-259. https://doi.org/10.1016/S0168-3659(99)00204-7
2. Deshpande AA, Rhodes CT, Shah NH, Malick AW. Controlled-release drug delivery systems for prolonged gastric residence: an overview. Drug development and industrial pharmacy. 1996;22(6):531-539. https://doi.org/10.3109/03639049609108355
3. Hwang SJ, Park H, Park K. Gastric retentive drug-delivery systems. Critical Reviews™ in Therapeutic Drug Carrier Systems. 1998;15(3):243–284. DOI: 10.1615/CritRevTherDrugCarrierSyst.v15.i3.20
4. Hemalatha K, Lathaeswari R, Suganeswari M, Kumar SV, Shering AM. Formulation and evaluation of metoclopramide hydrochloride microbeads by ionotropic gelation method. International Journal of Pharmaceutical and Biological Archive. 2011;2:921-925.
5. Patil JS, Kamalapur MV, Marapur SC, Kadam DV. Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: a review. Digest Journal of Nanomaterials and Biostructures. 2010;5(1):241-248.
6. Al-Kassas RS, Al-Gohary OM, Al-Faadhel MM. Controlling of systemic absorption of gliclazide through incorporation into alginate beads. International journal of pharmaceutics. 2007;341(1-2):230-237. https://doi.org/10.1016/j.ijpharm.2007.03.047
7. Takka S, Ocak O, and Acartu, F. Formulation and investigation of nicardipine HCl–alginate gel beads with factorial design-based studies. European Journal of Pharmaceutical Sciences. 1998; 6: 241–246. https://doi.org/10.1016/S0928-0987(97)10005-7
8. Silva CM, Ribeiro AJ, Figueiredo IV, Gonçalves AR, Veiga F. Alginate microspheres prepared by internal gelation: Development and effect on insulin stability. International journal of pharmaceutics. 2006;311(1-2):1-10. https://doi.org/10.1016/j.ijpharm.2005.10.050
9. Santucci E, Alhaique F, Carafa M, Coviello T, Murtas E, Riccieri FM. Gellan for the formulation of sustained delivery beads. Journal of controlled release. 1996;42(2):157-164. https://doi.org/10.1016/0168-3659(96)01373-9
10. Ross-Murphy SB. Rheological characterization of polymer gels and networks. Polymer Gels and Networks. 1994;2(3-4):229-237. https://doi.org/10.1016/0966-7822(94)90007-8
11. Kanapczyk J, Karnicki Z, Brzeski M, Bykowski P, Woytasz-Pajak A. Some aspects of chitosan hydrogel ointment base formulation. Chitin World, Karnicki, ZS; Brzeski, MM; Bykowski, PJ. 1994:512-519.
12. Yang JS, Xie YJ, He W. Research progress on chemical modification of alginate: A review. Carbohydrate polymers. 2011;84(1):33-39. https://doi.org/10.1016/j.carbpol.2010.11.048
13. Coradin T, Bah S, Livage J. Gelatine/silicate interactions: from nanoparticles to composite gels. Colloids and surfaces B: Biointerfaces. 2004;35(1):53-58. https://doi.org/10.1016/j.colsurfb.2004.02.008
14. Tao Y, Lu Y, Sun Y, Gu B, Lu W, Pan J. Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. International journal of pharmaceutics. 2009;378(1-2):30-36. https://doi.org/10.1016/j.ijpharm.2009.05.025
15. Stithit S, Chen W, Price JC. Development and characterization of buoyant theophylline microspheres with near zero order release kinetics. Journal of microencapsulation. 1998;15(6):725-737. https://doi.org/10.3109/02652049809008255
16. Pongjanyakul T, Puttipipatkhachorn S. Xanthan–alginate composite gel beads: molecular interaction and *in-vitro* characterization. International Journal of Pharmaceutics. 2007;331(1):61-71. https://doi.org/10.1016/j.ijpharm.2006.09.011
17. Hadjiioannou TP, Christian GD and Koupparis MA. Quantitative Calculations in Pharmaceutical Practice and Research. New York, VCH Publishers Inc. 1993; 343-348.
18. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. International journal of pharmaceutics. 1983;15(1):25-35. https://doi.org/10.1016/0378-5173(83)90064-9
19. Malakar J, Nayak AK, Pal D. Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion–gelation method. International journal of biological macromolecules. 2012;50(1):138-147. https://doi.org/10.1016/j.ijbiomac.2011.10.001
20. Daihom BA, Bendas ER, Mohamed MI, Badawi AA. Development and *in-vitro* evaluation of domperidone/Dowex resinate embedded gastro-floatable emulgel and effervescent alginate beads. Journal of Drug Delivery Science and Technology. 2020;59:101941. https://doi.org/10.1016/j.jddst.2020.101941
21. Hua S, Ma H, Li X, Yang H, Wang A. pH-sensitive sodium alginate/poly (vinyl alcohol) hydrogel beads prepared by combined Ca2+ cross-linking and freeze-thawing cycles for controlled release of diclofenac sodium. International journal of biological macromolecules. 2010;46(5):517-23. https://doi.org/10.1016/j.ijbiomac.2010.03.004
22. ICH guide lines Q1A(R2) 'guidelines for industry,stability testing of new drug substance and product' (Available on: http://www.ich.org)
23. Talukdar MM, Van den Mooter G, Augustijns P, Tjandra-Maga T, Verbeke N, Kinget R. *In-vivo* evaluation of xanthan gum as a potential excipient for oral controlled-release matrix tablet formulation. International journal of pharmaceutics. 1998;169(1):105-113. https://doi.org/10.1016/S0378-5173(98)00112-4
24. Boxenbaum H. Pharmacokinetic determinants in the design and evaluation of sustained-release dosage forms. Pharmaceutical research. 1984;1: 82- 88. DOIhttps://doi.org/10.1023/A:1016355431740
25. Srikanth MV, Janaki Ram B, Sunil SA, Sreenivasa Rao N. and Ramana Murthy KV. Gastro retentive drug delivery systems: Novel approaches and its evaluation a review. International Journal of Pharmaceutical Sciences Review and Research. 2011; 10(1): 203-216.
26. Timmermans J, Moës AJ. Factors controlling the buoyancy and gastric retention capabilities of floating matrix capsules: new data for reconsidering the controversy. Journal of pharmaceutical sciences. 1994; 83: 18-24. https://doi.org/10.1002/jps.2600830106
27. Bahrami GH, Mirzaeei SH, Kiani A. Determination of acyclovir in human serum by high-performance liquid chromatography using liquid–liquid extraction and its application in pharmacokinetic studies. Journal of Chromatography B. 2005; 816: 327–331. https://doi.org/10.1016/j.jchromb.2004.11.038
28. Emami J, Bazargan N, Ajami A. HPLC determination of acyclovir in human serum and its application in bioavailability studies. Research in Pharmaceutical Sciences. 2009; 4(1): 47-54.
29. Nayak AK, Pal D. Development of pH-sensitive tamarind

seed polysaccharide–alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. International journal of biological macromolecules.2011; 49:784-793. https://doi.org/10.1016/j.ijbiomac.2011.07.013
30. Nayak AK, Das B, Maji R. Calcium alginate/gum Arabic beads containing glibenclamide: development and *in-vitro* characterization. International journal of biological macromolecules. 2012;51: 1070-1078. https://doi.org/10.1016/j.ijbiomac.2012.08.021
31. González-Rodrıguez ML, Holgado MA, Sánchez-Lafuente C, Rabasco AM, Fini A. Alginate/chitosan particulate systems for sodium diclofenac release. International Journal of Pharmaceutics. 2002; 232:225-234. https://doi.org/10.1016/S0378-5173(01)00915-2
32. Malakar J, Nayak AK, Pal D. Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion–gelation method. International journal of biological macromolecules. 2012;50 :140-148. https://doi.org/10.1016/j.ijbiomac.2011.10.001
33. Kagan L, Hoffman A. Selection of drug candidates for gastroretentive dosage forms: pharmacokinetics following continuous intragastric mode of administration in a rat model. European journal of pharmaceutics and biopharmaceutics. 2008;69: 238–246.
34. Devhare LD, and Gokhale N. Antioxidant and Antiulcer Property of Different Solvent Extracts of Cassia Tora Linn. Research Journal of Pharmacy and Technology. 2022;15(3);1109-1113. DOI: 10.52711/0974-360X.2022.00185
35. Tiwari R, Mishra J, Devhare LD and Tiwari G. An updated review on recent developments and applications of fish collagen. Pharma Times. 2023;55(6):28-36.
36. Adimulapu AK, Devhare LD, Anasuya Patil A, Chachda NO, G. Dharmamoorthy. Design and Development of Novel Mini Tablet Cap Technology for the Treatment of Cardiovascular Diseases. International Journal of Drug Delivery Technology. 2023;13(3):801-806. DOI: 10.25258/ijddt.13.3.04
37. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2-(4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
38. Gnana RPM, Devhare LD, Dharmamoorthy G, Khairnar MV, Prasidha R. Synthesis, Characterisation, Molecular Docking Studies and Biological Evaluation of Novel Benzothiazole Derivatives as EGFR Inhibitors for Anti-breast Cancer Agents. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):475-480. DOI:10.25258/ijpqa.14.3.03
39. Sonule M, Devhare LD, Babu MN, Gunjal SD, Varalaxmi S. Microemulgel-based Hydrogel of Diclofenac Sodium using Lipidium sativum as a Gelling Agent. International Journal of Drug Delivery Technology. 2023;13(4):1235-1239. DOI:10.25258/ijddt.13.4.19
40. Shriram BK, Devhare LD, Mehrotra A, Deokar SS, Singh SP. Formulation and Evaluation of Mosquito Repellent Stick. International Journal of Drug Delivery Technology. 2023;13(4):1283-1286. DOI:10.25258/ijddt.13.4.26
41. Choudhary RK, Beeraka S, Sarkar BK, Dharmamoorthy G, Devhare L. Optimizing Verapamil Hydrochloride In-situ Delivery: A Strategic Formulation Approach using Box-Behnken Design for Enhanced Performance and Comprehensive Evaluation of Formulation Parameters. International Journal of Drug Delivery Technology. 2024;14(1):61-70. DOI: 10.25258/ijddt.14.1.11
42. Kumar KK, Kiran V, Choudhary RK, Devhare LD, Gunjal SD. Design Development and Characterization of Nicardipine Solid Lipid Nano-Particulars. International Journal of Drug Delivery Technology. 2024;14(1):71-78. DOI: 10.25258/ijddt.14.1.12
43. Priya MGR, Prasanth LML, Devhare LD, Yazdan SK, Gunjal S. Synthesis, DNA Binding, Molecular Docking and Anticancer Studies of Copper (II), Nickel (II), and Zinc (II) Complexes of Primaquine-based Ligand. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):69-75. DOI: 10.25258/ijpqa.15.1.10
44. Uplanchiwar VP, Raut SY, Devhare LD, et al. Pharmacological Assessment of Antiulcer Activity of Gloriosa Superba Linn Tubers In Experimentally Induced Gastric Ulcers. Journal of Medical Pharmaceutical and Allied Science. 2021;10(3);2852-2856.
45. Tiwari G, Gupta M, Devhare LD, & Tiwari R. Therapeutic and Phytochemical Properties of Thymoquinone Derived from Nigella Sativa. Current Drug Research Reviews. 2024;16(2):145-156. DOI: https://doi.org/10.2174/2589977515666230811092410