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Research Article

Metformin Hcl Loaded Sodium Alginate Floating Microspheres Prepared by Ionotropic Gelation Technique: Formulation, Evaluation and Optimization

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ABSTRACT

Floating alginate based microsphere system of Metformin Hcl was prepared by ionotropic gelation technique, with sodium bicarbonate as gas forming agent, swellable polymers Hydroxy propyl Methylcellulose (HPMC E50), Ethyl cellulose (EC) and calcium chloride gelling agent. Alginate microspheres were formed as alginate undergoes ionotropic gelation by calcium ions and carbon dioxide develops from the reaction of bicarbonate salt with acid which permeated through alginate matrix leaving gas bubbles or pores within, providing the buoyancy. The concentration of polymer used was optimized by using 3^2 factorial designs. A 3^2 factorial design was used to elucidate the effect of variables, concentration of polymers HPMC (X_1) and EC (X_2) as the independent variables. The prepared microspheres were evaluated for size analysis, drug loading, drug entrapment efficiency, buoyancy study, SEM and *in vitro* drug release. The size of alginate microspheres was found to increase with increase in concentration of polymers. Drug loading and drug entrapment efficiency and was found to be in acceptable range. All formulations possessed good floating properties with total floating time more than 12 hrs. From the study, it was concluded that spherical and free flowing microspheres of Metformin Hcl could be successfully prepared by ionotropic gelation technique with high entrapment efficiency and prolonged release characteristics.

INTRODUCTION

The novel design of an oral controlled drug delivery system should primarily be aimed at achieving more predictable and increased bioavailability of drugs. But there are several physiological difficulties, which include restraining/localizing the drug delivery system within the regions of the gastrointestinal tract and the high variable nature of gastric emptying process [1].

The major absorption zone, stomach or upper part of intestine, can result in incomplete drug release from the drug delivery system leading to diminished efficacy of the administered dose. Therefore, localizing the drug delivery in a specific region of the gastrointestinal tract due to its mucoadhesiveness increases the intimacy and duration of contact between the drug containing polymer and the mucous surface.

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Such a drug delivery system offers numerous advantages, especially for drugs exhibiting an absorption window or for drugs with a stability problem in the stomach. Overall, the intimate and prolonged contact of the drug delivery system with the absorbing membrane has the potential to maximize the rate of drug absorption [2, 3]. These considerations have lead to the development of oral controlled release microcapsules/microspheres possessing mucoadhesive properties.

Metformin HCl is used as monotherapy as an adjunct to diet for management of type 2 (noninsulin-dependent) diabetes mellitus (NIDDM) in patients whose hyperglycemia cannot be controlled by diet alone. Metformin HCl quite frequently causes gastrointestinal problems such as nausea, stomach pain, bloating, and diarrhoea. It has a short half life (1.5-4 hrs) and is absorbed from upper intestine within 6 hrs, So repeated administration is required to maintain effective plasma concentration [4, 5]. It

may therefore be more desirable to deliver the drug in a sustained release dosage form.

Factorial design is an efficient tool to obtain an appropriate mathematical model with minimum experiments for optimization of formulation design. Studies based on factorial design allow all the factors to be varied simultanously, thus enabling the evaluation of the effects of each variable at each level and showing interrelationship among them. The most important variables which affect the system function are selected and systemic experiments are then performed to the specified factorial design. The number of independent variables selected decides the number of experiments that are to be performed [6].

The objective of the present study was to develop alginate mucoadhesive microcapsules for sustained release of Metformin Hcl by ionic gelation technique, using Hydroxy propyl methyl cellulose (HPMC) as mucoadhesive polymer and ethyl cellulose (EC) as rate controlling polymer. A 3^2 full factorial design was employed to evaluate the effect of each of the selected variables and their interactions on the response.

MATERIALS AND METHODS

Metformin HCl was obtained as a gift sample from Zydus Cadila healthcare ltd., Ahmedabad, HPMC K4M was generously supplied by Dow Chemicals. Ethyl cellulose was gifted by Hi'media Ltd., Sodium alginate, Calcium chloride; Barium chloride and sodium bicarbonate were procured from S. D. Fine Chemicals Ltd., Mumbai.

Formulation of floating microspheres of Metformin Hcl [7, 8]

Floating alginate microspheres of Metformin Hcl were prepared by ionotropic gelation technique using different proportion of polymers as shown in Table 1. A 3% w/v solution of sodium alginate solution was added to weighed amount of ethyl cellulose dissolved in required quantity of ethanol.

Weighed quantity of drug and HPMC E50 was triturated to form fine powder, then added to above solution. Sodium bicarbonate, a gas-forming agent was added to this mixture and the resulting solution was stirred uniformly. Using a 26 G syringe needle the above solution was dropped into 100 ml of gently agitated calcium chloride (3% w/v) solution to obtain microspheres. The solution containing microsphere was stirred slowly using magnetic bead for about 10 min. The microspheres were further allowed to remain in the same solution for 20 min to improve mechanical strength. The formed microspheres were filtered, washed with distilled water, air-dried at room temperature and stored in desiccators.

Optimization of Floating microspheres of Metformin HCl [8, 9]

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels and experimental trials were carried for all nine possible combinations. The factors were selected based on preliminary study. The concentration of HPMC (X_1) and concentration of Ethyl cellulose (X_2) were selected as independent variables. The % drug release at 2, 6 and 10^{th} hours were Q_2, Q_6 and Q_{10} respectively selected as dependent variables.

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y_i) is measured for each trial.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where,
Y is the dependent variable,
b_0 is the arithmetic mean response of the nine runs and
b_1 is the estimated coefficient for the factor X_1.

The main effects (X1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed.

IR Study [10]

IR study was performed to study compatibility between drug and polymer. The IR spectrum of pure Metformin Hcl and drug loaded alginate beads were obtained using KBr disk method subjected to FTIR with a Tensor 27. The samples were analyzed between wave numbers 4000 and 600 cm^{-1}.

Characterization of microspheres

Microsphere size determination [7]

Microsphere size was determined using an optical microscope under regular polarized light. The mean microsphere size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Table 1: Composition of Metformin HCl microsphere formulation

Formulation Code	Metformin HCl (mg)	HPMC E 50 (mg)	Ethyl Cellulose (mg)	Sodium Bicarbonate (mg)
F1	500	100	100	100
F2	500	100	50	100
F3	500	100	150	100
F4	500	50	100	100
F5	500	50	50	100
F6	500	50	150	100
F7	500	150	100	100
F8	500	150	50	100
F9	500	150	150	100

Table 2: Micromeritic parameters of formulated floating microspheres

Formulation Code	Average particle size (μm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's Index (%)	Hausner's Ratio	Angle of repose (θ)
F1	646.75 ± 11.14	0.403 ± 0.03	0.469 ± 0.01	14.19 ± 2.1	1.14 ± 0.03	25°20′
F2	583.32 ± 15.82	0.341 ± 0.03	0.401 ± 0.02	15.04 ± 2.3	1.17 ± 0.03	21°25′
F3	700.56 ± 9.55	0.440 ± 0.03	0.521 ± 0.04	15.47 ± 1.1	1.18 ± 0.02	27°89′
F4	646.75 ± 11.14	0.400 ± 0.02	0.468 ± 0.02	14.53 ± 2.7	1.16 ± 0.03	23°96′
F5	576.60 ± 11.09	0.318± 0.03	0.364 ± 0.04	12.32 ± 1.6	1.13 ± 0.02	20°22′
F6	681.34 ± 8.41	0.412 ± 0.01	0.486 ± 0.02	15.07 ± 0.5	1.17 ± 0.01	27°75′
F7	651.55 ± 13.43	0.405 ± 0.03	0.467 ± 0.03	13.39 ± 2.1	1.15 ± 0.02	26°56′
F8	595.82 ± 9.11	0.352 ± 0.02	0.410 ± 0.03	14.06 ± 0.9	1.16 ± 0.01	21°80′
F9	730.36 ± 10.33	0.457 ± 0.04	0.531 ± 0.06	13.99 ± 1.3	1.16 ± 0.02	29°35′

Table 3: Percent Yield, Drug Entrapment Efficiency, Drug Loading, Percent Buoyancy, and Percent Cumulative Drug Release

Formulation Code	Percent Yield	% Drug Entrapment Efficiency	% Drug Loading	Percent Buoyancy	Percent Cumulative Drug Release (12 hrs)
F1	80.57	62.82 ± 0.49	33.05 ± 0.25	82.67	71.25
F2	84.47	70.24 ± 0.24	39.01 ± 0.12	84.67	82.92
F3	78.05	58.29 ± 0.42	28.91 ± 0.20	75.48	66.14
F4	85.55	75.33 ± 0.14	41.93 ± 0.15	86.90	77.95
F5	90.29	68.41 ± 0.49	40.21 ± 0.29	88.17	85.17
F6	81.05	60.99 ± 0.26	32.05 ± 0.15	77.87	70.78
F7	85.14	56.39 ± 0.28	28.17 ± 0.12	79.33	68.80
F8	83.24	69.00 ± 0.40	36.28 ± 0.20	80.88	73.12
F9	80.95	56.60 ± 0.29	26.91 ± 0.12	73.50	62.39

Table 4: Summary of regression analysis for all microsphere formulations

Coefficients	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
Q_2	28.98	-1.596	-0.841	0.452	-5.4	-6.585	0.9765
Q_6	48.853	-3.976	-2.935	0.33	-3.78	-9.645	0.9241
Q_{10}	65.921	-3.401	-3.191	0.697	-5.798	-11.128	0.9825

Morphological study using SEM [11]

The morphological study analysis was performed using Scanning Electron Microscope (SEM). The surface topography and cross section of optimized microsphere formulation was scanned and examined under Electron Microscope JEOL JSM 6360, Japan. The sample was loaded on to a copper sample holder and sputter coated with carbon followed by gold.

Micromeritics properties [12]

Angle of repose (θ)

The angle of repose of each powder blend was determined by glass funnel method, using following equation:

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

Where,
θ = angle of repose
h = height of the pile and
r = radius of the powder cone

Bulk density

Bulk density of formulated microspheres was determined by taking a known mass of microspheres in a 5 ml graduated measuring cylinder. The cylinder was dropped three times from a height of one inch at an interval of two seconds. The bulk density was calculated by following equation.

$$\text{Bulk density} = \frac{\text{Weight of microsphere in grams}}{\text{Bulk volume of microsphere in cm}^3}$$

Tapped density

Tapped density is the volume of powder determined by tapping using measuring cylinder containing weighed amount of sample. Tapped density of microspheres was calculated by following equation.

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}$$

Carr's compressibility index

This is an important property in maintaining uniform weight. It is calculated using following equation**,**

$$\% \text{ Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density X 100}}{\text{Tapped density}}$$

Hausner's ratio

Hausner's ratio can be calculated by formula,

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \text{ X 100}$$

Percentage Yield [13]

The prepared microspheres of all batches were accurately weighed. The measured weight of prepared microspheres was divided by total amount of all the excipients and drug used in preparation of the microspheres, which give the total percentage yield of floating microspheres. It was calculated by using following equation.

$$\textbf{PY (}\%) = \frac{\text{Actual Weight of product}}{\text{Total Weight of Excipients and Drug}}$$

Drug Loading and entrapment efficiency [7, 14]**:**

Microspheres (equivalent to 50 mg of the drug) were taken for evaluation. The amount of drug loaded and entrapped efficiency was estimated by crushing the microspheres and extracting with aliquots of 0.1 N Hcl, repeatedly. The extract was diluted to 100 ml in a volumetric flask using 0.1 N Hcl. The solution was filtered and the absorbance measured at 237 nm against appropriate blank.

The drug loading in microspheres was estimated using following formula,

$$\text{DL (\%)} = \frac{W_D}{W_T} \text{ X 100}$$

Where,
DL = Drug loading,
W_D = the total weight of the drug loaded in the microspheres
W_T = the total weight of the microspheres.

$$\text{EE (\%)} = \frac{W_A}{W_T} \text{ X 100}$$

Where,
EE = Entrapment Efficiency,
W_A = Actual drug content and
W_T = Theoretical drug content

Percentage Buoyancy [11, 15]

Microspheres (0.3g) were spread over the surface of a USP XXIV dissolution apparatus (type II) filled with 900 ml 0.1 N Hcl. The medium was agitated with a paddle rotating at

100 rpm for 12 hrs. The floating and the settled portion of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres.

$$\% \text{ Floating Microspheres} = \frac{\text{Weight of floating microspheres at time t X 100}}{\text{Initial weight of microspheres}}$$

In vitro drug release studies [16]

The *in vitro* dissolution studies were carried using USP XXIV paddle type dissolution apparatus. Weighed amount of drug loaded floating microspheres were introduced into 900 ml dissolution medium of pH 1.2 maintained at 37±0.5 °C at a rotation speed of 100 rpm. 2 ml of aliquots were withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The samples were analyzed spectrophotometrically at 237 nm to determine the concentration of drug present.

Release Kinetics [17]

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into, Zero order, First order, Higuchi matrix, and Peppas model. The 'r' values obtained were compared to judge the best fit model.

RESULT AND DISCUSSION

The IR spectra of pure Metformin Hcl and drug loaded alginate beads, shown in the Fig.1, in which no significant shift in the peaks were observed, indicating the stability of the drug during encapsulation process.

Micromeritic studies reveal that the prepared microspheres were faint brown, discrete, spherical and compact. Mean particles size of formulated microspheres was found to be in the range of 576.60±11.09 to 730.36±10.33 µm. The mean particle size of microsphere was found to increase with the polymer concentration, due to increase in viscosity of the polymer solution which increased the droplet size. The value of angle of repose determined ranges between 20º to 29º, bulk density and tapped density of formulated microspheres was found to be in range of 0.318±0.03 to 0.457±0.04 and 0.364±0.04 to 0.531±0.06 respectively. The Carr's index and Hausner's ratio was found to be in range of 12 to 16, 1.13±0.02 to 1.18±0.02 respectively .Upon considering the micromeritic properties of all formulations, F5 showed the best flow properties and compressibility, as indicated by angle of repose (20º22'), lowest Carr's index (12.32±1.6), and Hausner's ratio (1.13±0.02) suitable for formulation of solid dosage form. Results tabulated in Table 2.

The scanning electron microscope images of microsphere illustrate spherical shape with thin and incomplete calcium surface coat with porous nature and rough texture as shown in the Fig.2. The porous structure may be due to rapid escape of the carbon dioxide during formulation. Inward dents observed in formulation indicate collapse of the walls of the microspheres during the in situ drying process.

It was found that the average percentage yield was greater than 80 % for all formulations. The drug loading was found to be in range of 26.91 to 41.93 %. Formulation F4 showed highest loading of 41.93 % while lowest drug loading of 26.91 % was observed in formulation F9. Overall the drug loading was found to decrease with increase in the polymer concentration due to its higher viscosity which affects the diffusion coefficient of drug. Drug entrapment was attributed to the permeation characteristics of polymers used, that could facilitate the diffusion of part of entrapped drug to the surrounding medium during preparation of floating microspheres. Depending upon the drug to polymer ratio the drug entrapment was found in the range of 56.60 to 75.33%. The drug loading was found to decrease with increase in polymer concentration due to its higher viscosity which affects the diffusion coefficient of drug. *In vitro* buoyancy studies reveal that in spite of stirring the dissolution medium for more than 12 hours about 73 to 88% microspheres of batches F1 to F9 still continued to float without any apparent gelation. This indicates that the microspheres exhibit excellent buoyancy which may be attributed to the pores and cavities present in them. Formulation F9 showed least percentage buoyancy of 73.50 %, while F5 showed highest buoyancy of 88.17 % attributed to the relative density of microspheres in higher polymer concentrations. *In vitro* drug release studies it was found that with increase in concentration of HPMC E50 and Ethyl cellulose the drug release decreases. The increased density of the polymer matrix at higher concentration results in an increased diffusional path length. This may decrease the drug release from the polymer matrix.

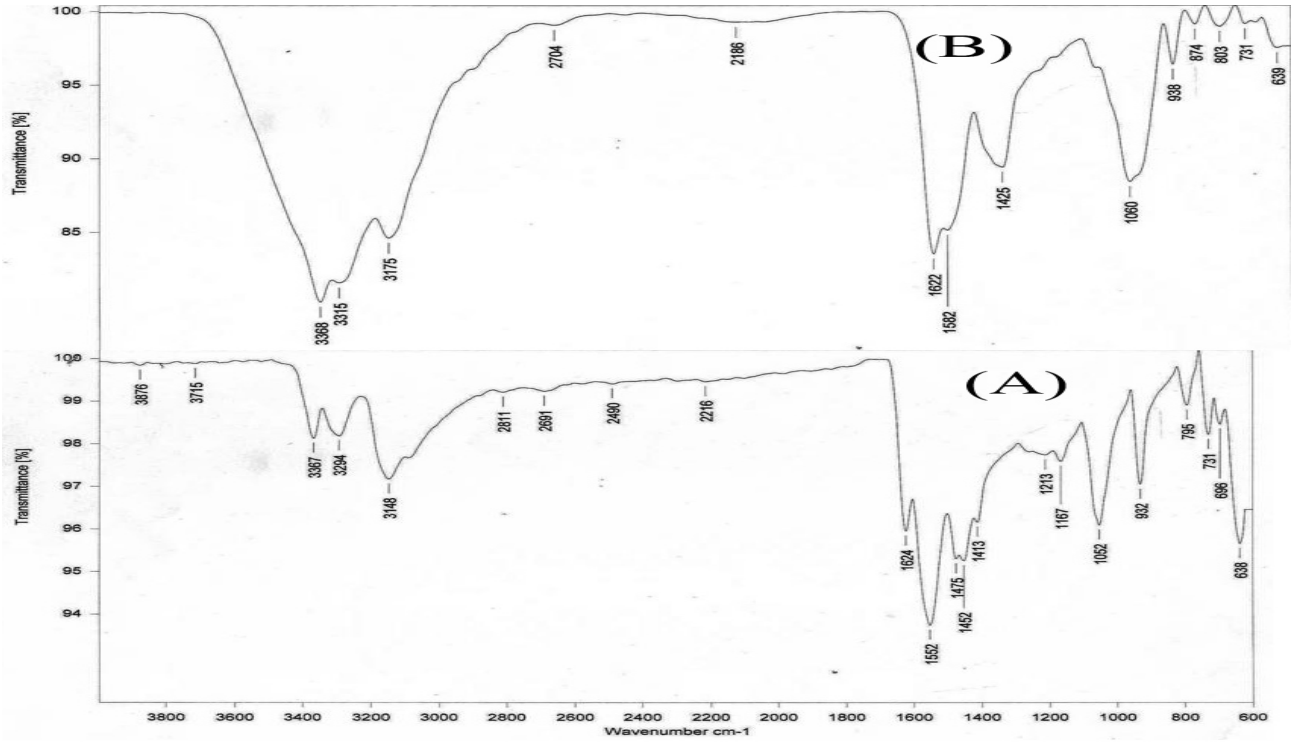

Figure 1: (A) IR spectra of pure Metformin HCl, (B) IR spectra of optimized formulation

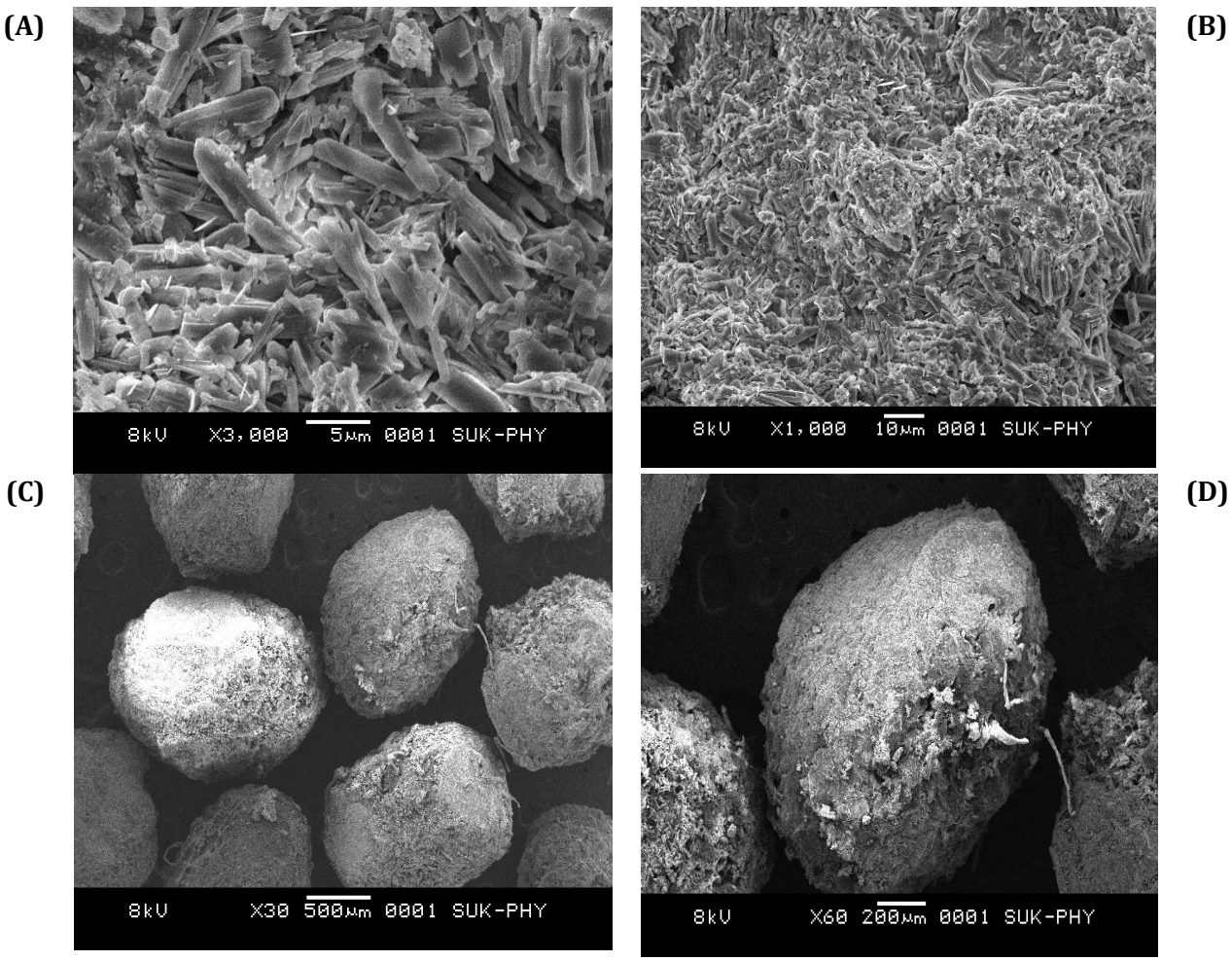

Figure 2: (A) Surface Topography (3000 X), (B) Surface Topography (1000 X), (C) Spherical and Rough, (D) Rough and Porous nature

The *in vitro* release profile obtained indicated a biphasic pattern with initial burst effect followed by polymer controlled slower release. From the value of r obtained, all formulation follow the Higuchi matrix model which is diffusion controlled. Results tabulated in Table 3.

The result of regression analysis showed that all the co-efficients bear a different sign, which indicated that both the polymers show different effect on the release of drug. Drug release at 2nd h (Q_2) gives correlation co-efficient 0.9765. The P value for variable X_1 and X_2 were 0.0970 and 0.2973 respectively (P<0.05), indicating that X1 and X2 variables did not show significant effect on drug release and combination co-efficient was positive but the P value was not less than 0.05, which indicated that combination of independent variable showed no significant effect at 2nd h release as depicted in Fig. 3.

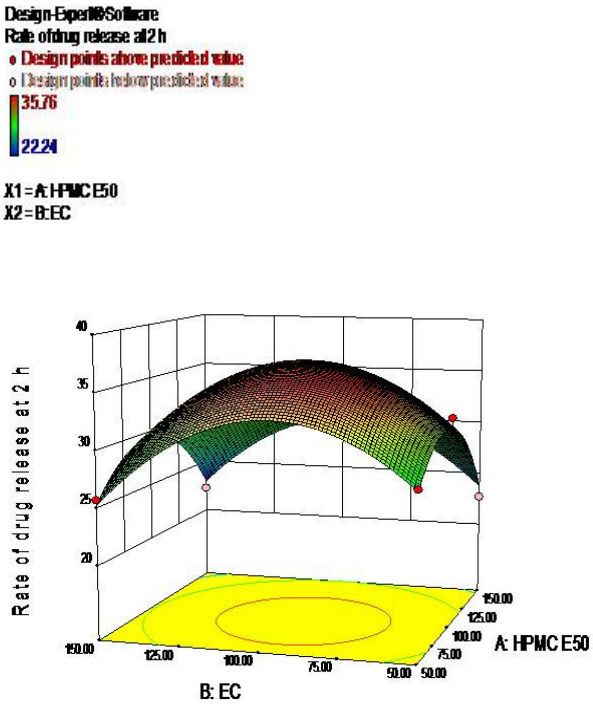

Figure 3: *In vitro* Drug release at 2 hrs

$Q_2 = 28.98 - 1.596X_1 - 0.841X_2 + 0.452X_1X_2 - 5.40X_1^2 - 6.58X_2^2$

Drug release at 6 h (Q_6) showed less linearity compared to Q_2 with correlation co-efficient 0.9241. The P value for variable X_1 and X_2 were 0.1211 and 0.2113 (P<0.05), indicating that variables X1 and X2 fail to show any significant effect even after 6 h; The combination co-efficient was positive but the P value was not less than 0.05 indicating that the combination of independent variable did not show significant effect at 6 h release. The co-efficient of X_1 and X_2 were negative indicated that when concentration of both the variable increased, drug release decreased as depicted in Fig. 4.

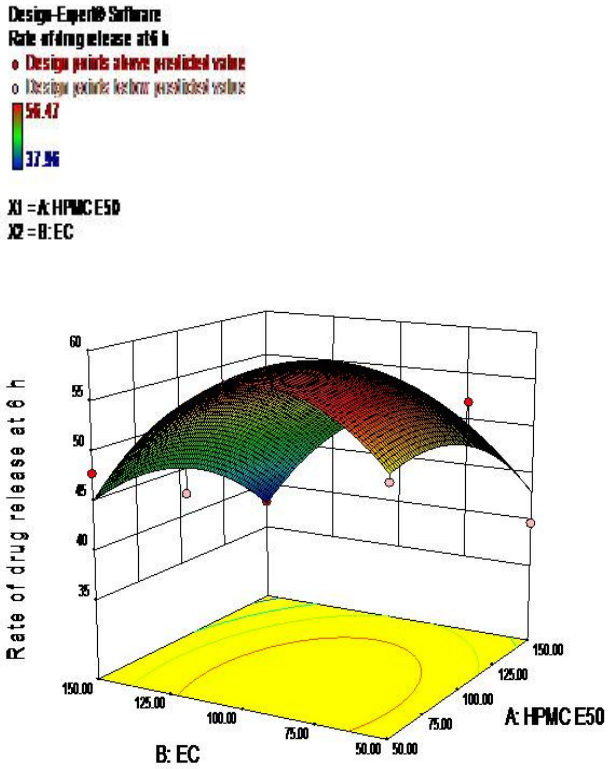

Figure 4: *In vitro* Drug release at 6 hrs

$Q_6 = 48.853 - 3.976X_1 - 2.935X_2 + 0.33X_1X_2 - 3.78X_1^2 - 9.645X_2^2$

Drug release at 10 h (Q_{10}) showed the P value for variables X_1, X_2 and X_1X_2 were 0.036, 0.042, 0.588 respectively; indicating that variables X1 and X2 had significant effect; whereas combination of variables failed to show significant effect on drug release at 10 h. The co-efficient of X_1 and X_2 were negative indicating that when concentrations of both the variables increased, drug release decreased as depicted in Fig.5.

$Q_{10} = 65.921 - 3.401X_1 - 3.191X_2 + 0.69X_1X_2 - 5.79X_1^2 - 11.12X_2^2$

The Q_2, Q_6, and Q_{10} for all the batches F1 to F9 varied from 22.24 % to 35.76%, 37.96% to 56.47%, and 45.40% to 68.02% with correlation coefficients 0.9765, 0.9241 and 0.9825 respectively. Results tabulated in Table 4.

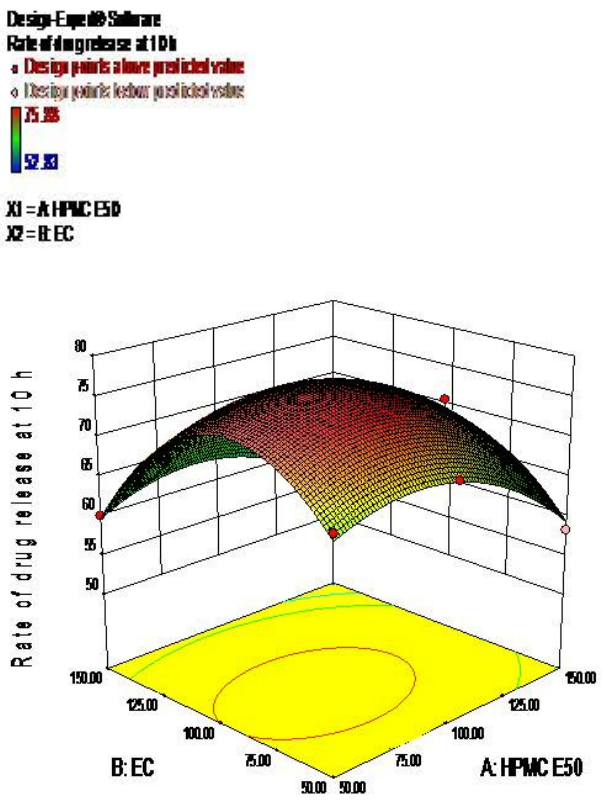

Figure 5: *In vitro* Drug release at 10 hrs

CONCLUSION

Metformin Hcl loaded alginate microspheres were successfully prepared by inotropic gelation method for use in floating drug delivery system. Concentration of polymer ratio influenced drug loading, entrapment efficiency, buoyancy and drug release profile of microspheres. A systematic study using a 3^2 factorial design helped in optimizing the suitable polymer concentration for desired drug release.

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