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Research Article

PIOGLITAZONE LOADED CHITOSAN HYDROGEL BEADS: FABRICATION AND *IN-VITRO* CHARACTERIZATION

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Abstract

The objective of the present research work is to formulate pioglitazone loaded chitosan polyelectrolyte complex (PEC) hydrogel beads by ionotropic gelation technique using sodium tripolyphosphate (TPP) as an ionic cross linking agent. These PEC hydrogel systems are based upon the fact that their biodegradable polymeric network can capture the drug to release in a sustained manner. Different formulations of pioglitazone loaded microbeads were characterized for particle size, surface texture analysis, drug entrapment efficiency, *in vitro* drug release studies and kinetics and *in vitro* mucoadhesion test etc. The optimized formulation was found to be spherical with rough surface, free flowing and white in colour. The particle size range was found to be from 570±33.60µm to 705±34.44µm with entrapment efficiency in the range of 64.51±1.44% to 88.23±1.35%. The optimized formulation showed a better release profile of 84.39% among all the five formulations at the end of 12 h and follows Higuchi release kinetics. Thus, it can be concluded that the Pioglitazone loaded chitosan microbeads could be productively formulated by ionotropic gelation technique with high entrapment efficiency and prolonged release characteristics.

Keywords: - : Pioglitazone, Chitosan, hydrogel beads,sodium tripolyphosphate, microbeads.

Introduction

Hydrogels are of particular concern in controlled release drug delivery because of their soft tissue biocompatibility, the ease with which drugs are dispersed in matrix and the high degree of control achieved by selecting the physical and chemical properties of polymer network [1]. Hydrogel from marine origin mucopolysaccharide such as chitosan and their derivative are being extensively employed for sustained drug release from dosage form. Chitosan is a copolymer of glucosamine and N-acetyl glucosamine [2] and considered as an important natural biocompatible polymer because of its nontoxic, biodegradable, mucoadhesive and easily bioabsorbable properties. Furthermore, chitosan possess antacid and antiulcer activities which avoid or weaken drug irritation in the stomach. All of these remarkable properties of chitosan make it an ideal polymer for formulating drug delivery devices [3]. Microbeads are spherical microcapsule that serves as the solid substrate in which the drug is entrapped or encapsulated in the core of the beads. These beads can provide sustained drug delivery with more uniform distribution of drugs within the gastrointestinal tract. Drug loaded beads offer an inert environment within the matrix and encapsulation is usually achieved in a media free of organic solvents [4]. The chitosan beads provides as depot reservoir that will allow the continuous gradual release of small amounts of drug in solution form to the upper part of the small intestine (the main site of absorption) which leads to the higher and more consistent therapeutic blood levels of the drug. Thus adverse effects are expected to be highly reduced. The special behaviour of using chitosan as a drug delivery system includes sustained release, decreased particle size leading to increases surface area and hence increased therapeutic achievement, increased efficacy and decreased toxicity [5]. Pioglitazone (Fig 1) is a thiazolidinediones antidiabetic agent that proceeds via diminishing insulin resistance. This agent is profitably used in the management of type 2

diabetes mellitus (also known as non-insulin-dependent diabetes mellitus [NIDDM] or adult-onset diabetes) [6,7].

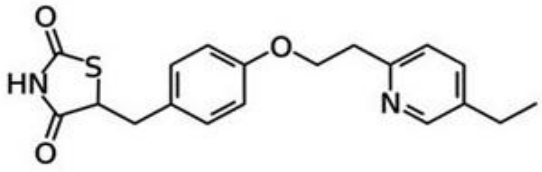

Fig 1: Structure of Pioglitazone

Pioglitazone act by binding and activating the peroxisome proliferation-activated receptor-γ (PPAR-γ) and do not stimulate insulin release. Pioglitazone has an oral bioavailability of 83% and peak plasma concentrations of pioglitazone are achieved in 2–2.5 h. Elimination half life of Pioglitazone is 3-7 h [8]. The goal of the present research work was to develop multiparticulate delivery system consisting of hydrogel beads for site specific delivery of pioglitazone using natural polysaccharide [9] (chitosan) and sodium tripolyphosphate as an ionic cross linking agent by ionotropic gelation technique.

MATERIALS AND METHODS

Materials

Pioglitazone was received as a gift sample from Lincoln Pharmaceuticals ltd, Ahmedabad, India. Chitosan and sodium tripolyphosphate were purchased from Indian Sea Foods, Cochin and Himedia laboratories pvt.ltd, Mumbai respectively. All other chemicals, reagents and solvents used were of pharmaceutical or analytical grade.

Fabrication of pioglitazone loaded chitosan hydrogel beads

Drug loaded chitosan hydrogel beads were prepared by the unique ionotropic gelation technique using TPP as the gelling counter ion. For chitosan hydrogel beads, different concentration of chitosan solution were prepared by dissolving the desire mass of chitosan in 1% (v/v) acetic acid and stirring for about 60 min. Meanwhile 30 mg of core material, pioglitazone, was dissolved separately in 2 ml of ethyl alcohol. Then, 8 ml of chitosan solution and 2 ml of drug solution were mixed together to obtain 10 ml of different concentration of drug polymer solution. The gelling medium was obtained by dissolving the desire amount of TPP in 100 ml of distilled water. The beads were formed by dropping the 10 ml of bubble free drug polymer solution by using a disposable plastic syringe with a 24 gauze needle into the gently agitated various concentration of TPP solution (1%, 2%, 3% w/v). The beads were separated after 2 h of curing time by decantation and were rinsed twice with distilled water. These beads then dried at room temperature (25°) for 12 h and were used as such for further studies [10]. The formulation compositions of various batches are shown in Table 1.

Table 1: Composition of pioglitazone loaded chitosan hydrogel beads.

Formulation code	Drug (mg)	Polymer concentration (%w/v)	Cross-linking agent concentration (%w/v)	Curing time (h)
F1	30	1.0	1	2
F2	30	1.5	2	2
F3	30	2.0	2	2
F4	30	2.5	3	2
F5	30	3.0	3	2

Characterization of Drug Loaded Chitosan Hydrogel Beads

Particle size analysis:

The particle size analysis of prepared chitosan hydrogel bead was carried out by an optical microscope fitted with an ocular and stage micrometer. Fifty randomly chosen hydrogel beads were taken to measure their individual size. Stage micrometer (Mittotuyo micrometer, NSK Co., Japan) was used to calculate calibration factor. 10 deviation of stage micrometer was matched with the deviation of ocular disc and calibration factor was calculated. The particle size was calculated by multiplying the number of deviation of ocular disc occupied by particle with calibration factor [4].

Morphological analysis:

Surface and cross-sectional morphology of hydrogel beads were examined by scanning electron microscopy (SEM- JEOL, model-JSM, 35CF, Japan at magnification of 80X, 500X and 5 KX) using gold sputter technique.The vacuum dried beads coated drug released Vs log time).

with gold palladium using prior to microscopy. A working distance of 18 mm,10.5 mm, 9 mm respectively, a tilt of zero-degree and accelerating voltage of 20 kV were the operating parameters.

Drug entrapment efficiency:

The pioglitazone content in the chitosan hydrogel beads was determined by digestion method. For this, 10 mg of drug loaded beads were pulverized and incubated in 10 ml 0.02M phosphate buffer (pH 7.4) at room temperature for 24 h. Next day it was stirred for 5 min and filtered. The supernatant was assayed spectrophotometrically for pioglitazone content at the wavelength of 269 nm using double beam UV-Visible spectrophotometer (UV 1601, Shimadzu, Japan). All analyses were carried out in triplicate [11,12]. The entrapment efficiency (EE) was calculated according to the following formula.

EE(%) = Actual drug content / Theoretical drug content ×100%

***In vitro* drug release study:**

In-vitro drug release study was carried out for all the formulations of hydrogel bead using a USP Type II dissolution apparatus (Electro lab-TDT 06P, Mumbai) at 100 rpm, maintained at a temperature of 37± 0.5°C for a period up to 12 h followed by phosphate buffer saline pH 7.4 as simulated intestinal fluid (SIF). 5 ml of sample was withdrawn at a predetermined time points (0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 h) and at the same time equal amount of fresh dissolution media was replaced to maintain sink condition. The samples were passed through a 0.45 µm membrane filter and absorbance was measured by using double beam UV-Visible spectrophotometer (UV 1601, Shimadzu, Japan) at 269 nm [13]. The release data were fitted to various mathematical models to know which model is best fitting the obtained release profile.

Kinetic analysis of dissolution data:

To study the mechanism and release kinetics of drugs, data obtained from the *in vitro* drug release studies were plotted in various kinetic models such as Zero order (cumulative amount of drug released vs. time), First order (log cumulative percentage of drug remaining vs. time), Higuchi's model (cumulative percentage of drug released vs. square root of time), and Korsmeyer-Peppas (log cumulative percentage of

In vitro wash-off method for mucoadhesion testing:

The mucoadhesive properties of various formulations of pioglitazone-loaded chitosan hydrogel beads were evaluated by the in vitro wash-off method. In this method, freshly excised pieces of goat intestinal mucosa (1×1 cm, collected from a slaughter house) were mounted on a glass slide (7.5×2.5 cm) by using thread. About 50 beads were spread over the wet rinsed tissue specimen and then immediately hung from the arm of the tablet disintegration test apparatus (PLT-280 TANCO). The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of phosphate buffer (pH 7.4) maintained at 37±0.5°C. The adherence of beads was regularly observed. The number of beads that remained adhered to the tissue was counted at regular intervals for up to 12 h [14,15].

RESULT AND DISCUSSION

Particle size and morphology:

The particle size and morphology of pioglitazone loaded chitosan hydrogel beads prepared with various concentrations of chitosan and TPP were investigated. The results in Table 2 reveal that the mean particle size was different for all the formulations and found to be in the range of 570±33.60µm to 705±34.44µm. The effect of polymer concentration on the size of beads were studied and was found that as the polymer concentration increased, the particle size is proportionately increased (Fig 2). This could be attributed to an increase in relative viscosity at higher concentration of chitosan results in the formation of larger particles [15]. The scanning electron micrographs (SEM) and optical photomicrographs of chitosan hydrogel beads of pioglitazone with their surface morphology are illustrated in Fig 3. Chitosan hydrogel beads were found to be well spherical, with uniform size distribution under optical microscope. It can be revealed from SEM that the formulated chitosan hydrogel beads were almost spherical, discrete and covered continuously with the polymers.

The morphology of beads surface affects by the different concentration of chitosan and TPP. As the concentration of chitosan increased, the viscosity of chitosan solution increased which leads to the formation of relatively strong walls of beads upon cross linked with TPP.

Table 2: Evaluation parameter of pioglitazone loaded chitosan hydrogel beads

Formulation code	Particle size (n=30) (Mean± SD)	% Drug entrapment efficiency (Mean± SD) (n=3)	% Mucoadhesion after 12 h
F1	570±33.60	64.51±1.44	54±0.11
F2	643±20.11	67.74±0.57	63±0.08
F3	621±22.13	75.29±0.80	69±2.0
F4	678±51.15	88.23±1.35	72±1.32
F5	705±34.44	87.15±1.09	74±0.09

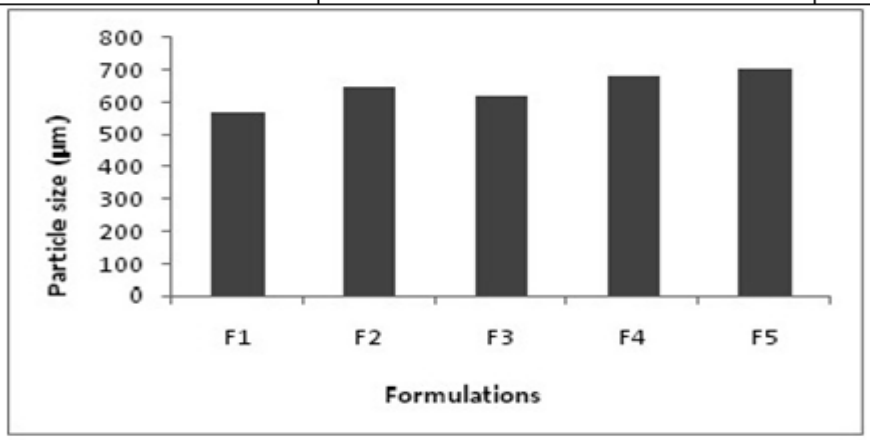

Fig 2: Effects of chitosan concentration on the mean particle size of drug loaded chitosan hydrogel beads

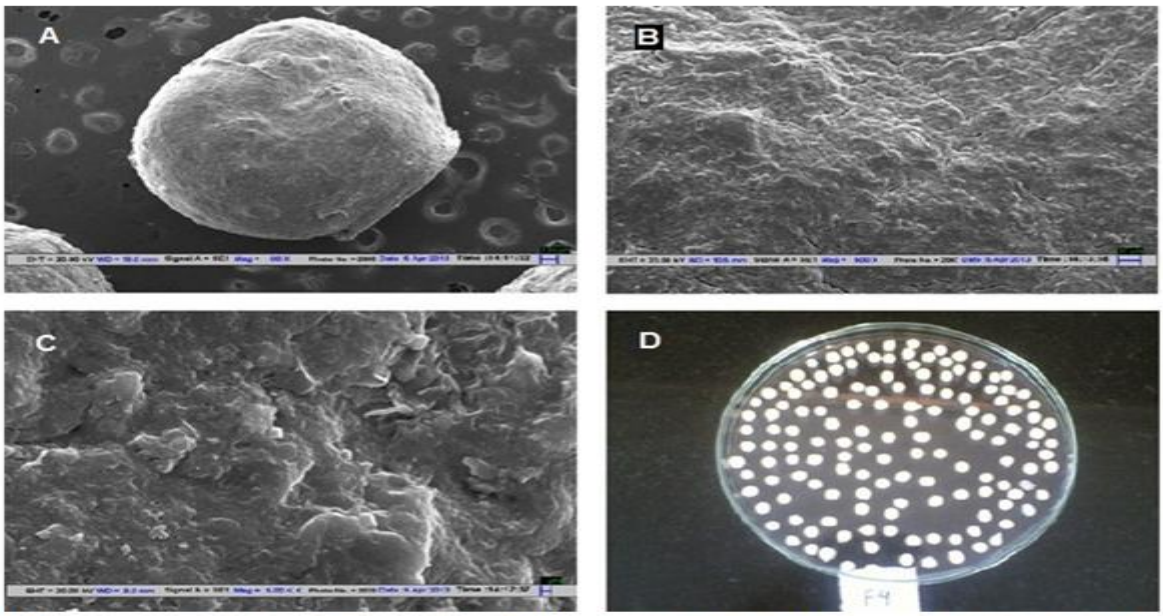

Fig 3: SEM images of the optimized pioglitazone loaded chitosan hydrogel beads at magnification of A-80X, B-500X, C-5 KX and D-Optical photomicrographs of chitosan hydrogel beads.

Drug entrapment efficiency:

Drug entrapment efficiency gives the information about the percentage of entrapped drug with respect to the total drug introduced into polymer solution. Chitosan concentration was having considerable effect on drug entrapment efficiency. Fig 4 illustrates that the drug entrapment efficiency increased as the concentration of chitosan polymer increased because of the higher viscosity of the polymer solution at the highest polymer proportion would be expected to decrease the diffusion of the drug into the external phase which would result higher entrapment efficiency [14,15]. Drug entrapment efficiency also depends upon the ratio of drug to polymer, concentration of sodium tripolyphoshphate and curing time. Drug entrapment efficiency decreases as curing

time increases considerably because of the high cross linking of polymer. From the Table 2, for formulation F1 to F5 as concentration of chitosan was increased the entrapment efficiency was to be increased from 64.51±1.44% to 88.23±1.35%.

***In vitro* dissolution study:**

The *in vitro* dissolution profile of drug loaded chitosan hydrogel beads with different concentration of polymer is shown in Table 3. As the concentration of chitosan increased, the rate and extent of drug release from prepared beads decreased significantly. This could be attributed to the increase of chitosan matrix density and in the diffusion path length which the drug molecules have to traverse (by formation of bigger sized beads). The drug release from these microbeads was characterized by an initial phase of high release i.e. burst effect. As gelation proceeded the remaining drug from the beads was released at a slower rate followed by a phase of moderate release. This initial burst effect was reduced with the increase in chitosan concentration. The reason behind the decrease in burst effect is that the chitosan beads resulted in better entrapment efficiency and formed a thick layer around the beads. The best formulation F4 among the five formulations showed 84.39±0.53% drug release after 12 h. The graphical representation of cumulative in vitro drug release for all the formulations are shown in Fig 5.

Kinetic assessment of the in vitro drug release from the prepared chitosan hydrogel beads:

The in vitro dissolution data were analyzed by different kinetic models in order to find out the n value, which describes the drug release mechanism, the data were fitted to models representing zero-order, first order, Higuchi is square root of time model and Korsmeyer-Peppas model. Table 4 shows data analysis of drug release profile according to different kinetics models. The regression value (r^2) were calculated and observed that the kinetic data was best fitted to higuchi's model and had good regression coefficient for F4 formulation. When the data was fitted according to the Higuchi model there was high r^2 value 0.969, indicating that release from drug loaded chitosan hydrogel beads followed diffusion mechanism because of the hydrogel matrix behavior of the polymer used. Higuchi model for pioglitazone release and Korsmeyer-Peppas model for mechanism of drug release from developed beads are shown in Fig 6 and 7 respectively.

***In vitro* wash off method for mucoadhesion testing:**

Mucoadhesive property was studied on the prepared formulations of chitosan beads by *in vitro* wash off test method. The percentage of drug loaded chitosan hydrogel beads attached to the goat intestinal mucosa after 12 h is shown in Table 2. Result indicated that drug polymer ratio had a significant effect on mucoadhesive property. It was observed that as time increases mucoadhesion capacity decrease which was shown by decrease in adhered drug loaded particles. Moreover as the concentration of chitosan increases mucoadhesion increases it may be due to increase in mucoadhesion capacity.

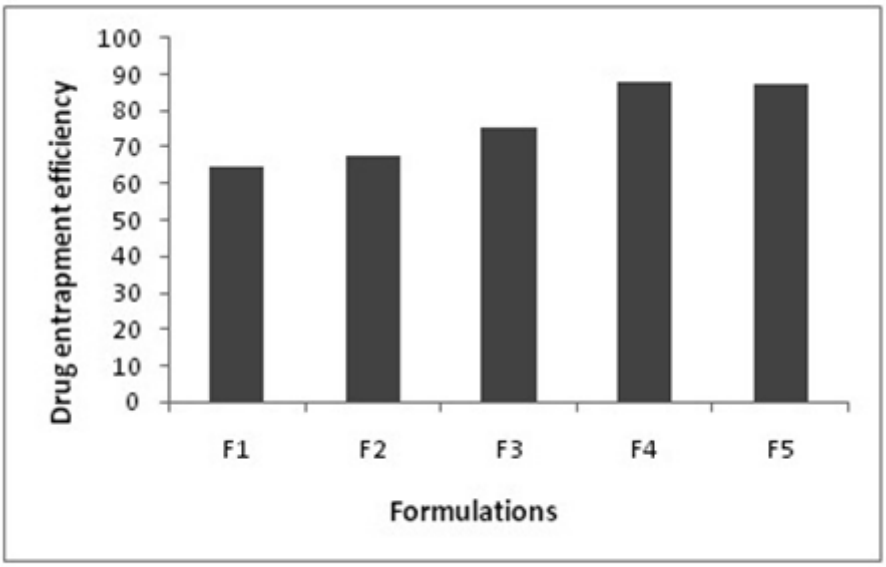

Fig 4: Drug entrapment efficiency of formulation F1-F5

Table 3: *In vitro* cumulative % drug release of formulation F1-F5

Time (h)	Cumulative percent drug release				
	F1	F2	F3	F4	F5
0	0±1.12	0±0.44	0±0.54	0±0.01	0±1.09
0.25	5.52±0.32	3.42±0.17	4.13±3.12	2.21±0.48	1.42±3.11
0.5	12.87±1.09	7.34±2.23	11.44±0.98	6.47±0.55	5.66±0.78
1	27.34±2.22	19.67±1.19	20.39±1.65	14.86±1.23	17.09±1.15
2	45.21±0.98	28.04±0.32	24.42±1.55	27.42±1.45	29.48±1.22
3	56.18±0.89	45.11±0.16	39.60±1.43	45.03±1.33	48.95±1.03
4	73.62±3.55	59.96±0.66	51.89±0.17	56.11±1.44	52.46±0.33
6	86.30±2.76	72.25±1.34	59.31±0.12	71.02±1.00	64.81±0.98
8	86.34±0.17	76.48±1.76	62.18±0.19	76.99±0.56	67.08±1.32
10	-	78.32±0.87	68.23±1.32	81.54±1.26	69.12±1.05
12	-	78.44±3.22	69.71±1.24	84.39±0.53	71.89±.19

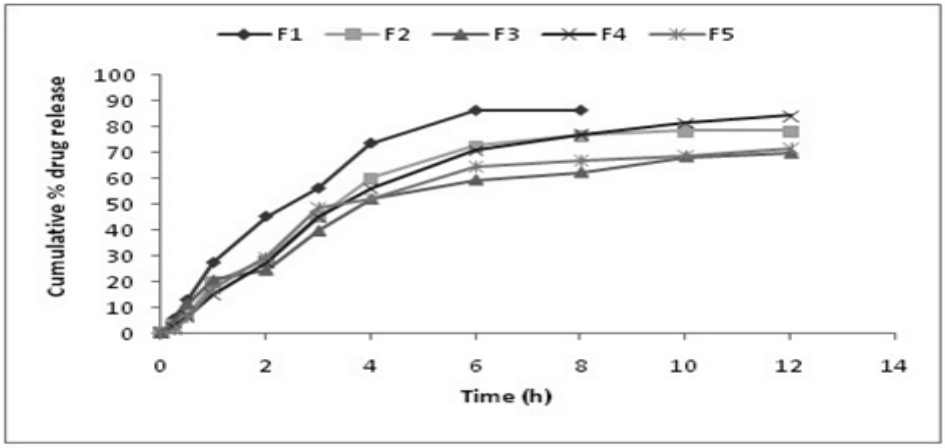

Figure 5: *In vitro* cumulative percent drug release profile of formulation F1-F5

Table 4: *In vitro* drug release kinetic data for formulation F1-F5

Formulation code	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas	
				(r^2)	n-value
F1	0.886	0.955	0.972	0.985	0.775
F2	0.845	0.912	0.954	0.968	0.740
F3	0.864	0.932	0.970	0.978	0.682
F4	0.889	0.96	0.969	0.951	0.733
F5	0.820	0.889	0.946	0.909	0.774

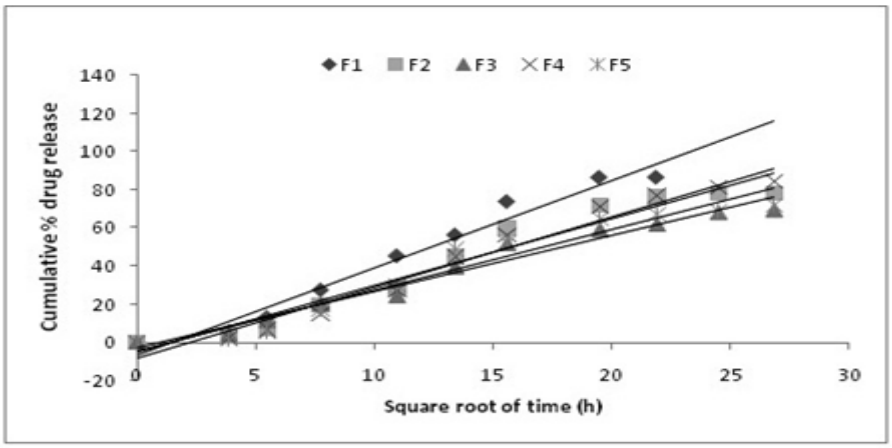

Fig 6: Higuchi kinetic model of formulation F1-F5

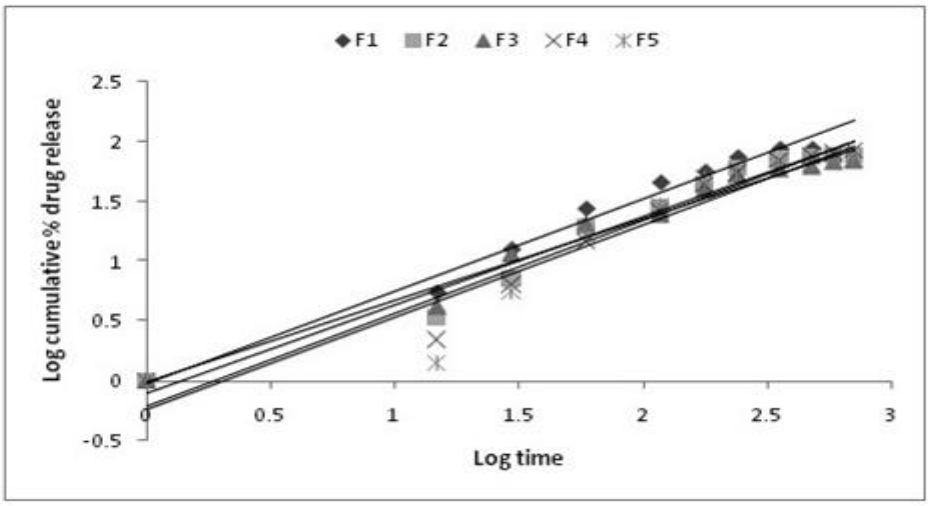

Fig 7: Korsmeyer-Peppas kinetic model of formulation F1-F5 for mechanism of drug release

CONCLUSION

Chitosan hydrogel beads containing pioglitazone was found to be potential, cost effective and satisfactory formulation. The proper selections of formulation are important to achieve high drug encapsulation efficiency and to sustain the drug release from chitosan beads. Among the five formulations, microbeads containing chitosan 2.5% cross linked with 3% TPP (F4) was found to be the most promising formulation because it has higher drug entrapment efficiency of 88.23±1.35% and drug release upto 84.39±0.53% at 12 h. Drug release from the beads was affected by varying chitosan concentration and the cross linking agent. By increasing the polymer concentration entrapment efficiency increased and drug release exhibited more sustained effect. The addition of different cross linking agents was observed to alter the drug entrapment and release characteristics.

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