Fabrication and Evaluation of Fluvastatin Hydrogel Beads

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ABSTRACT

The aim of the present work is to provide a therapeutic amount of Fluvastatin into the proper site in the body and also to achieve and maintain the desired concentration. Fluvastatin is slightly soluble in water. It is having half-life of 2hrs and low bioavailability. In the present study nine formulations were fabricated by using several polymers like HPMC K15M, Carbapol 934P and HPMC K4M in different proportion. The FTIR Spectra revealed that, there was no interaction between polymers and Fluvastatin. As the polymer ratio was increased, the mean particle size of Fluvastatin hydrogel beads was also increased. From the results it can be inferred that there was a proper distribution of Fluvastatin in the hydrogel beads and the deviation was within the acceptable limits. On the basis of released data and graphical analysis formulation F9 containing Carbapol 934P in higher proportion showed a good controlled release profile up to 12hrs with maximum entrapment efficiency because of high polymer concentration and follows zero order kinetics with super case II transport mechanism.

Keywords: Fluvastatin, Hydrogel beads, Carbopol 934P, FTIR, HPMC K15M, HPMC K4M.

INTRODUCTION

In the past few years, the advances in the ground of biomaterials has led to various studies on alternative biocompatible materials and the development of these materials focusing on properties, benefits, limitations, and the use of substitute resources (such as polysaccharides and proteins) for its preparations. Among the most studied biomaterials, hydrogels (HGs) have been standing out owing to their advantages, like biocompatibility, biodegradability, mechanical properties, and responsiveness[1-2]. Hydrogels are soft materials composed of three dimensional networks of hydrophilic polymers, which can swell either in water or in biological fluids. Because of this behavior, great attention was devoted to these systems for biomedical applications. Indeed, by tuning the physicochemical properties of the hydrogels

with varying the degree of crosslinking either by chemical or physical or physical-chemical means these networks can be made suitable as the modulated drug delivery devices[3-4].

Microspheres are little circular particles, with measurements in the micrometer range (ordinarily 1 μm to 1000 μm). Microspheres provide consistant and prolonged therapeutic effect, diminishes the dosing recurrence and thereby improve the patient compliance, they could be infused into the body because of the circular shape and more modest size, better medication use will improve the bioavailability and decrease the rate or power of unfriendly impacts, microsphere morphology all owes a controllable variability in degradation and drug release[5]. Many authors have reported the preparation and evaluation of hydrogel beads using various active pharmaceutical ingredients [6-10].

Fluvastatin is an antilipemic specialist that seriously hinders hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the change of HMG-CoA to mevalonic acid, the rate-restricting step in cholesterol biosynthesis. Fluvastatin has a place with a class of drugs called statins and is utilized to lessen plasma cholesterol levels and forestall cardiovascular illness. It is likewise the main totally manufactured HMG-CoA reductase inhibitor and is basically particular from the contagious subordinates of this restorative class. Fluvastatin is a racemate including equimolar measures of (3R,5S)- and (3S,5R)- fluvastatin[11]. Stucture of fluvastatin is shown in Fig 1. When orally administered, fluvastatin is primarily excreted in the faces (~90%) as metabolites, with less than 2% present as unchanged in the urine and half life of fluvastatin is 3 hrs.

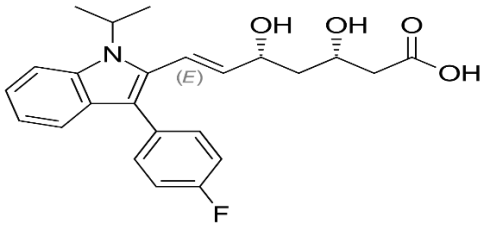


Fig 1.Stucture of fluvastatin

MATERIALS AND METHODS

Fluvastatin was gift sample from Metrochem API Private Limited, India. Sodium alginate, HPMC K15M, Calcium chloride, Carbapol 934P, Sodium CMC and HPMC K4M were purchased from Loba chemie Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade and were procured from S.D. Fine Chemicals, Mumbai, India.

Solubility:

Solvency of fluvastatin was settled in water, pH 1.2, pH 6.8 and pH 7.4 phosphate buffer. Dissolvability studies were performed by taking suitable quantity of Fluvastatin in various volumetric flasks containing the solvents. The formulations were shaken for 24 hrs at customary spans. The arrangements were sifted by utilizing whattmann's channel paper grade no. 41. The sifted arrangements were examined spectrophotometrically at 304 nm (table 2 and table 3).

Determination of λ_{max}:

A solution of Fluvastatin containing the concentration 10 µg/ ml was ready in 6.8pH buffer and UV spectrum was taken utilizing Shimadzu (UV-2550) double beam spectrophotometer. The solution was scanned in the scope of 200 – 400 nm.

Calibration curve of Fluvastatin in 0.1NHCL:

10mg of Fluvastatin was precisely weighed and transferred into 10ml volumetric flagon. It was dissolved and diluted to volume with 0.1 N HCL to give stock arrangement containing 1000µg/ml. The standard stock arrangement was then sequentially diluted with 0.1 N HCL to get 2 to 12 µg/ml of. The absorbance of the arrangement was estimated against 0.1 N HCL as blank at 304nm utilizing UV spectrophotometer. The absorbance esteems were plotted against concentration (µg/ml) to acquire the standard calibration bend.

Calibration curve of Fluvastatin in 6.8pH phosphate buffer:

10mg of Fluvastatin was accurately weighed and transferred into 10ml volumetric flask. It was dissolved and diluted to volume with 6.8pH phosphate buffer to give stock solution containing 1000µg/ml. The standard stock solution was then serially diluted with 6.8pH phosphate buffer to get 2 to 12 µg/ml of. The absorbance of the solution was measured against 6.8pH phosphate buffer as blank at 304nm using UV spectrophotometer. The absorbance esteems were plotted against concentration (µg/ml) to acquire the standard calibration curve.

Drug polymer interaction (FTIR) study

Drug polymer interactions were studied by FT-IR spectroscopy. 1 to 2 mg of Fluvastatin alone, mixture of drug and polymer, beads were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR- spectrum of the pellet from 400–4000 cm^{-1} was recorded taking air as the reference and compared to study any interference.

Preparation of Fluvastatin Hydro gel beads[12]

Ionotropic gelation method:

Exact amount of polymer was broken up in 25ml of refined water and blended to form dispersion. Medication was added to the above scattering and again blended for uniform dissemination. Further more, blended until a homogenous combination was obtained. The combination was expelled through a 23G needle into calcium chloride arrangement (2% w/v). The beads were permitted to stay in a similar solution for 30 min to work on their mechanical strength. The shaped beads were isolated, washed with water and permitted to dry at room temperature overnight. Formulation design for fluvastatin hydrogel beads using different ratios of drug and polymers was shown in
table 1.

Table 1 : Formulation Design For Fluvastatin Hydrogel Beads Using Different Ratios of Drug and Polymers.

Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Fluvastatin	500	500	500	500	500	500	500	500	**500**	500	500	500
Sodium Alginate	500	500	500	500	500	500	500	500	**500**	500	500	500
Sodium CMC	500	750	1000	-	-	-	-	-	**-**	-	-	-
HPMC K4M	-	-		500	750	1000	-	-	**-**	-	-	-
Carbapol 934P	-	-	-	-	-	-	500	750	**1000**	-	-	-
HPMC K15M	-	-	-	-	-	-			**-**	500	750	1000
Calcium chloride (%)	2	2	2	2	2	2	2	2	**2**	2	2	2

Evaluation parameters:

Surface morphology (SEM) :

Scanning electron microscopy has been utilized to decide molecule size distribution, surface geography, surface, and to inspect the morphology of fractured or separated surface. SEM is presumably the most usually utilized technique for portraying drug delivery systems, owing in huge to simplicity of sample preparation and simplicity of activity. SEM studies were done by utilizing JEOL JSM T-330A filtering microscope (Japan). Dry Fluvastatin gel beads were

placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Fluvastatin hydrogel beads were taken by random scanning of the stub.

Percentage yield:

Percentage practical yield of Fluvastatin hydrogel beads was calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of Fluvastatin beads recovered from each batch in relation to the sum of starting material.

The percentage yield of Fluvastatin beads prepared was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Drug Content:

To decide the medication content and encapsulation effectiveness of the globules, 25 mg weight comparable hydrogel beads were squashed utilizing a porcelain mortar and a pestle, and dipersed in reasonable solvent. The dispersion was sonicated for 15 minutes and left overnight for 24 hrs., then, at that point, the dispersion was separated. A 1 ml test was taken and diluted with reasonable solvent, and drug content was examined utilizing an UV-apparent spectrophotometer at λmax of 304 nm, table 4.

Drug Entrapment Efficiency:

The drug entrapment efficiency of prepared beads was decided by using the following equation.

EE (%) = Actual Drug Content/ Theoretical Drug Content X 100

In-vitro dissolution studies[13]:

Procedure for In-vitro dissolution study

The release rate of Fluvastatin Hydrogel beads was determined by employing USP XXIII apparatus I (basket method). The dissolution test was performed using 900 ml 0.1N HCL, for 2hours and at 6.8pH buffer for remaining hours, iat 37 ±0.5°C at 50 rpm. Fluvastatin hydrogel beads equivalent to 25 mg of Fluvastatin was used for the study. At different time points (hourly) 5ml of the sample solution was withdrawn from the dissolution apparatus for upto 12 hrs, and the samples were replaced with fresh dissolution medium. The samples were filtered and the absorbance was determined at 287nm.

Kinetics of drug release:

To inspect the medication discharge energy and system, the aggregate delivery information

were fitted to models addressing zero order (Q v/s t), first order [Log(Q0-Q) v/s t], Higuchi's square root of time (Q v/s t1/2) and Korsemeyer Peppas double log plot (log Q v/s log t) individually, where Q is the combined level of medication delivered at time t and (Q0-Q) is the total percentge of medication staying after time t.

In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows. Cumulative percentage drug release Vs. Time (zero order rate kinetics), log cumulative percentage drug retained Vs. Time (first order rate kinetics), cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation), log of cumulative percentage drug release Vs. log Time (Peppas exponential equation.)

RESULTS AND DISCUSSION

From the solubility studies it was observed that Fluvastatin was found to be more soluble in 6 .8pH buffer among buffers (Fig.2). λmax of Fluvastatin in 6.8pH buffer (10µg/ml) was shown in Fig 3. Calibration curve of Fluvastatin was constructed in 0.1 N HCl at maximum wavelength of 304 nm and analyzed for regression analysis (Fig.4). Regression analysis was selected because it minimizes the deviation and correct the variance heterogeneity. The regression line was defined by its slope (m) and its intercept (C) for normal regression analysis was found as 0.062 and 0.006, respectively, with regression coefficient of 0.999 respectively. Calibration curve of Fluvastatin was constructed in 6.8pH buffer at maximum wavelength of 304 nm and analyzed for regression analysis (Fig.5). Regression analysis was selected because it minimizes the deviation and correct the variance heterogeneity. The regression line was defined by its slope (m) and its intercept (C) for normal regression analysis was found as 0.079 and 0.002, respectively, with regression coefficient of 0.999 respectively.

Table 2: **Solubility Studies of Fluvastatin.**

Solvent	Solubility (µg/ml)
0.1N HCL	0.493
6.8pH buffer	0.894
7.4pH buffer	0.581
Water	1.124

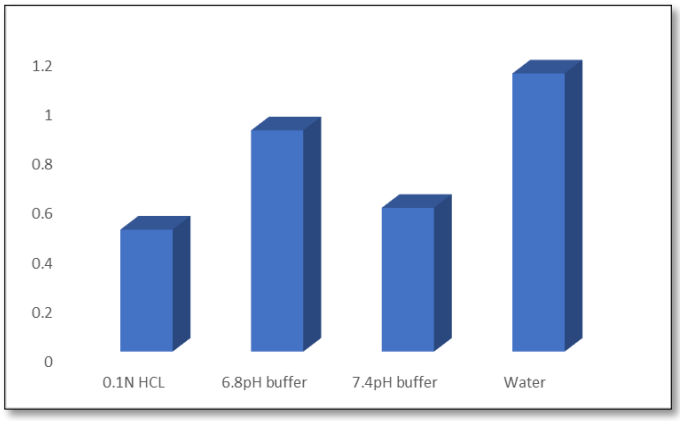


Figure 2: Solubility studies of Fluvastatin

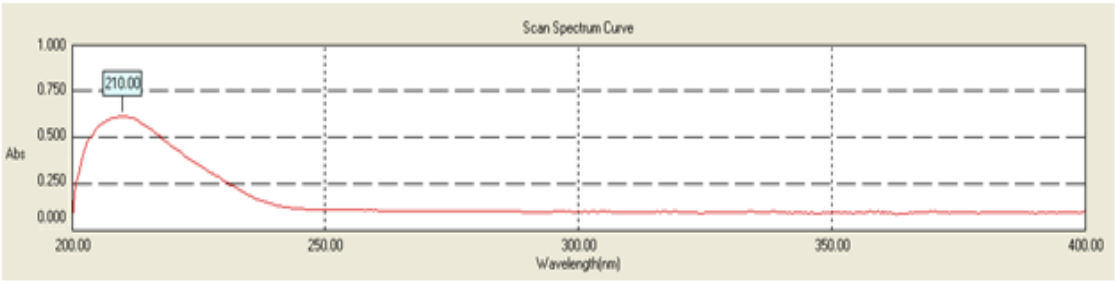


Figure 3: λmax of Fluvastatin in 6.8pH buffer (10µg/ml)

Table 3: Standard calibration data of fluvastatin in 0.1N HCL. And 6.8 pH buffer.

Concentration (µg/ml)	Absorbance	
	0.1N HCL	6.8 pH buffer
0	0	0
2	0.131	0.131
4	0.254	0.254
6	0.381	0.381
8	0.499	0.499
10	0.623	0.623
12	0.741	0.741

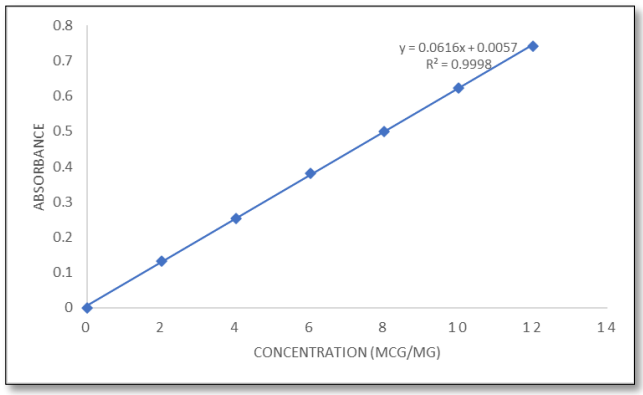


Figure 4: Standard calibration curve of Fluvastatin in 0.1N HCL.

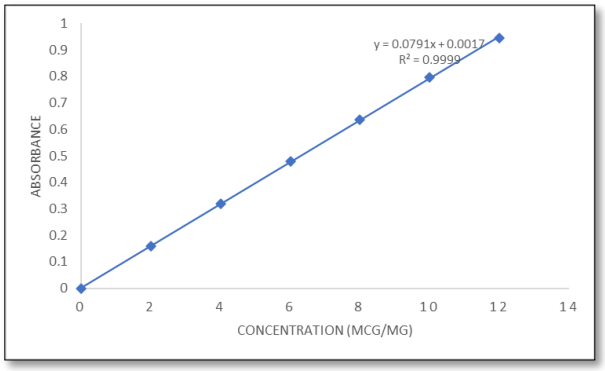


Figure 5: Standard calibration curve of Fluvastatin in 6.8pH buffer.

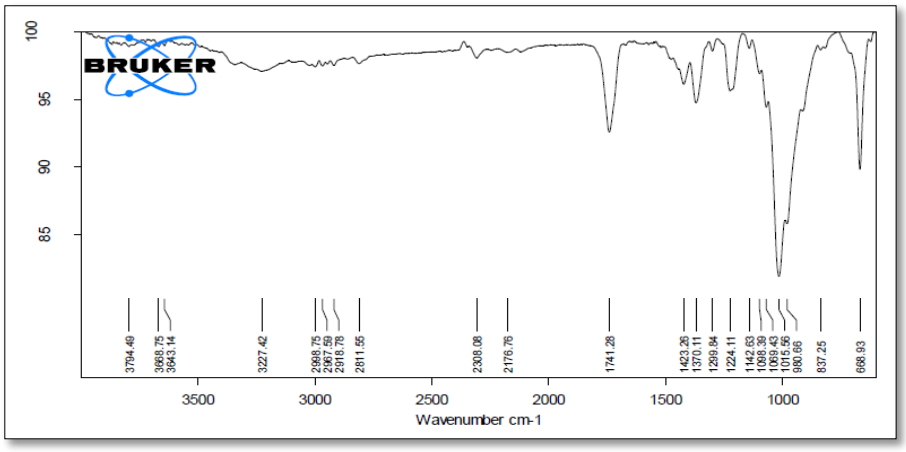


Figure.6: IR spectra of Pure Fluvastatin:

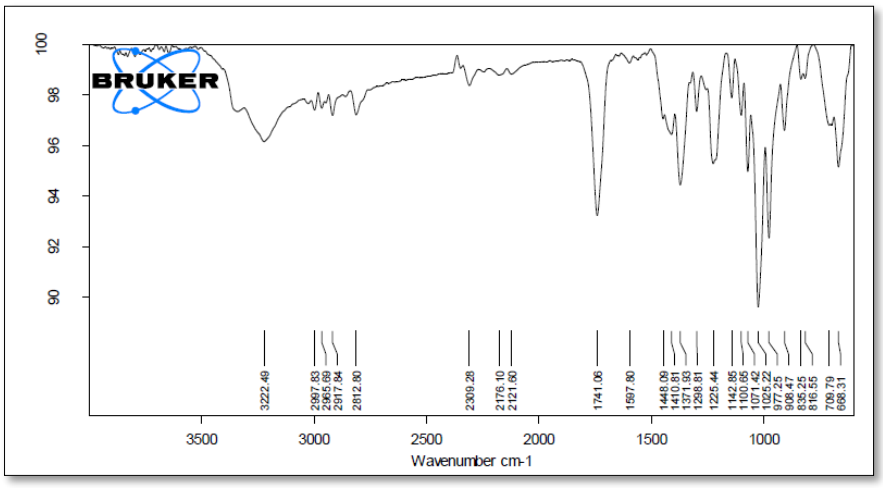


Figure.7: IR spectra of Fluvastatin and Excipients

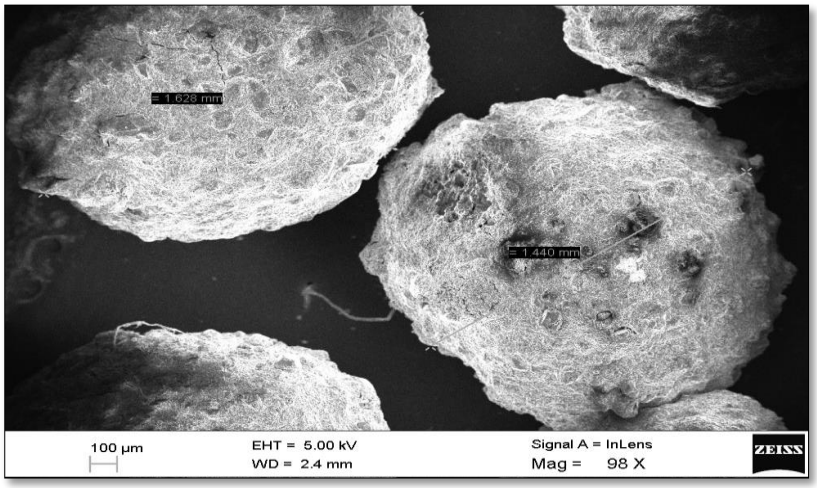


Figure 8: SEM photographs of Hydro gel beads.

From the spectra of Fluvastatin, physical mixture of Fluvastatin and polymer, Fluvastatin and blank beads, it was observed that all characteristic peaks of Fluvastatin were present in the combination spectrum, thus indicating compatibility of the Drug and polymer. From the compatibility studies it was concluded that the functional groups that were present in the pure drug were also found in the optimized formulation with very minute changes, from this we can conclude that the drug and excipients have no interactions (Fig.6 and Fig.7). The percentage yield of all formulation was found in the range of 81 to 95%. The drug content estimations showed the values in the range of 95 to

99% which reflects good uniformity in drug content among the formulations F1 to F12 and indicates these values were within specified range as per USP (±15% of label claim was acceptable) . SEM photographs of Hydro gel beads Fig.8

Table 4: Drug content and percentage yield of fluvastatin hydrogel beads.

Formulation Code	Percentage Yield	Drug content (%)
F1	82.56	95.36
F2	86.49	96.48
F3	90.36	95.02
F4	93.18	99.46
F5	86.46	97.16
F6	93.49	98.42
F7	91.75	96.35
F8	81.65	95.72
F9	89.35	98.06
F10	92.57	96.74
F11	90.72	99.67
F12	95.01	97.82

Drug release kinetics:

The *in vitro* performance of Fluvastatin hydrogel beads showed prolonged and controlled release. The results of the *in vitro* dissolution studies show controlled and predictable manner as the polymer concentration increases the drug release from the hydrogel beads decreases. %Drug Release of F1-F12 was shown in Fig 9. Among all the three polymers used for formulation of hydrogel beads of Fluvastatin, the formulations prepared by using Carbapol 934P shows maximum drug release at the end of 12hrs. So F9 formulation was considered as the optimized formulation, the drug release kinetics were performed for the F9 formulation. From the drug release kinetics of the Fluvastatin hydrogel beads it was concluded that the formulation F9 follows Zero order release with super case II transport mechanism Fig.10.

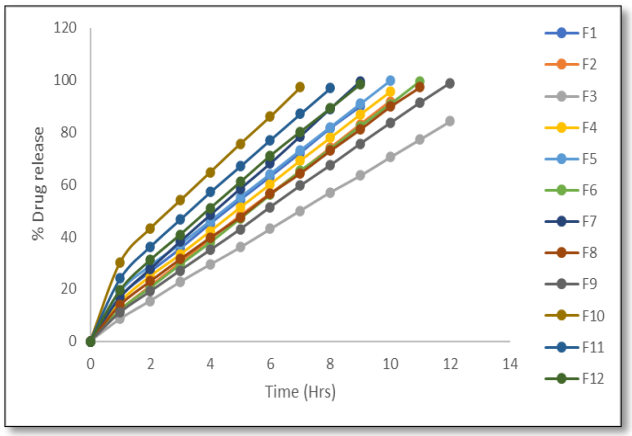
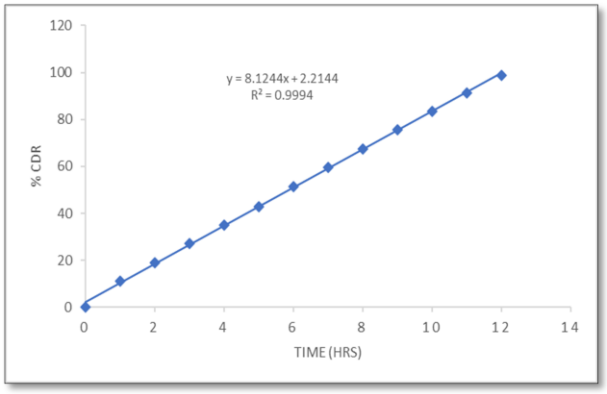
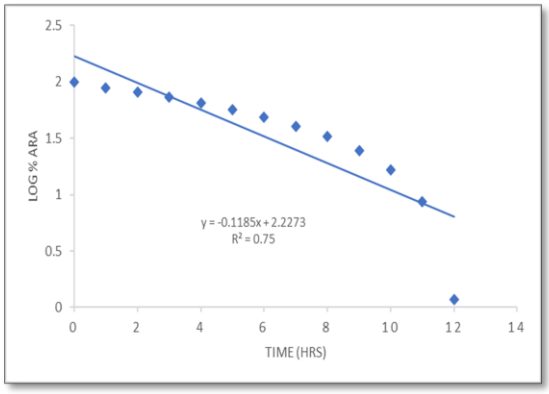


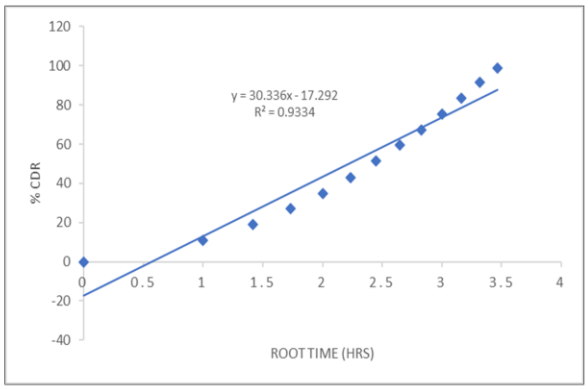
Figure 9:%Drug Release of F1-F12.



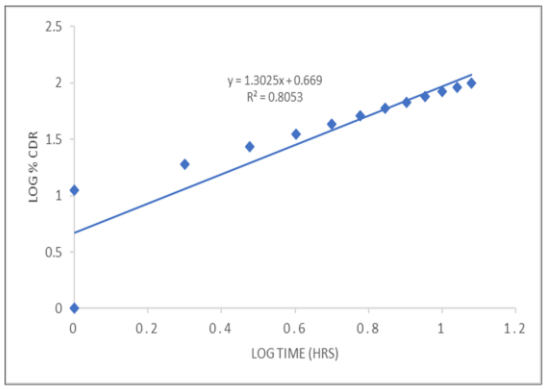
Zero order graph of optimized formulation.



First order graph of optimized formulation



Higuchi plot of optimized formulation



Peppas plot of optimized formulation

Figure.10: Zero order, First order, Higuchi plot and Peppas plot of optimized formulation.

TABLE 5: **Drug Release Kinetics.**

Batch	Zero Order	First Order	Higuchi	Peppas	Peppas(n)
F9 (r^2)	0.999	0.750	0.933	0.805	1.303

CONCLUSION

An attempt was made to prepare hydrogel beads of Fluvastatin by using sodium alginate and rate retarding natural polymers like HPMC K15M, HPMC K4M, Sodium CMC and Carbopol 934P. In the present study nine formulations were formulated by using ionotropic gelation method. All the formulations were subjected for Preformulation evaluation. The FTIR Spectra revealed that, there was no interaction between polymers and Fluvastatin. Entrapment efficiency was increased within polymer concentration. From the results it can be inferred that there was a proper distribution of Fluvastatin in the beads and the deviation was within the acceptable limits. On the basis of release data and graphical analysis formulation Fluvastatin showed a good controlled release profile with maximum entrapment efficiency because of high polymer concentration (Carbopol 934P) with sodium alginate than other polymers. The invitro dissolution data for best formulation F9 were fitted in different kinetic models i.e., zero order, first order, Higuchi and korsemeyer-peppas equation. Optimized formulation F9 shows R^2 value 0.999. As its value nearer to the '1' it is conformed as it follows the zero-order release. The mechanism of drug release is further confirmed by the korsemeyer and peppas plot, the 'n' value is 1.303 for the optimized formulation (F9) i.e., n value was >0.89 this indicates Super case transport. Hence, from the above obtained data it can be summarized that it is possible to formulate hydrogel beads to achieve a controlled release using sodium alginate and Carbopol 934P.

Conflict of interest:

The author declares no conflict of interest.

ACKNOWLEDGMENT

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