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Evaluation of Acyclovir Loaded oil entrapped Calcium alginate Beads Prepared by Ionotropic Gelation Method

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Abstract: The purpose of this research was to prepare acyclovir floating alginate beads to improve its poor, variable oral bioavailability and patient compliance by prolonging the residence time in the gastrointestinal tract. Oil entrapped floating beads prepared by the emulsion gelation method were optimized for polymer: cross linking agent ratio (sodium alginate/ Calcium chloride), oil concentrations (10%, 20% and 30% w/w) and drug: polymer (D: P) ratios (1:1, 2:1 and 3:1). The prepared beads were evaluated for diameter, surface morphology, encapsulation efficiency, buoyancy and in-vitro release. The results clearly indicated that the percentage of oil plays an important role in controlling the floating of oil-entrapped Calcium alginate beads. *In vitro* drug release in the fed state conditions demonstrated sustained release of Acyclovir for 8 h, which best fitted the Higuchi model with $n < 0.5$. Calcium alginate beads containing 20% oil and 2:1 D: P ratio showed an optimum DEE (89.54 %). Scanning electron microscopy revealed that the beads were spherical in shape with rough surface. These results demonstrate that the oil entrapped gel beads can be used as floating drug delivery system for systemic drug delivery

Key-words: Floating drug delivery system; Emulsion gelation; Calcium alginate; olive oil; Acyclovir.

INTRODUCTION:

The most convenient method for controlled delivery is undoubtedly oral but oral controlled release formulations that exhibit greater absorption in stomach and upper small intestine have not been successfully prepared with conventional oral approach[1]. Absorption window in the proximal gut can limit the bioavailability of orally administered compounds and can be a major obstacle to the development of controlled release formulations for important drugs[2, 3]. Two main approaches are presently being explored: (I) bio adhesive dosage forms that have a slow intestinal transit; and (2) the gastro retentive dosage system[4]. These systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients [5, 6, 7].

Acyclovir is a potent antiviral drug with low toxicity belonging to the deokiguanosin family. It is widely prescribed for the treatment of herpes simplex virus infections as well as in the treatment of varicella zoster infection[8]. It has maximum absorption in stomach and upper part of small intestine. Due to low gastric retention, the bioavailability of drug is low (10-20%) as large portion of drug misses the absorption window when given orally owing to an important first pass metabolism. The recommended adult dosage of acyclovir is 200mg twice daily or 400mg once daily[9]. The effective treatment of genital herpes simplex requires administration of 1000mg of acyclovir in 5 divided doses a day. An alternative dose of 800mg leads to plasma fluctuations, thus a sustained release dosage form of acyclovir is described. The short biological half life of drug (~2-3 hours) also favours development of a sustained release floating formulation[9].

The aims of this study were to develop oil-entrapped Calcium alginate beads and to investigate their morphology, floating behavior entrapment efficiency and in- vitro release. The effect of selected factors, such as polymer: cross linking agent ratio (sodium alginate/ Calcium chloride), oil concentrations (10%, 20% and 30% w/w) and drug: polymer (D: P) ratios (1:1, 2:1 and 3:1) on bead size, morphology, floating properties and in-vitro release was also investigated. Published literature on this method of GRDDS has been outlined in Table 1.

MATERIALS AND METHODS:

Materials:

Sodium alginate was purchased from Sigma-Aldrich Chemicals (India); Acyclovir was generous gift sample of Kwality Pharma Pvt Ltd, Amritsar. Light mineral oil was of standard pharmaceutical grade and all other chemicals used were of analytical grade.

Methods:

Preparation of Acyclovir floating calcium alginate beads:

Formulations F1-F16 were prepared by emulsion gelation method[10]. Sodium Alginate (1% 2% 3% and 4%w/v) was dissolved in distilled demineralized water with agitation. Acyclovir and different concentrations of olive oil (10%, 20% and 30% w/w) were added to the polymer solution to make 100-g mixtures. To ensure emulsion stabilization, the mixtures were homogenized at 10,000 rpm using a homogenizer (Erweka, type 4R401, Germany) for 10 min.This solution was dropped through 23 G needle in to 1%,2%,3% and 4%w/v calcium chloride (250 ml) and left at room temperature for 20 minutes. The resultant beads were washed twice with distilled water and kept for drying at room temperature up to 12 hours.

Optimization of floating calcium alginate beads:

In this study three factor namely, concentration of sodium alginate (1%2%3%and4%), concentration of calcium chloride as a cross linking agent (1%2%3%and4%) and percentage of oil were selected as independent variables while bead size, shape, buoyancy, drug entrapment efficiency, density, flow properties and non leakage of oil were the dependent variables used for optimization of process variables (independent variables) in preparation of floating calcium alginate beads.

Evaluation of acyclovir loaded floating alginate beads:

Micromeritic studies:

Study of Size and uniformity of beads:

To prepare uniform beads (i.e. of the same size and density) it is essential that synthesis conditions such as viscosity, rate of falling of drops, stirring rate and distance between syringe and gelation medium, be maintained constant during the course of the formation of beads. Variation in any of these parameters during the bead formation process may result in the production of non-homogenous and non-uniform beads, affecting the overall results to an appreciable extent[11]. The process homogeneity can be greatly influenced by emulsion homogenization which yields fine dispersion of oil and water with size uniformity. Without homogenization, the oil might separate out from the solution and uneven sized beads may be formed[12]. In order to test the product uniformity, the individual diameters of 20 dried Calcium alginate beads were measured with a calliper[13]. The results are expressed as the mean diameter (mm) ± standard deviation.

Density measurement: [14]

The mean weight and diameter of the beads was measured and used to mathematically calculate the densities of the spherical calcium alginate beads using the following equations:

$D=M/V$

$V=4/3\pi r^3$

Where *D* is the density of the beads; *M* is the weight of the beads; *V* is the volume of the beads; *r* is the radius of the beads.

Buoyancy of the Floating beads:

The floating ability was determined using USP dissolution tester apparatus II (Paddle method). Fifty beads were put in the vessel and the paddles were rotated at 50 rpm in 500 ml 0.1 N HCL pH 1.2, maintained at 37±0.5 °C for 12 hours. The floating and the settled portion of beads were recovered separately. Buoyancy percentage was calculated as the ratio of the number of beads that remained floating and total number.The floating ability of the beads was measured by visual observation and the percent of floating beads was taken as the average of three determinations. The preparation was considered to have buoyancy, only when all beads floated on the test solution immediately or within a lag time which did not exceed 2 min[14].

Determination of drug entrapment efficiency (DEE):[14]

An accurately weighed amount of 200 mg of acyclovir loaded Calcium alginate beads was dissolved in 250 ml of 0.1N HCL pH 1.2 by stirring for 6 h using magnetic stirrer. The resulting solution was then filtered using 0.45 m Millipore filter (Sartorius, GmbH, Germany) Acyclovir content was determined spectrophotometrically (Shimadzu UV–visible 1601 PC, Kyoto, Japan) at the predetermined λ max (255 nm). The determinations were made in triplicate and

DEE was calculated according to the following equation:

$$DEE\ (\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Scanning electron microscopy (SEM):

Morphological examination of the surface and internal structure of the dried Calcium alginate beads was carried out using a scanning electron microscope (JEOL JEM-1200 EX II, Japan) equipped with secondary electron detector at an accelerating voltage of 10 kV. The samples were coated with gold to a thickness of about 30 nm in a vacuum evaporator. The internal structure of beads was examined by cutting them with a steel blade.

In vitro Acyclovir release studies[14]:

The in vitro release studies were carried out using USP rotating basket apparatus-Pharma test, type PTW-2, Germany (apparatus I). Amounts of beads equivalent to 200 mg acyclovir were introduced into the baskets which were rotated at 50 rpm in 900 ml 0.1 N HCL (pH 1.2)and in simulated gastric fluid(pH 3.12) maintained at 37 ± 0.5 °C. Aliquots of 5 ml of the solution were withdrawn at predetermined time intervals and replaced by fresh dissolution medium. The withdrawn samples were analyzed for acyclovir content spectrophotometrically at λmax (255nm).None of the ingredients used in the bead formulations interfered with the assay. The results were expressed as the mean of three experiments.

Kinetic of drug release[15]:

The result of in-vitro dissolution studies of beads were fitted with various kinetics models, like zero order (% cumulative drug release vs. time), first order (log %drug remaining vs. time), Higuchi's model (% cumulative drug release vs. square root of time) but these models failed to explain drug release mechanism due to swelling (upon hydration) along with gradual erosion of the matrix. Therefore the dissolution data were also fitted to well-known Korsmeyer and Peppas semi-empirical model to ascertain the mechanism of drug release.

$$\log (Mt/M\infty) = \log k + n \log t$$

Where, $M\infty$ is the amount of drug release after infinite time; k is the release rate constant which considers structural and geometric characteristics of the beads; and n is the diffusional exponent; indicative of the mechanism of drug release. Table2 shows an analysis of diffusional release mechanism obtained by various value of n. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test. The data were processed for regression analysis using MS EXCEL statistical function.

Biodegradability studies of gel beads:

The biodegradability studies were carried out using USP rotating basket apparatus-Pharma test, type PTW-2, Germany (apparatus I). Amounts of beads (200 mg) were introduced into the baskets which were rotated at 50 rpm in 900 ml of different pH buffer solution (5.0, 6.8, 8.0) maintained at 37 ± 0.5 °C.

TABLE -1: CALCIUM ALGINATE BEADS PREPARED BY IONOTROPIC GELATION METHOD

S.No	Major Area of research	Drug
1	Floating sodium alginate/dextran-based hydrogel beads	
2	Oil entrapped floating gel beads	Amoxicillin trihydrate, Metformin hydrochloride, loratadine, Famotidine, metronidazole,
3	Calcium-alginate-phosphate composite gel beads	Diclofenac Sodium, Hydrocortisone,
4	Floating Calcium alginate gel beads	Riboflavin, Theophylline, Diclofenac, Metronidazole, nicardipine HCl, Propranolol hydrochloride, paracetamol
5	Tamarind gum and sodium alginate composite gel beads	
6	Calcium alginate and chitosan treated calcium alginate beads	Verapamil hydrochloride, Diclofenac hydroxyethylpyrrolidine, macromolecular drug

TABLE -2: RELEASE MECHANISM WITH VARIATION OF N VALUES

n value	Mechanism
n≤0.5	Quasi-fickian diffusion
0.5	fickian diffusion
0.5≤n≤1.0	Anomalous(non-fickian) diffusion
n≥1.0	Non –fickian super case 11
1	Non –fickian case 11

RESULT AND DISCUSSION:

Optimization of floating calcium alginate beads:

Optimization of sodium alginate and calcium chloride ratio:

Table 3 shows that the DEE increased by increasing sod. Alg. concentration from 1 to 3% w/v. Increasing Sod. Alg. Concentration above 3% w/v increased the viscosity of the alginate solution to the extent that the formation of drops was strongly hindered. Thus 3% w/v of Sod. Alg. was maintained in all the subsequent formulations. Further inspection of the results it was found unexpectedly that increasing Cal. Chloride concentration (1, 2, 3 and 4% w/v) decreased the DEE in the beads (63.13, 58.16, 55.52 and 46.84% respectively). Thus Cal. Chloride concentration was maintained at 1% w/v in all next formulation. Formulation that showed 63.13 % DEE and consisting of 3% w/v Sod. Alg. and 1% w/v Cal. Chloride was selected for further investigations.

Optimization of oil concentration:

The formation of gel beads of calcium alginate using oils is a simple and rapid process. The incorporation of oil into the drug-alginate solution was done with and without homogenization. Without homogenization, the oil separated out and uneven sized beads were formed. On increasing the homogenization time, the size of the beads formed decreased and size uniformity was obtained. The concentration of drug and polymer throughout the study was kept constant but concentration of olive oil utilized was altered and the data is tabulated in table-4. The results show that DEE reached its maximum by increasing oil concentration up to 20% (63.93) without oil leakage and showed excellent floating ability. The mean diameter of oil entrapped formulations ranged from 1.61mm (10%oil), 175mm (20%oil) and 2.16 mm (30%oil). It was observed that amount of oil affected the morphology of beads. An increase in concentration of oil caused increase in size and sphericity of the beads, which could be due to their density and volatility. Formula that showed floating immediately without oil leakage and showed maximum DEE was selected for further investigations.

TABLE- 3: OPTIMIZATION OF SODIUM ALGINATE AND CALCIUM CHLORIDE RATIO:

Formulation code	Calcium chloride (%w/v)	Sodium alginate (%w/v)	DEE (%)	Floating property (0.1N HCL)	Spherical Shape	Free flowing
F1	1	1	46.01	>12hr	yes	yes
F2	2	1	42.46	> 12hr	yes	yes
F3	3	1	37.56	> 12hr	yes	yes
F4	4	1	33.86	>12hr	yes	yes
F5	1	2	56.71	>12hr	yes	yes
F6	2	2	54.47	> 12hr	yes	yes
F7	3	2	50.96	>12hr	yes	yes
F8	4	2	47.28	> 12hr	yes	yes
F9	1	3	63.13	>12hr	yes	yes
F10	2	3	58.16	>12hr	yes	yes
F11	3	3	55.52	>12hr	yes	yes
F12	4	3	46.84	> 12hr	yes	yes
F13	1	4	Not available	>12hr	No	No
F14	2	4	Not available	>12hr	No	No
F15	3	4	Not available	>12hr	No	No
F16	4	4	Not available	> 12hr	No	No

*Olive oil concentration remained constant (20%) in all the above formulation

TABLE-4: OPTIMIZATION OF OIL CONCENTRATION:

Formulation Code	Oil concentration (%W/W)	Drug: Polymer Ratio (%W/W)	Mean Diameter(mm) (mean ±SD)	Density (g/m3)	Drug Entrapment Efficiency (%) (mean ±SD)	Buoyancy	Oil leakage
F9A	10	1:1	1.61±0.16	0.1737	47.08±0.97	S	No
F9B	20	1:1	1.75±0.15	0.2328	63.93±1.12	S-F	No
F9C	30	1:1	1.83±0.16	0.2636	61.13±0.85	F	Yes

Abbreviations: S = sink, F = float (immediately, and still afloat for at least 24 h), S→F = sink immediately and then gradually float,

Optimization of drug: polymer ratio:

Table 5 shows that the DEE increased from 53.14% to 89.54% by increasing the acyclovir: Sod. Alg. ratio from 1:1 to 3:1, respectively. Due to the statistically non-significant difference in DEE observed between the acyclovir: Sod. Alg. ratio 2:1 and 3:1 respectively, the smaller ratio (2:1) was chosen in all the next formulae. Formula that showed 89.54% DEE and consisting of 3% w/v Sod. Alg. and 1% w/v Cal. Chloride at 2:1 D/Sod. Alg. ratio was selected for further investigations.

Study of Size and uniformity of beads:

The mean diameters of the Acyclovir-loaded calcium alginate beads with small values of standard deviation are shown in Table4, confirmed high process uniformity regarding homogenization efficiency and low variability in processing conditions. Further inspection of the results reveals that the mean diameter of the calcium alginate beads with olive oil ranged between 1.61±0.09 and 1.83±0.07 mm. It was found that the particle size distribution of each formulation was within a narrow range. Different % w/w of Olive Oil to alginate were used to determine the effect of the Olive Oil on the size of the floating Beads. It was observed that an increase in oil concentration led to an increase in beads size. This might be due to the increased droplet viscosity by higher oil content; Droplets of lower viscosity were efficiently stirred, with a reduction in emulsion droplet size leading to smaller bead formation. Similar results were reported[16].It should be pointed out that, by visual inspection; the dried Calcium alginate beads were of spherical geometry.

Density measurement:

The results obtained for the diameter and weight of calcium alginate beads were used to calculate their density**.** Table-4 shows that the calculated densities of all the prepared beads were less than the density of 0.1 N HCL (i.e. 1.004 g cm^3) imparting their flotation .Their values ranged from 0.1737 to 0.2636. It is to be noted that formula prepared with 10% olive oil displayed lower beads-densities values compared to those prepared with 20%and30% olive oil. The results therefore suggest that the calcium alginate beads should float when placed in aqueous medium.

TABLE-5: OPTIMIZATION OF DRUG: POLYMER RATIO:

Formulation Code	olive Oil concentration (%W/W)	Drug: Polymer Ratio (%W/W)	Drug Entrapment Efficiency (%) (mean ±SD)
F9B1	20	1:1	53.14%±1.05
F9B2	20	2:1	89.54%±0.93
F9B3	20	3:1	87.89%±0.99

TABLE- 6: RELEASE KINETIC EQUATION VALUES OF THE OPTIMIZED FORMULATIONS

Formulation	Zero order		First order		Higuchi		Korsmeyer-peppas	
F9B2	K	R^2	k	R^2	k	R^2	n	R^2
	9.949	0.980	-0.115	0.976	35.89	0.981	0.636	0.978

Determination of the beads buoyancy:
Table 4 shows how the oil loadings affect the buoyancy of the alginate beads. All non-oily beads failed the buoyancy test as several specimens began sedimentation either upon contact with the SGF (simulated gastric fluid) or soon after agitation started. In contrast, all oily samples stayed afloat for a 24 h test cycle. Table also lists the buoyancy of the drug loaded beads. The results show that the buoyancy decreased for the beads with less oil inclusion or more drug incorporation. Different results were obtained when acyclovir was loaded in Conventional Calcium alginate beads, that is, the acyclovir -loaded Calcium alginate beads containing olive oil (10%), did not float immediately. This is probably due to the increased density of the beads when the drug was added. However, the beads were then a float after the drug was released from the beads. Additionally, the amount of oil required to keep the beads a float was increased. The acyclovir -loaded Calcium alginate beads containing olive oil (20%) floated immediately in SGF. Formula that showed floating immediately without oil leakage and consisting 20 % olive oil was selected for further investigations.

Determination of drug entrapment efficiency (DEE):
The inspection of the results (Table-4) reveals that for calcium alginate beads, on keeping D: P ratio constant, DEE reached its maximum by increasing oil concentration up to 20% and The DEE of calcium alginate beads with olive oil(20%) ranged between 53.14% and 89.54% as shows in table-5 .Further inspection of the results(Table-5) reveals that, at given oil concentration(20%), increasing the initial drug loading resulted in a significant increase in DEE up to 2:1 D: P ratio. Higher D: P ratio (3:1) did not affect DEE significantly. From these findings, it could be concluded that 2:1 D: P ratio has the optimum DEE with 20% olive oil concentrations in calcium alginate beads.

Scanning electron microscopy (SEM):
Samples were taken from optimized formulations and operating conditions for SEM observation. The oil-entrapped Cal. alginate structure formed showed the sponge-like structure where the oil was entrapped. This sponge-like structure may correspond to the egg-box structure of calcium alginate (as proposed in Figure 3), which was rigid and water insoluble. The pores of the oil-entrapped Cal. Alg. beads represented the oil droplets, and their size was influenced by concentration of oil.Figure1and Fig 2 shows the external and internal morphology of an oil-entrapped Cal.alg. Bead made of 20% olive oil. The morphology of the external and internal structure was identical. The surface of oil-entrapped cal. Alg. beads showed small pores containing oil droplets dispersed all over the structure. These are unique to the floating calcium alginate beads and enable floatation.

In-vitro Acyclovir release studies:
In vitro drug release study of acyclovir gel beads was carried out both in the fasted state, pH 1.2, and in the fed state, pH 3.12, for a period of 8 h. In the fasted state, gel beads exhibited a biphasic release profile as an initial rapid drug release phase (burst effect) was followed by a slower, gradually declining drug release phase after two hour extending up to 8 h (Fig. 1). F9B2 released 77.46 ± 2.0% acyclovir within two hours, followed by a sustained release profile for 8 h. Marketed tablet formulation containing 200 mg of acyclovir, when dissoluted under similar conditions, released more than 88% of the drug within 1 h but could not sustain the release over the following 8 h, but rather exhibited a rapid first-order decline. This release behaviour substantiates the use of acyclovir emulsion gel beads as a drug delivery system for modifying the release characteristics of the drug. Under simulated fed state conditions, acyclovir gel beads (F9B2) did not exhibit a burst release and sustained drug release was observed. The cumulative amount of drug released in a sustainable manner, from F9B2 was 87.89±1.0%, at the end of 8 h (Fig.1). Absence of burst effect in fed state may be correlated to the chemical nature of the drug. Being basic in character, it readily dissolves at lower pH values of the fasted state, but as the pH increases (fed conditions) the solubility reduces. The dissolution profiles obtained in the fed state indicate concomitant administration of meals as an essential aspect to obtain the desired controlled release of the gastro retentive formulation of acyclovir.

Kinetic of drug release:
The data were processed for regression analysis using MS EXCEL statistical function. The results are shown in Table 6 and graphs in figure 5 to 8.The values of kinetic constant (k) and correlation coefficient (R^2) calculated from equations are presented in Table 6. As observed from the table, correlation coefficients (R^2) of optimized formulations were high enough to evaluate the drug dissolution behaviour using equation (R^2: 0.976- 0.981).The *in-vitro* drug release of optimized formulation in fed state showed the highest regression coefficient values for higuchi model(0.981), thus indicating absolute correlation between the two variables for the Higuchi model. Optimized formulations followed Higuchi's equation proving that the release is by diffusion mechanism. The values of release exponent (n) were calculated from Korsmeyer and Peppas equation and the 'n' values was determine

to be 0.636 indicating Anomalous (non-fickian) diffusion.

Biodegradability studies of gel beads:

Biodegradability studies revealed that the alginate beads F9B2 was found to disintegrate and dissolve in intestinal pH within 3 hours (Figure 9). Formulation F9B2 seemed to completely biodegrade in intestinal fluid, and it is the pH of media, which is responsible for slow dissolution of the beads in intestinal fluid. This indicates that after gastric emptying the regular shaped beads, gradually become rough with an irregular surface and thereafter was degraded. Thus the alginate beads proved to be suitable gastro retentive dosage form, as they have a rigid structure that resist biodegradation in gastric pH but exhibit complete biodegradation in phosphate buffer pH 8.0.

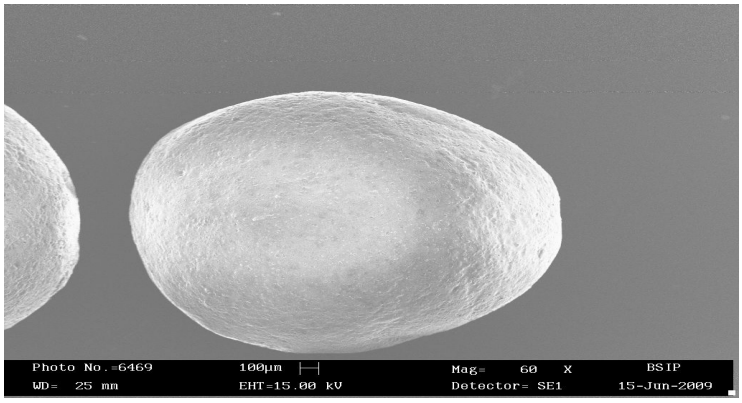

Fig.1: Scanning electron microscope (SEM) photograph of Optimized Formulation (External Structure).

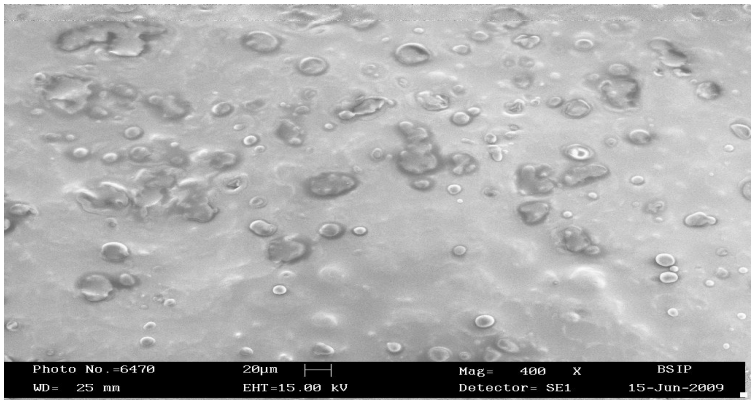

Fig. 2: Scanning electron microscope (SEM) photograph of Optimized Formulation (Enlarged External Structure).

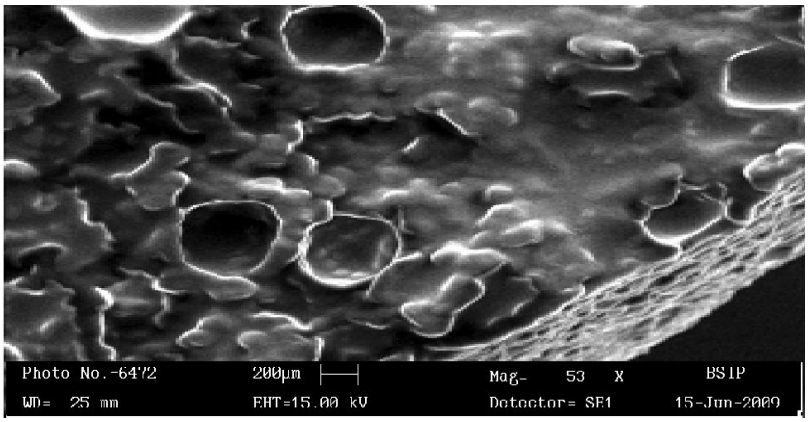

Fig. 3: Scanning electron microscope (SEM) photograph of Optimized batch (Internal Structure).

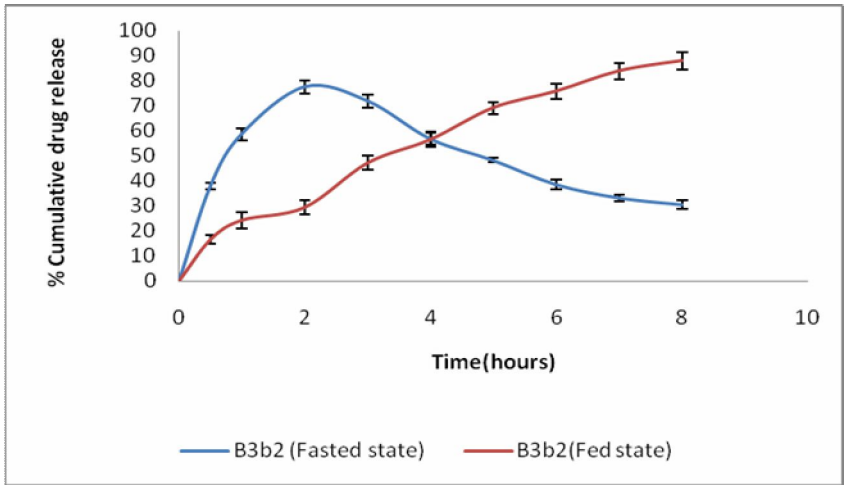

Fig.4. Drug release profiles of optimized formulation F9B2 in fasted and fed states (Mean± SD, *n* = 3).

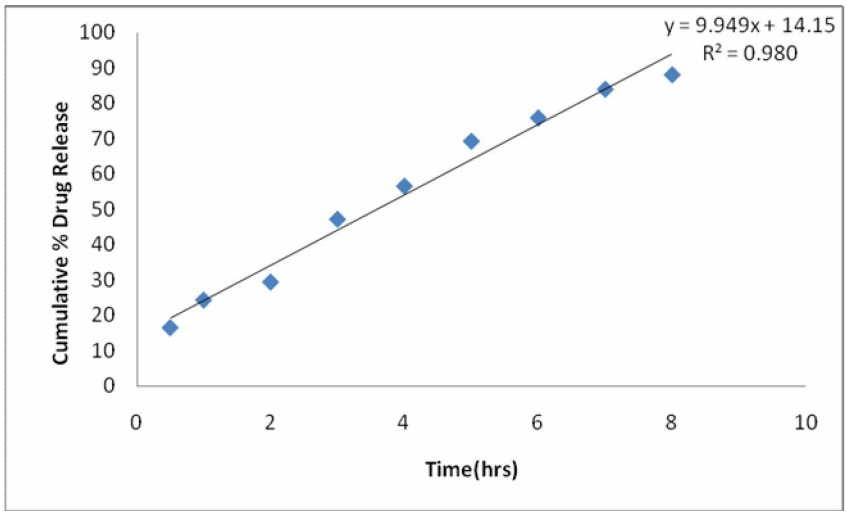

Figure 5: Zero order release kinetics of optimized formulation (F9B2)

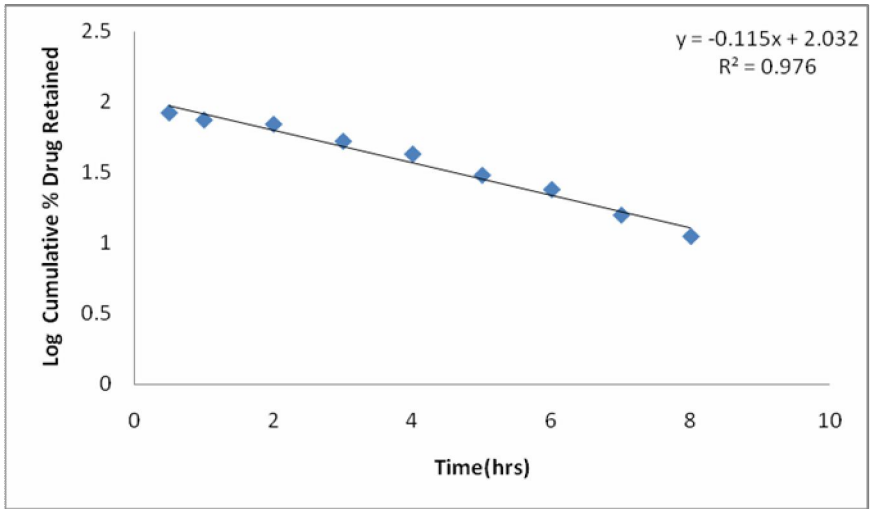

Figure 6: First order release kinetics of optimized formulation (F9B2)

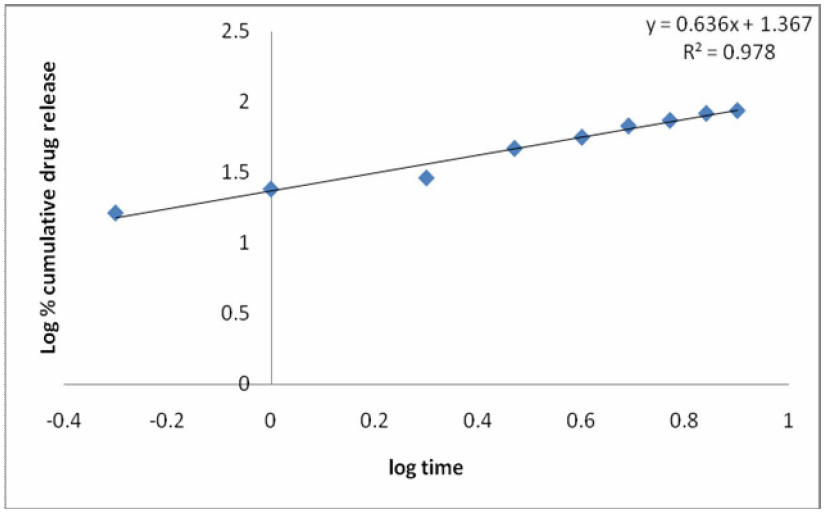

Figure 7: Korsmeyer and Peppas release kinetics of optimized formulation (F9B2)

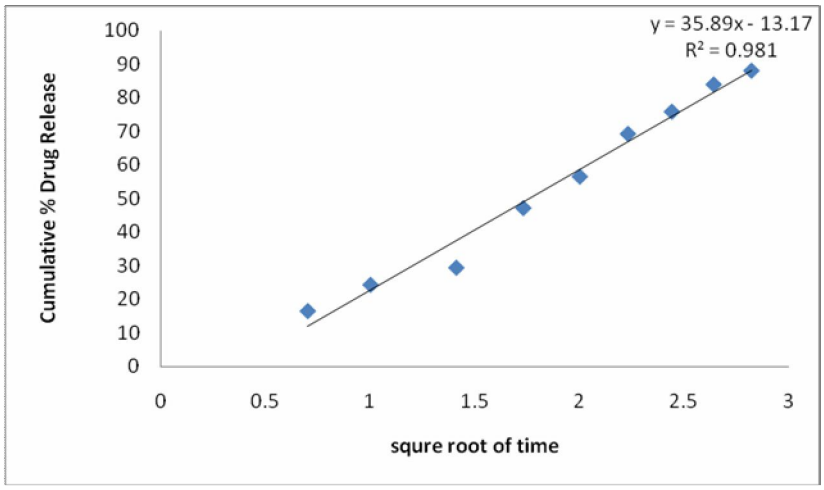

Figure 8: Higuchi matrix release kinetics of optimized formulation (F9B2)

pH 5.0 **pH 6.8** **pH 8.0**

Figure 9: Images of complete biodegradation of Optimized Formulation after 3 hours

CONCLUSION:

A new sustained release system of oil entrapped calcium alginate beads were designed and prepared by an emulsion gelation method and it's morphological and release characteristics were studied. The prepared beads were easy to prepare and the mean diameter of beads increased with increase in the amount of the oil phase. The pore size of oil-entrapped beads was affected by concentration of the oil. The beads showed excellent sustaining properties as compared to the conventional beads. The designed therapeutically efficacious gastro retentive formulation of acyclovir combining an excellent buoyant ability and suitable drug release pattern could possibly be advantageous in terms of increased bioavailability of acyclovir. Biodegradability studies in phosphate buffer conclude complete biodegradation of formulation thus avoiding accumulation in the body. Diffusion was found to be the main release mechanism. Thus oil entrapment technique can become a useful tool for systemic drug delivery.

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