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Mucoadhesive microspheres of oral anti diabetic drug-Glipizide using different polymers

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ABSTRACT

The objective of the present investigation was to formulate and evaluate the mucoadhesive microsphere of Glipizide using Hydroxyl Propyl Methyl Cellulose K4M and Carboxy Methyl Cellulose as polymers. Glipizide microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross linking agent. Twenty preliminary trial batches, F1-F20 batches of microspheres were prepared by using different volume 10 to 70 ml of glutaraldehyde as cross linking agent, cross linking time 1 to 4 hours and 3:1 ratio of polymer-to-drug with two different polymers. From these twenty batches of each polymer, the optimized formulation is selected based on the percentage of mucoadhesion, Drug entrapment efficiency and sphericity of microspheres. A 3^2 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X1), and stirring speed (X2) on dependent variables percentage of mucoadhesion, drug entrapment efficiency, swelling index and invitro drug release study. The drug polymer compatibility studies were carried out using FTIR and the stability studies were conducted for the optimized formulation. Among the two polymers, the best batch was Hydroxy propyl methyl cellulose K4M exhibited a high drug entrapment efficiency of 69% and a swelling index 1.16 % mucoadhesive after 1hour is 70% and the drug release was also sustained for more than 12 hours. The polymer-to-drug ratio had a more significant effect on the dependent variables.

Keywords: Microspheres, Glipizide, Hydroxy Propyl Methyl Cellulose K4M, Carboxy Methyl Cellulose, Glutaraldehyde.

INTRODUCTION

A primary object of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the GI tract. Stability problem in the intestinal fluid can be overcome. Therapeutic effect of drugs insoluble in the intestinal fluids can be improved[5]. Mucoadhesive microspheres carrier systems are made from the biodegradable polymers in

sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems[1-3]. Microspheres form an important part of such novel drug delivery systems. They have carried applications and are prepared using assorted polymers[1]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[6-9]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site[10-13].

Glipizide is a second-generation oral anti-diabetic drug used in type-2 diabetes (Non-Insulin dependent diabetes mellitus) that can acutely lower the blood glucose level in humans by stimulation the release of insulin from the pancreas. Its shot biological half life (0.3+0.7 hours) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg of per day[18, 20-21]. HPMC K4M and CMC are synthetic good mucoadhesive and biodegradable polymers.

Thus the development of controlled-release dosage forms would clearly be advantageous. Moreover, the site of absorption of Sulfonyl ureas in the stomach. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Thus, an attempt was made by using synthetic mucoadhesive polymers HPMC K4M and CMC by using Glipizide as a drug. On the basis of the preliminary trials a 3^2 full factorial design were employed for all the polymers batches, to study the effect of independent variable X_1 polymer-to- drug ratio and the stirring speed X_2 on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and t80. The drug polymer compatibility studies were carried out using FTIR. The stability studies were conducted for the optimized formulation.

MATERIALS AND METHOD

Glipizide was obtained as gift sample from Madras Pharmaceuticals, Chennai. Hydroxy propyl methyl cellulose K4M was obtained from Orchid Lab, Chennai. Carboxy methyl cellulose was obtained from AET, Laboratories, Hyderabad. Span 85(0.5%w/v) was obtained from Loba Chemical Pvt. Ltd, Mumbai. Acetic acid, Petroleum ether 80:20, Light and heavy Liquid paraffin, Glutaraldehyde (25% v/v aqueous solution) of analytical grade are used.

Preparation of microspheres

Microspheres were prepared by simple emulsification phase separation technique by using two different polymers HPMC K4M and CMC. The different volume of cross linking agent glutaraldehyde was used as per method described in Thanoo et al[14].

Polymer (1.5gms) was dissolved in 150ml of 1% v/v aqueous acetic acid solution and 500mg of drug was dispersed in the polymer solution in F1-F20 batches. The resultant mixture will be extruded through a syringe (No.20) in 1lit of liquid paraffin (Heavy and light 1:1 ratio). Containing 0.5% Span 85 and stirring was performed using propeller stirrer at different stirring speed. After 15 min cross linking agent glutaraldehyde was added and stirring was continued. The amount of cross linking agents (10-70mL) and cross linking times were varied (1-4hrs), respectively, as showed in Table 1.In factorial design batches B1-B9, the optimized amount of

glutaraldehyde was used as a cross linking agent and cross linking time. The Polymer–to-Drug ratio (1:1, 3:1 and 6:1) and Stirring speed (500, 1000 and 1500rpm) were varied in batches B1-B9 was showed in Table II. Microspheres thus obtained were filtered and washed with Petroleum ether (80:20) to remove traces of oil. They were finally washed with water to remove excess of glutaraldehyde. The microspheres were then dried at room temperature at 25^0C & 60% RH for 24 hours.

Evaluation of microspheres

Drug content

According to literature review the assay for second generation oral-anti diabetic drugs like Glipizide was estimated by ultraviolet visible (UV/VIS) spectrophotometric method. Aqueous solution of drug was prepared in phosphate buffer (pH 7.4) and absorbance is measured on ultraviolet visible spectrophotometer at 276 nm[22]. The method is validated for linearity, accuracy and precision. The method obeys Beer's law in the concentration range of 5- 50 mcg/ml, a standard drug solution was analyzed repeatedly, the mean error (accuracy) and relative standard deviation (Precision) were determined.

Drug entrapment efficiency

50 mg of microspheres were crushed in a glass mortar and pestle, and the powdered microspheres was suspend in 10 ml of phosphate buffer solution (pH 7.4). After 24 hours, the solution filtered and the filtrate is analysed for the drug content. The drug entrapment efficiency is calculated using the following formula;

Practical drug content/Theoretical drug content x 100.

Particle size

The particle size of the microspheres was determined by using optical microscopy method[23]. Approximately 50 microspheres are counted for particle size using a calibrated optical microscope.

Swelling Index of Microspheres

For estimating the swelling index, the 100 microspheres was suspended in 5ml of simulated gastric fluid USP (pH 1.2)[24]. The particle size would be monitored by microscopy technique every 1 hour using an optical microscope. The increase in particle size of the microspheres will be noted for up to 8 hours and the swelling index is calculated as per method described by Ibrahim[25].

In-Vitro Wash-off test for Microspheres

The mucoadhesive properties of the microspheres are evaluated by in-vitro wash-off test reported by Lehr et al[26]. A 1cm by 1cm piece of rat stomach mucosa was tied onto a glass slide (3inch by 1inch) using thread. Microspheres are spread onto the wet rinsed tissue specimen, and the prepared slide is hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue is counted.

Drug release study

The drug release study will perform using USP XXIV basket apparatus[22] at 37^0C+0.5^0C and 50 rpm using 900ml of phosphate buffer (pH7.4) as dissolution medium. Microspheres equivalent to 10 mg of glipizide were used for the test. Five ml of sample was withdrawn at predetermined time intervals and filtered through a 0.45 micron membrane filter, diluted suitably and analyzed. Spectrophotometrically an equal amount of fresh medium was replaced immediately after withdrawn of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lamberts-Beer's law equation. The t80 was calculated using the weibullequation [27].

Scanning electron microscopy

A scanning electron photomicrograph of drug-loaded mucoadhesive microspheres was taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber. The scanning electron photomicrograph is taken at the acceleration voltage of 20kv chamber pressure or 0.6mm Hg, Original magnification X 800[11].

Stability testing

Optimized formulations of microspheres were tested for stability studies. Both the formulations were divided into 3 sample sets and stored at 4 ± 1^0C, 25± 2^o C & 60 ± 5% RH and 37± 2^oC & 65 ± 5% RH. After 30 days, in vitro drug release studies and percentage entrapment efficiency were determined.

Release kinetics and mechanism

To know the release mechanism and kinetics of Glipizide, optimized formulation was attempted to fit in to mathematical models and n, r^2 values for zero order, First order, Higuchi and Peppas models.The peppas model is widely used, when the release mechanism is not well known or more than one type of release could be involved. The semi-empirical equation.

$$Mt/M\infty = ktn$$

Where, *Mt*/*M*∞ is fraction of drug released at time't', k represents a constant, and n is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-fickian release, the value of n falls between 0.5 and 1.0; while in case of fickian diffusion, n = 0.5; for zero-order release (case II transport), n = 1; and for supercase II transport, n > 1. Observation of all the r^2 values indicated that the highest r^2 (0.9756) value was found for Zero order release. According to 'n' value it is one, so it follows non-fickian diffusion with zero order release (case II transport).

Factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

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

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and bi is the estimated coefficient for the factor X_i. The main effects (X_1 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. The polynomial

terms (X_1^2 and X_2^2) are included to investigate non-linearity. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables i.e. drug:polymer ratio (X_1) and the stirring speed at rpm (X_2) on dependent variables % mucoadhesion, drug entrapment efficiency, particle size and the time required for 80% drug dissolution (t80).

RESULTS AND DISCUSSION

The glipizide mucoadhesive microspheres were prepared by simple emulsification phase separation technique using HPMC K4M and CMC. Acetic acid from 1% to 8% v/v was used to prepare polymer solution. But there is no effect in concentration of acetic acid was observed on percentage mucoadhesion or drug entrapment efficiency, therefore 1% v/v of acetic acid was used.

Polymer concentration is an important factor, mentioned in Lee et al based on Viscosity of polymers solution. Three different concentrations 0.5,1 and 2% v/v were selected for trial batches, from this 1% concentration showed a maximum sphericity was observed so we select 1% w/v of polymer in 1% v/v acetic acid was found to be the optimum concentration and 1:1 Heavy and light paraffin was used as dispersion medium and 0.5% v/v of Span 85 is added as anionic surfactant to dispersion medium was found to be essential to minimize aggregation of microspheres.

Forty preliminary trail batches F1-F20 of microspheres were prepared by using HPMC K4M and CMC as polymers, the volume of cross linking agent 10-70ml and stirring speed were varied from 500, 1000 and 1500 rpm shown in Table I. From these forty batches, the F1-F4 batches of HPMC K4M and CMC were prepared by using 10 ml glutaraldehyde showed very irregular shaped microspheres and percentage of mucoadhesion also good but drug entrapment efficiency is not good. Batches F5-F8 prepared by using 20ml of glutaraldehyde showed good mucoadhesion properties and drug entrapment efficiency.

Batches F9-F12 of HPMC K4M and CMC was prepared by using 40ml of glutaraldehyde showed spherical free flowing microspheres and also shows 63 to77% and62 to 76% mucoadhesion, 53 to 58% and 59 to 54% of drug entrapment efficiency. Batches F13-F16 of HPMC K4M and CMC showed 59 to 79% and 64 to 86% of mucoadhesion, also showed 59 to 64% and 66 to 71% of drug entrapment efficiency. The batches F17-F20 was showed spherical free flowing microspheres and showed 71 to 67% and 76 to 67% of drug entrapment efficiency. The cross linking agent increase means the mucoadhesivenes is decreases and cross linking time did not show a significant effect on the percentage of drug entrapment efficiency, shown in Table I. From these twenty batches HPMC K4M and CMC the best optimized formula was F17 and F14 shown in table I. Thus, we conclude the cross linking time did not have a significant effect on the percentage drug entrapment efficiency.

On the basis of the preliminary trials 3^2 full factorial design were employed, to study the effect of independent variable X_1 (polymer-to- drug ratio 1:1, 3:1 and 6:1) and the stirring speed X_2 (500, 1000 and 1500rpm) on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and t80.The results depicted in Table II clearly indicate that all the dependent variables are strongly dependent on the selected independent variable as they show a wide variation among the nine batches. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries

(i.e. positive or negative).The dependent variables indicate a good fit. All the nine batches of two polymers HPMC K4M and CMC B1-B9 were prepare by using 70ml and 60ml of glutaraldehyde and 1 and 2 hours crosslinking time shown in Table II. Invitro wash of test for percentage mucoadhesion after 1 hour of HPMC K4M and CMC varied from 29 to 70% and 46 to 73% showed good correlation coefficient. These, indicates that the effect of X_1(polymer-to-drug ratio) is more significant thanX_2 (stirring speed). Moreover, stirring speed had a negative effect on percentage mucoadhesion (the stirring speed increased means the % of mucoadhesion is decreased).This finding may be attributed to the change in particle size that affects mucoadhesion. Similar results were obtained for swelling index. Thus, the polymer concentration increased the swelling index also increased. The swelling index varied from 0.297 to 1.160 and 0.667 to 1.637 showed good correlation coefficient. Thus, we can conclude that the amount of polymer and stirring speed directly affect the percentage mucoadhesion and swelling index .The drug entrapment efficiency varied from 36 to 69% and 46 to 74% showed good correlation coefficient. Indicates that the effect of X_1(polymer-to-drug ratio) is more significant thanX_2 (stirring speed). Moreover, stirring speed had a negative effect on drug entrapment efficiency (the stirring speed increased means the particle size and drug entrapment efficiency was decreased). Mucoadhesive microspheres of all the nine batches shows spherical and free flowing. They range in particle size from 42 to 68 and 47 to 95. The stirring speed has negative effect on t_{80} because as the particle size increased the drug release decreases. Batches B7 and B5 is the optimized formulation and they are spherical free flowing shown in Fig 1.

The stirring speed and polymer ratio was increased; the % of mucoadhesion and the drug entrapment efficiency was decreased. From these nine formulations of HPMC K4M and CMC the best optimized formula was B7 and B5 batches, shown in Table II.

The In vitro drug release studies were carried out the percentage drug dissolved at different time interval was calculated using the Lambert's-Beer's equation. The t80 was calculated using the weilbull equation. The average values of t80 for two polymer batches B1 to B9 are shown in Table III, IV.

The release mechanism and kinetics of Glipizide, optimized formulation was attempted to fit in to mathematical models and n, r^2 values for zero order, First order, Higuchi and Peppas models.The peppas model is widely used, when the release mechanism is not well known or more than one type of release could be involved and the report was given in Graphs I and II, Invitro Zero order dissolution studies and Hixon-Crowell models in Table V, Graphs III and IV.

The stability studies were carried out by storing the optimized formulations at $4^0 \pm 1^0$c, 25 $\pm 2^0$c & 60 $\pm$ 5% RH and 37$\pm 2^0$c & 65$\pm$5% for one month. The percentage entrapment efficiency and invitro release studies were carried out. The drug release of HPMC K4M and CMC microspheres at 4$\pm 1^0$c showed 92.25% and 89.23% , percentage entrapment efficiency 68.78% and 70.12%, the drug release at 25 $\pm 2^0$c & 60 $\pm$ 5% RH showed 96.41 % and 93.5% , percentage entrapment efficiency 65.36 % and68.20% and the drug release at 37 $\pm 2^0$c & 65 $\pm$ 5% RH showed 98.65% and 93%, percentage entrapment efficiency 63.71 %, 65.76. The FTIR spectroscopy indicates there was no interaction took place between drug and the polymer.

Table I. Preliminary Trials of glipizide mucoadhesive microsphere by using HPMC K4M and CMC

Batchcode	Vol. of glutaraldehyde (ml)	Cross linking time(h)	% Mucoadhesion after 1 hr.		Drug Entrapment Efficiency (%)		Sphericity of microsphere
			CMC	HPMC K4M	CMC	HPMC K4M	
F1	10	1	89	90	35	34	Very Irregular
F2	10	2	83	84	37	36	
F3	10	3	78	79	39	38	
F4	10	4	76	73	41	40	
F5	20	1	85	86	48	47	Slightly Irregular
F6	20	2	79	80	52	51	
F7	20	3	72	73	54	53	
F8	20	4	66	67	57	56	
F9	40	1	76	77	54	53	Spherical from following
F10	40	2	70	71	56	55	
F11	40	3	63	64	58	57	
F12	40	4	62	63	59	58	
F13	60	1	86	79	66	59	
F14	60	2	84	70	70	60	
F15	60	3	72	65	71	62	
F16	60	4	64	59	71	64	
F17	70	1	62	79	67	71	
F18	70	2	55	56	69	68	
F19	70	3	48	49	72	67	
F20	70	4	42	43	76	67	

Note: All batches were prepared by polymer to drug ratio of 3:1 at 1000 rpm speed

Table II. Formulation of glipizide mucoadhesive microsphere by using HPMC K4M and CMC loaded glipizide microsphere by using 3^2 full Factorial designs

Low (-1) - 1:1 – 500 rpm, Medium (0) - 3:1-1000 rpm and Low (+1) - 6:1 – 1500 rpm.

Batch Code	Variable levels in coded from		% Mucoadhesion After1hr		Drug Entrapment Efficiency (%)		Swelling Index		Particle Size		T80 (min.)	
	X1	X2	HPMC K4M	CMC	HPMC K4M	CMC	HPMC K4M	CMC	HPMC K4M	CMC	HPMC K4M	CMC
B1	-1	-1	47	55	58	51	0.674	0.743	68.1	57.0	243	234
B2	-1	0	32	49	41	49	0.366	0.679	44.5	55.2	236	230
B3	-1	1	29	46	36	46	0.297	0.667	42.5	47.2	223	218
B4	0	-1	51	73	47	69	0.589	1.637	57.4	64.1	211	202
B5	**0**	**0**	45	**68**	43	**68**	0.438	**1.170**	53.7	**61.2**	232	**229**
B6	0	1	42	65	39	63	0.326	0.937	49.3	57.8	241	248
B7	**1**	**-1**	**70**	83	**69**	74	**1.160**	1.297	**68.4**	95.0	**218**	492
B8	1	0	66	75	54	70	0.707	1.153	63.5	86.8	448	465
B9	1	1	58	70	48	67	0.726	1.097	59.8	71.4	401	376

Variables level --Drug-to-polymer ratio (X_1) and Stirring speed (X_2)

Table III. In-vitro Release profile of Glipizide mucoadhesive microsphere HPMC K4M –B7.

Time	Root Time	Log time	Abs	CDR	% CDR	Log % CDR	% Drug Retained	Log % Drug Retained	(%Retained)$^{1/3}$
1	1	1	0	0.0286	4.89	24.45	1.388	75.55	1.878
2	2	1.414	0.3010	0.0332	6.246	31.23	1.494	68.77	1.837
3	3	1.752	0.4771	0.0374	7.544	37.72	1.576	62.28	1.794
4	4	2	0.6020	0.0414	8.828	44.14	1.644	55.86	1.747
5	5	2.236	0.6989	0.0466	10.446	52.23	1.717	47.77	1.679
6	6	2.441	0.7781	0.0516	12.068	60.34	1.780	39.66	1.598
7	7	2.645	0.8450	0.0603	14.682	73.41	1.865	26.59	1.424
8	8	2.828	0.9030	0.0672	16.9	84.5	1.926	15.5	1.190

Table IV. In-vitro Release profile of Glipizide mucoadhesive microsphere CMC-B5.

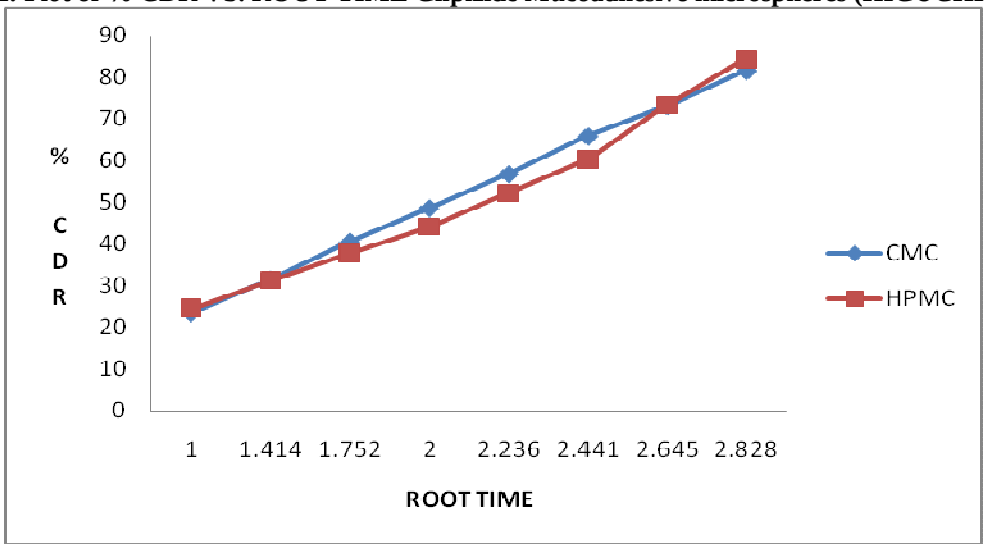
Time	Root Time	Log time	Abs	CDR	% CDR	Log % CDR	% Drug Retained	Log % Drug Retained	(% Retained)$^{1/3}$
1	1	0	0.0278	4.698	23.49	1.370	76.51	1.883	4.245
2	1.414	0.3010	0.0335	6.306	31.53	1.498	68.47	1.835	4.091
3	1.752	0.4771	0.0398	8.128	40.64	1.608	59.36	1.773	3.900
4	2	0.6020	0.045	9.736	48.68	1.687	51.32	1.710	3.716
5	2.236	0.6989	0.0501	11.366	56.83	1.754	43.17	1.635	3.508
6	2.441	0.7781	0.0557	13.18	65.9	1.818	34.1	1.532	3.242
7	2.645	0.8450	0.0596	14.638	73.19	1.864	26.81	1.428	2.992
8	2.828	0.9030	0.0644	16.322	81.61	1.911	18.39	1.264	2.639

Table V. Model Fitting for the Release Profile of Glipizide Muco adhesive Microspheres HPMC K4M-B7 and CMC-B5

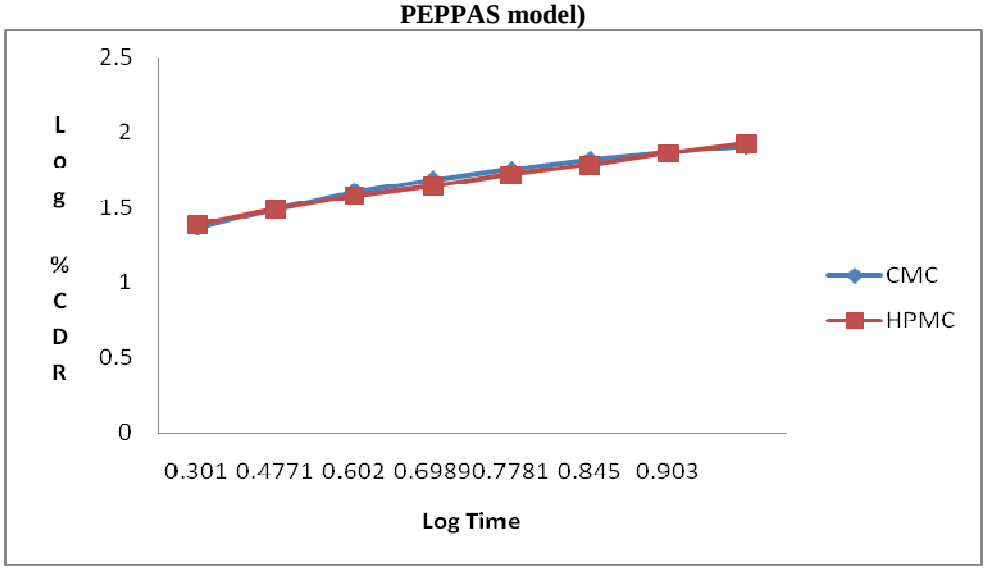
Formulation Code	Zero Order	First Order	Higuchi Matrix	Korsmeyer-Peppas		Hixon-Crowell	Best Fit Model
	R	R	R	R	N	R	
HPMC K4M	0.981 0	0.882	0.932	0.947	0.587	0.925	Zero
CMC	0.999	0.963	0.983	0.987	0.609	0.984	Zero

R= correlation coefficient; n= slope (≤0.5 – fickian diffusion; 0.5<n<1 – non fickian diffusion; 1 – Case – II transport; >1 – super case –IItransport)

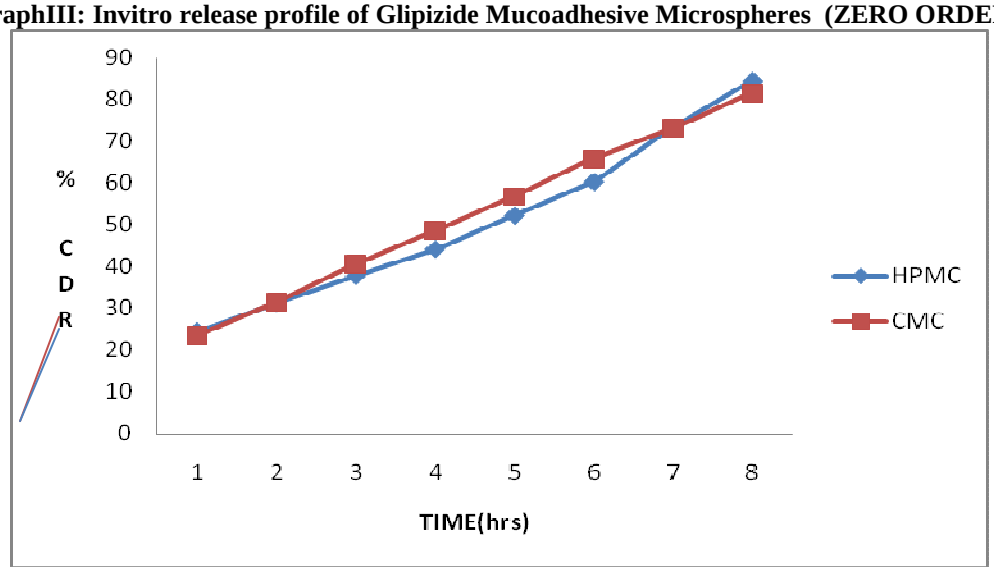
Graph I: Plot of % CDR VS. ROOT TIME Glipizide Mucoadhesive microspheres (HIGUCHI model)



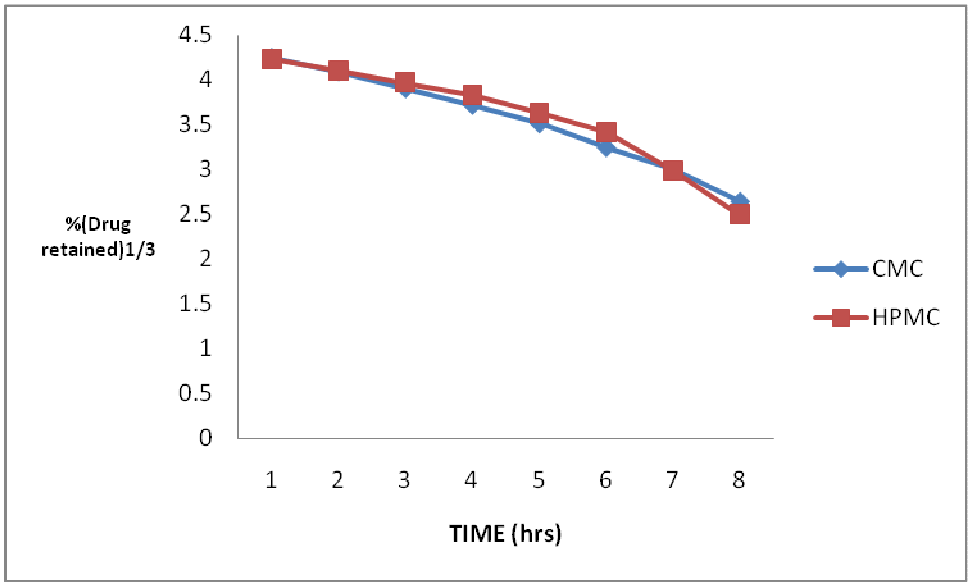
Graph II: Plot of Log %CDR VS. LOG TIME Glipizide Mucoadhesive Microspheres (KORSMEYER-PEPPAS model)



GraphIII: Invitro release profile of Glipizide Mucoadhesive Microspheres (ZERO ORDER).



Graph IV: Plot of (%drug retained) 1/3 vs. time for Glipizide mucoadhesive microspheres (Hixon-Crowell)



CONCLUSION

The results of a 3^2 full factorial design revealed that the polymer-to-drug ratio and stirring speed significantly affected the dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and swelling index. As the concentration of glutaraldehyde increases, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negetive effect on t_{80}. Among these two polymers HPMC K4M microspheres exhibited a high percentage mucoadhesion of 70% after 1 hour and 69% drug entrapment efficiency. The microsphere of glipizide could sustain the release of the drug for more than 12 hours. The t_{80} of 218 minutes indicates that the mucoadhesive microspheres could sustain the release of the drug for more than 12 hours.

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