Formulation And Evaluation of Enteric Coated Microspheres of Acetaminophen

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ABSTRACT

Microspheres are novel drug delivery systems that are used to control the release of dosage form. The present work is to formulate and evaluate the enteric coated microspheres loaded Acetaminophen using inotropic gelation method for the controlled drug delivery of Acetaminophen. Enteric coated microspheres were prepared by using 2% sodium alginate (SA) aqueous solution with three different concentration (10%, 15%, 20% w/v)of cross linking agent (Cacl2). Followed by coating with 2% hydroxypropyl methylcellulose phthalate. Three formulations were formulated and evaluated for micrometric properties like angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index, drug content, In-vitro drug release, SEM analysis and release kinetics. Among all three formulation, formulation 3(F3) showed maximum percentage drug release at 8 hrs (82.20%). So the formulation 3 (F3) is selected as optimized formulation. Thus it can be concluded that all the coated microspheres prolonged and extended the drug release. Therefore, these coated microspheres can be used to control and prolong the drug release effectively and reducing the dosing frequency of Acetaminophen.

Keywords: Enteric coated microspheres, Sodium alginate, Calcium chloride, Hydroxyl propyl Methylcellulose

Introduction:

Microspheres are free-flowing powders made up of biodegradable proteins or synthetic polymers with diameters ranging from 1-1000nm [1]. A well-designed controlled drug delivery system can solve some of the drawbacks of traditional therapy while also improving a medicine's therapeutic efficacy. To achieve optimum therapeutic efficacy, the chemical must be delivered to the target area in the correct amount and at the proper time, resulting in minimal toxicity and adverse effects [2, 3]. Microspheres are free-flowing powders made up of biodegradable protein or

synthetic polymers with particle sizes of less than 200 micrometers [4]. Microspheres as drug carriers, for example, have become a controlled release dosage form in an unique drug delivery system. Microspheres are defined as a "monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) a structure composed of a continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level[5]. The short biological half-life (2.5 hours) and decreased absorption after a single dose are two drawbacks of Acetaminophen [6]. Microsphere enteric coating using an appropriate coating material is another essential technique in microsphere formulation for modifying microsphere properties, particularly in-vitro release profile [7]. As a result, the current study aims to develop acetaminophen loaded alginate microspheres prepared with three different concentrations of cross linking agent ($Cacl_2$), followed by enteric coating with Hydroxyproyl methylcellulose phthalate (coating agent) to modify the dissolution profile and provide a controlled release of drug, resulting in reduced dosing frequency. The microspheres that had been prepared were put to the test. The micrometric features of the enteric coated microspheres, as well as the drug content, in vitro drug release, SEM analysis, and release kinetics, were all evaluated.

Materials And Methods

Acetaminophen was received as a Oxford lab fine chem. Llp. Sodium alginate and calcium chloride were purchased from Spectrum chemicals Ltd and Hydroxypropyl methylcellulose phthalate were purchased from global scientific company .All other chemical were of analyticnhal grade.

Preparation Of Stock Solution Preparation Of Solutions For Calibration Curve

Stock solution 1:

Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of drug in 100 ml solution of methanol and phosphate buffer pH 6.8 in 100 ml volumetric flask (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.

Stock solution 2:10 ml of this (stock solution 1) is diluted to 100ml with phosphate buffer pH 6.8 to get a stock solution containing 100 µg/ml of drug.

Stock solution 3:1ml of this (stock solution 2) is diluted to 10 ml with phosphate buffer pH 6.8 to get a stock solution containing 10 µg/ml of drug.

Preparation of sample solution

Different dilution of stock solution with phosphate buffer pH 6.8 were made to obtain solution have in concentration 2, 4, 6,8,10, µg/ml. absorbance was measured at 245nm space against phosphate buffer pH 6.8 as blank, using UV spectrophotometer. Standard curve was plotted with concentration on X-axis and absorbance on Y-axis

Formulation Of Enteric Coated Microspheres Of Acetaminophen

The microspheres of Acetaminophen were prepared by Ionotropic gelation method using sodium alginate as polymer and calcium chloride as cross linking agent .A 2% sodium alginate solution was prepared by dissolving weighed amount of sodium alginate in 100 ml distilled water with stirring on a magnetic stirrer .Weighed amount of Acetaminophen was dispersed in the sodium alginate solution .cross linking solutions of various concentration (10% , 15%, 20%) w/v were prepared by dissolving calcium chloride in distilled water .Sodium alginate solution was filled in the syringe and dropped into the solution of cross linking agent from a height of 6 inches with speed of about 50 drops per minute. Microspheres were prepared due to the cross linking of the cross linking of the polymer by the calcium ions [8].The prepared microspheres were collected by decantation followed by centrifugation of the solution, air dried overnight and then stored in vacuum desiccators. For the coating of prepared microspheres a fraction of uncoated microspheres were subjected to the process of coating with 2% Hydroxy Propyl

Methyl Cellulose Phthalate (HPMCP) in water. The prepared microspheres were dipped into the enteric coating solution for 30 minutes and collected by decantation followed by centrifugation ,air dried for overnight and then stored in microspheres prepared with different concentration (10,15,20%) of cross linking agent were coated as formulation F1, F2, F3,

Formulation Of Enteric Coated Microspheres

Table 1: Formulation of enteric coated microspheres

S. NO	FORMULATION	ACETAMINOPHN (Mg)	SODIUM ALGINATE % (W/V)	CALCIUM CHLORIDE % (W/V)	HYDROXYL PROPYL METHYL CELLULOSE PHTHALATE (HPMCP) %(w/v)
1	F1	500	2	10	2
2	F2	500	2	15	2
3	F3	500	2	20	2

Figure 1: acetaminophen loaded microspheres

Evaluation Of Enteric Coated Microspheres of Acetaminophen

Micromeritic Propertis [9, 10, 11]

1. Bulk density: A weighed amount of microspheres were filled into a measuring cylinder and the volume (Vo) occupied by the microspheres was noted and the bulk density was calculated as followed

Bulk Density = Mass of the microspheres (W) / Bulk volume of the microspheres (Vo).

2. Tapped density: A weighed quantity of microspheres was filled in a measuring cylinder and the cylinder was tapped against a wooden surface at regular interval for 100 times, then the volume occupied by the microspheres was noted down and tapped density was calculated as followed.

Tapped Density = Mass of the microspheres (W) / Tapped Volume of the microspheres (V_f).

3. Flow properties: Carr's compressibility index and Hausner's ratio were calculated for the uncoated microspheres using the following equations.

4. Carr's index: Carr's index was determined using bulk density and tapped density.

Carr's index= Tapped density- Bulk density / Tapped density× 100

5. Hausner's ratio: Hausner's ratio is used for predicting the flow characteristics.

.**Hausner's ratio = Tapped density/ Bulk Density**

6. Angle of repose :[12] It is a measure of resistance to flow and calculated by funnel method. Weighed quantity of microspheres was passed through the funnel and the heap was formed on the paper. The area of the heap was encircled and diameter of the circle and the height of the heap were measured and the angle of repose was calculated as followed.

$$\theta = \tan^{-1}(h/r)$$

Where,

θ= the angle of repose

h = height of pile

r = radius of base of the pile

In-Vitro Drug Release Study:

In-vitro release study of the microspheres was carried out using USP rotating basket method A weighed amount of microspheres was placed in the basket, and then put into the 900ml dissolution medium of phosphate buffer pH 6.8 (after 8h) at maintained 37 ± 0.5˚C with a paddle rotation speed of 50 rpm. At 1,2,3,4,5,6,7, and 8hrs, 5ml Samples were withdrawn at Different intervals and an equal volume of the fresh dissolution medium was introduced into the apparatus. Each sample was diluted suitably with dissolution medium and analyzed with UV spectrophotometer at 245 nm for determining the drug release.

SEM Analysis:

The surface morphology of the formulated beads was analyzed by scanning electron microscope (SEM) (Carl ZEISS EVO 18-Germany)-operating modes secondary electron (SE) and Backscattered electron (BSD) modes. Up to 200 nm resolution depends up on sample. it is attached with AMETEK Team V.4.3 EDS detector.

Drug content:

An amount of microspheres containing a quantity equivalent to 100 mg Acetaminophen were weighed, crushed after 24 hrs diluted appropriately and analyzed Spectrophotometerically at 245 nm for determination of the drug content.

DRUG RELEASE KINETIC STUDY

Drug Release Kinetics [13, 14, 15]

Drug release kinetics was performed using model dependent method in which the dissolution profile of each formulation has been subjected various kinetics like Zero order, First order, Higuchi's and Korsmeyer-Peppas model. The data obtained from in vitro drug release studies were plotted in various kinetic models; as mentioned below zero order (Equation 1) as cumulative amount of drug released vs time, first order(Equation 2) as log as cumulative percentage of drug remaining vs time, and Higuchi's model (Equation 3)as cumulative percentage of drug released vs square root of time.

$$Q_t = Q_0 + k_0 t \quad \text{(Equation 1)}$$

Where Q_t is the amount of drug dissolved in time t,Q_0 is the initial amount of drug in the solution and K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axis.

$$\text{LogC} = \log C_0 - kt/2.303 \quad \text{(Equation 2)}$$

Where C_0 is the initial concentration of drug is the first order constant, and t is the time.

$$Q_t = K_H t^{1/2} \quad \text{(Equation 3)}$$

Where K_H is the constant reflecting the design variables of the system and t is the time in hours. Hence drug release rate is proportional to the reciprocal of the square root of time. Drug release were plotted in korsmeyer equation (Equation 4) as log cumulative percentage of drug released vs. log time, and the exponent n was calculated through the slope of the straight line.

$$M_t/M_\infty = Kt^n \quad \text{(Equation 4)}$$

Where M_t/M_∞ is the fractional solute release, t is the release time, K is a kinetic constant.

Table 2: Diffusion Exponent and Solute Release Mechanism

Diffusion exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0 .45<n<0.89	Anomalous (non-Fickian) diffusion
0.89	Case-II transport
n>0.89	Super case-II transport

Results And Discussion

Standard calibration curve for Acetaminophen:

The UV Spectrophotometric method was used to analyze Acetaminophen. The absorbance of the drug in phosphate buffer (pH 6.8) was measured at a wavelength of 245nm. The results are given in table 5 and Figure 14.

Table 3: standard calibration curve for Acetaminophen

S.No	Concentration	Absorbance at 245 nm
1	0	0
2	2	0.16
3	4	0.32
4	6	0.44
/5	8	0.63
6	10	0.79

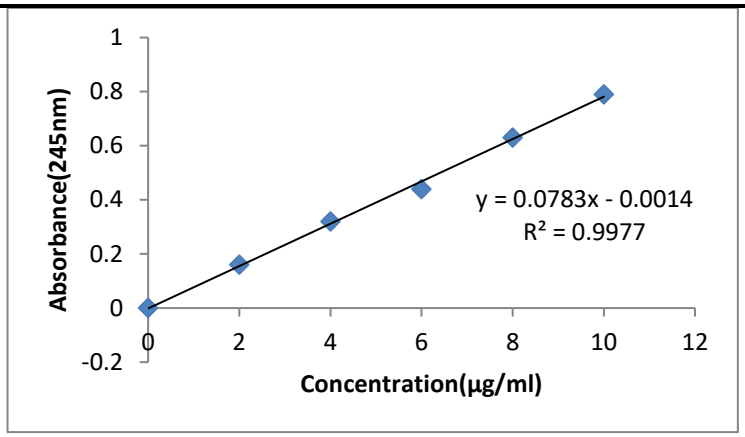

Figure 2: Standard calibration curve Acetaminophen

Micromeritic Properties

Flow properties:

Table 4: Flow properties of Acetaminophen **microspheres**

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (0)	Carr's index	Hausner's ratio
F1	0.729	0.833	7.125	24.9	1.332
F2	1.269	1.428	7.463	22.1	1.285
F3	0.939	0.970	11.309	6.2	1.067

In- Vitro Dissolution Study

Cumulative percentage release of enteric coated microspheres carried out in 6.8 pH Phosphate buffer for two hours and the release rate was decreased by increasing the polymer concentration. The rapid release was obtained in formulation F3 due to optimum concentration of polymer.

Table 5: *In-vitro* dissolution profile of enteric coated microsphere Mean Cumulative Percentage Drug Release (%)

Time in hours	%CDR		
	F1	F2	F3
0	0.00	0.00	0.00
1	2.38	3.22	8.10
2	12.54	7.56	17.20
3	32.05	18.72	24.63
4	40.33	34.43	36.13
5	56.26	58.18	51.21
6	59.09	60.66	63.17
7	63.73	65.68	73.62
8	67.78	70.08	82.26

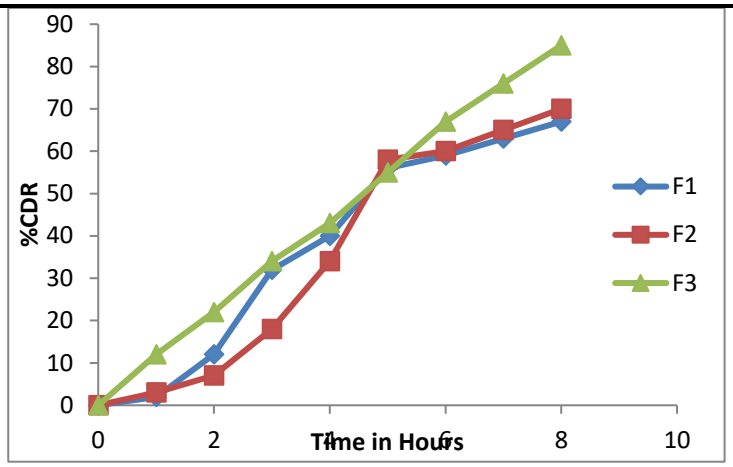

Figure3*: In-vitro* dissolution profile of enteric coated microsphere Mean Cumulative Percentage Drug Release (%)

SEM analysis:

Morphological analysis of the microspheres was carried out using Carl ZEISS EVO 18-Germany scanning electron microscopy the surface and morphological characteristics for the given optimum formulation (F3)

Figure 4: SEM Analysis for Formulation (F3)

Drug release kinetics

The release profile obtained from the best optimized formulation was fitted to various kinetic equations to know the mechanism of drug release as indicated by the maximum r^2 value

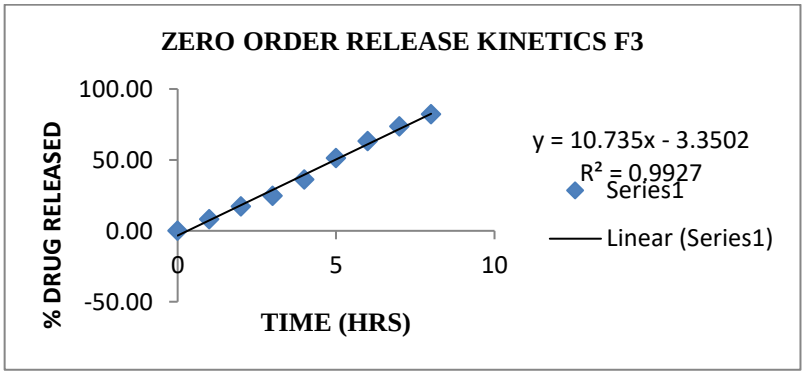

Figure 5: Zero order kinetics for Optimized Formulation F3

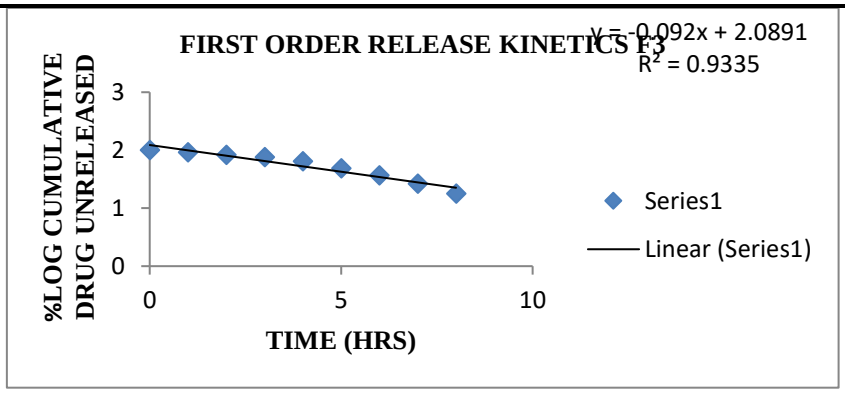

Figure 6: First order kinetics for Optimized Formulation F3

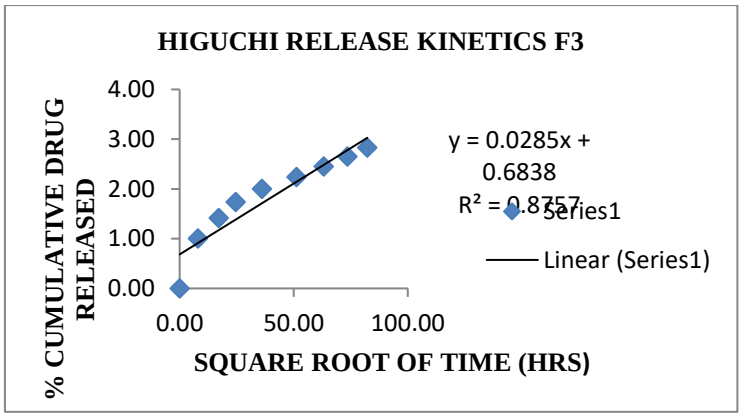

Figure 7: Higuchi order kinetics for Optimized Formulation F3

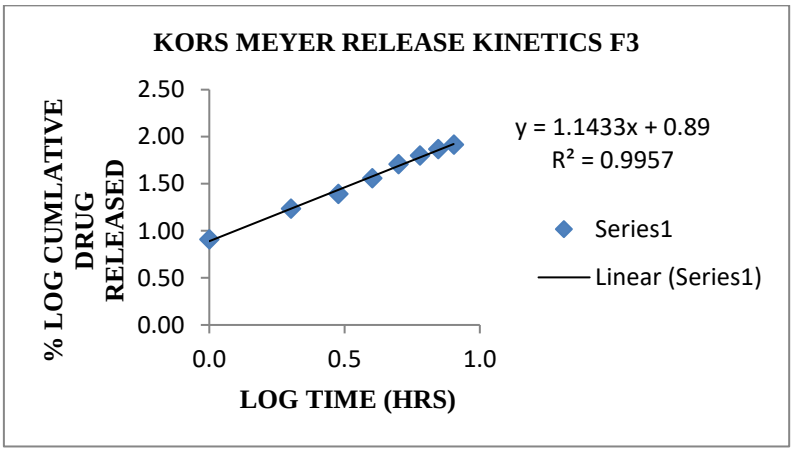

Figure 8: Korsmeyer peppas order kinetics for Optimized Formulation F3

From the release kinetic data the optimized formulation was fitted with various kinetics equations. From the graphical representation it can be understood that this layer is best fit into Zero Order kinetics which has shown a regression coefficient (r^2) of 0.9927 and Higuchi model (r^2=0.8757) and Peppas equation was used to analyze the pattern of the formulation and the value of "n" was found to be 0.45< n < 0.89, indicating the drug release follows Non-Fickian diffusion.

Table 6: Kinetics analysis of dissolution data for formulation

Formulation	Model	R^2	Slope	K
F3	Zero Order	0.9927	10.735	3.3502
	First Order	0.9335	0.092	2.0891
	Higuchi Model	0.8757	0.0285	0.6838
	Korsmeyer Model	0.9957	1.1433	0.89

Conclusion

The enteric coated microspheres were prepared by inotropic gelation method technique using three different formulations of three enteric coated microspheres of Acetaminophen were prepared by inotropic method. Micromeritics result of microspheres showed that they have good flow property. Various evaluation parameters were done for all three formulations such as percentage of drug content in –vitro drug release studies, SEM analysis study and drug release kinetics study. Among all three formulations, Formulation 3 (F3) showed maximum percentage drug release at 8 hours. So the formulation 3 (F3) is selected as optimized formulation. From the In-vitro drug release data of F3 was fitted with various kinetic equations and it follows non-fickian diffusion. Thus it can be concluded that all the coated microspheres prolonged and extended the drug release. Therefore, these coated microspheres can be used to control and prolong the drug release effectively and reducing the dosing frequency of Acetaminophen.

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