Int J Pharma Res Health Sci. 2016; 4 (6): 1483-1488
DOI:10.21276/ijprhs.2016.06.10
Ananad K *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Formulation and Evaluation of Glipizide Mucoadhesive Microspheres

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ARTICLE INFO

Received: 24 Nov 2016
Accepted: 28 Dec 2016

ABSTRACT

Objective: Mucoadhesive microcapsule have the potential to be used as controlled drug delivery systems to provide efficient absorption and enhanced bioavailability by making intimate contact with the mucus membarane due to high surface to volume ratio, keeping an objective glipizide is selected as model drug.
Experimental approch: Glipizide mucoadhesive microcapsules were prepared by emulsification solvent evaporation method using colophony/chitosan alone and in different ratios. The microcapsules were characterized by FTIR, DSC, particle size, SEM, encapsulation efficiency, swelling index, *in vitro* wash off test and *in vitro* drug release.
Findings: The microcapsules were spherical, discrete, free flowing and exhibit good mucoadhesion and swelling indices. The *in vitro* study suggests drug release was diffusion controlled and follows zero order. The best fit model was Korsemeyer Peppas with 'n' values greater than 0.5 indicating the drug release mechanism was non fickian.
Disucssion: The presence of chitosan/colophony as mucoadhesive polymer in the microcapsules resulted in controlled drug release. This can be attributed to poor swelling of chitosan as well as low errosion of colophony. Increase in chitosan/colophony concentrations resluts in reduced porosity, different drug release profiles. Increase in concentrations of colophony the drug release was faster due to low ionic interaction and faster solubilization of microcapsules. At high concentrations of chitosan the drug release was retarded for longer duration of time.
Conclusions: The results indicated colophony/chitosan are selected as promising mucoadhesive carriers for controlling drug delivery of glipizide.
Keywords: Microcapsules, Glipizide, Colophony, Chitosan, *In vitro* dissolution

1. INTRODUCTION

Mucoadhesive microcapsules include microparticles and microspheres having a diameter of 1-1000µm and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Microcapsules, in general have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microcapsules as additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact

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with the mucus layer, specific targeting of the drug to the absorption site.

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years 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. Microspheres form an important part of such novel drug delivery systems[1-3]. They have varied applications and are prepared using assorted polymers[4]. 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[5-8]. 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[9-12]. Chitosan (obtained by deacetylation of chitin) is a cationic polymer that has been proposed for use in microsphere systems by a number of authors[13-17]. Chitosan was selected as a polymer in the preparation of mucoadhesive microcapsules because of its good mucoadhesive and biodegradable properties. Glipizide is a second generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat type II diabetes (non-insulin-dependent diabetes mellitus). Its short biological half-life (3.4±0.7hr) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day[18]. Thus, the development of controlled release dosage forms would clearly be advantageous. Researchers have formulated oral controlled release products of glipizide by various techniques[19,20]. Moreover, the site of absorption of glipizide is 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 in this investigation to use chitosan/colophony as a mucoadhesive polymer and prepares microcapsules. The microcapsules were characterized by *in vitro* stuides.

2. MATERIALS AND METHODS

Materials: Glipizide was obtained as gift sample from M/s Lupin laboratories, Pune. Chitosan (central house Mumbai) and Colophony were procured from commercial sources.

Preparation of colophony: Colophony was extracted repeatedly with solvent ether (10g colophony 4X50ml ether), the ether extract was concentrated to dryness. The dried lump was powdered and passed through the mesh 120 and used. All other reagents used were of analytical grade. Distilled water was used throughout the study.

Method: The microspheres containing glipizide were prepared using colophony/chitosan alone and in combination as coat material by an emulsification solvent evaporation method. The required quantity of colophony/chitosan/mixture of two was dissolved in 10 ml of chloroform. The resulting chloroform mixture was emulsified by adding dropwise (1ml/min) 200 ml of 0.5% w/v sod CMC through a syringe with a needle no 23 in a mechanical stirrer rotating at 1000 RPM for 3hr. The obtained microspheres were washed repeatedly with water and are collected by vaccum filtration. The spherical, rigid microspheres were dried at room temperature for 12h and stored in suitable storage conditons for further evaluation. Different formulae of glipizide microspheres were given in table 1.

Evaluation methods

Entrapment efficiency: Microcapsules eqivivalant to 50mg were taken crushed in a glass mortar and pestle and the powdered microcapsules were extracted with 50ml of methanol and the solution was kept for 1h with occasional shaking. Further 1ml solution was diluted to 100 ml with phosphate buffer pH 7.4. The drug content was determined spectrophotmetrically at 223nm method and the drug entrapment efficiency was calculated by using the following formula.

Entrapment efficiency = Drug entrapped / Theoretical drug content X 100

Particle size: The particle size of the microcapsules was determined by using range of standard sieve 30/40, 40/60. Amount of microcapsules retained on different sieves were weighed and average size of microcapsules was calculated using the formula,

$D_{avg} = (\sum Xi\ fi) / fi$

Where, Xi → Mean weight

fi→Percentage material retained on the smaller sieve in the size range.

Shape and surface morphology: The external morphology of microspheres was analyzed by scanning electron microscopy (SEM). Microspheres were fixed on aluminum studs and coated with gold using a sputter coater SC 502, under vacuum [0.1 mm Hg]. The microcapsules were then analyzed by scanning electron microscopy (SEM) [Model JSM-840 A, Joel. Japan].

Swelling index: Swelling index was determined by measuring the extent of swelling of microspheres in phosphate buffer.The microspheres equivalent to 50mg were placed in glass vial containing 10ml of phosphate buffer pH 7.4 at 37°C ± 0.5°C in the incubator with occasional shaking. The microspheres were removed periodically, blotted with filter paper and observed for weight changes. Weight was measured during the swelling until equilibrium was obtained. The degree of swelling was calculated by the following formula,

Degree of swelling = We-Wo/Wox100

Where, Wo is initial weight

We is weight of swollen microcapsules

***In vitro* wash-off test:** Mucoadhesive property of the microcapsules was evaluated by an *in vitro* adhesion testing known as the wash-off test. The intestinal mucosa of 1cm^2 area was tied to a glass slide (3X1 inch) with thread. Microcapsules were spread (~50) onto wet and rinsed tissue specimen. The slide was then hung onto grooves of the USP tablet disintegrating test apparatus. The tissue specimen was given a slow, regular up-and-down movement in a beaker containing phosphate buffer pH 7.4 (500ml) at 37°C. The number of microcapsules still adhering to tissue was calculated at the end of 30min, 1h and at the hourly interval up to 8h.

***In vitro* dissolution studies:** The *in vitro* dissolution studies were performed at two pH levels viz., 1.2 pH (simulated gastric fluid) and 7.4 pH phosphate buffer (simulated intestinal pH). The glipizide release study was performed using USP type I basket apparatus at 37°C± 0.5°C and at 50 rpm. An accurately weighed microspheres equivalent to 10 mg of glipizide were used for the study. The dissolution is carried out in 900ml 0.1N (pH 1.2) for 2hr and after 2hrs the dissolution medium was replaced with pH 7.4 phosphate buffer and continued the dissolution. In each case 5ml sample solution was withdrawn and replacing equal volume of respective dissolution mediums at predetermined time intervals, filtered, diluted suitably and analyzed spectrophotometrically at 223nm. The *in vitro* release data was computed and intrepreated by using dissolution software PCP Disso V3.0.

3. RESULTS AND DISCUSSION

The compatibility between drug and polymer was confirmed by using FTIR spectral data figure. FTIR spectra of glipizide shows characteristic peaks at 3326 and 3251 cm^{-1} for NH-CO-NH and CONH stretching; 3030 cm^{-1} for aromatic C-H stretching; 2855, 2915 cm^{-1} for C-H stretching of CH_2 groups; 1689 cm^{-1} Co of NH-CO-NH; 1650 cm^{-1} CO of CONH; 1598 cm^{-1} C=N stretching; 1583,1527,1484 cm^{-1} for C=C aromatic ring stretching; 1409 cm^{-1} for C-N stretching and 840 cm^{-1} for 1,4 di substituted phenyl ring. The appearance of strong absorption bands at cm^{-1} 3327, 3251 and 2943, 2854, 1684, 1651, 1598 and 1583 indicates that there is no interaction between the drug glipizide and polymer colophony. FTIR spectra glipizide, chitosan and mixture of glipizide and polymer chitosan clearly resembles the glipizide and the bands corresponding to glipizide were strongly observed in the spectra which indicates no interaction between drug and polymer chitosan. FTIR spectra of pure glipizde, chitosan, colophony and mixture of glipizide and two polymers colophony, chitosan respectively clearly indicates that no interaction between glipizide and polymer because all the characterstics absorption bands of glipizide were present figure1.

DSC thermogram of pure glipizide it shows a strong endothermic peak at 214.950C indicates the melting point of glipizide with onset at 211.61^{0}C. DSC thermogram of colophony shows broad endothermic peak at 87.72^{0}C indicates the melting point of colophony with onset at 87.72^{0}C. The DSC thermogram for mixture of glipizide-colophony-chitosan indicates that there is a slight interaction between glipizide with added polymers represented by broad endothermic peak at 175^{0}C .The DSC data clearly indicates there is no interaction between glipizide and added polymers figure 2, these results were justified by FTIR studies.

Table 1: Formulae of glipizide mucoadhesive microcapsules at different coat: core ratios

Batches	Coat : Core	Quantity (mg)		
		Chitosan	Colophony	Glipizide
F1	1:2	600	-	1200
F2	1:2	---	600	1200
F3	(1:1):2	300	300	1200
F4	(1:2):2	200	400	1200
F5	(1:3):2	150	450	1200
F6	(2:1):2	400	200	1200
F7	(3:1):2	450	150	1200

Table 2: Evaluation parameter data of glipizide mucoadhesive microcapsules

Batches	Yield %	Mean particle size in µm	Encapsulation effeciency %	Drug content %
F1	92.22±1.44	495.23 µm	60.21±1.12	98.97±2.12
F2	91.32±0.73	443.23 µm	58.23±1.43	99.82±1.23
F3	92.67±1.09	464.32 µm	72.21±0.99	98.82±1.42
F4	95.61±2.11	423.32 µm	64.23±2.11	99.89±1.44
F5	93.44±1.47	432.23 µm	69.12±0.89	97.67±1.98
F6	95.33±2.13	487.34 µm	78.21±1.58	97.04±2.31
F7	94.98±1.56	542.32 µm	76.23±1.82	98.99±1.11

Table 3: Relative swelling index of glipizide mucoadhesive microcapsules

Batches	F1	F2	F3	F4	F5	F6	F7
Time in hours	Relative swelling	Relative swelling	Relative swelling	Relative swelling	Relative swelling	Relative swelling	Relative swelling
0	0	0	0	0	0	0	0
0.5	0.2	0.18	0.4	0.46	0.4	0.44	0.46
1	0.4	0.38	0.52	0.68	0.54	0.74	0.78
2	0.6	0.46	0.6	0.84	0.76	0.8	0.84
3	0.68	0.48	0.74	0.90	0.82	0.98	1
4	0.72	0.58	0.88	0.91	0.88	1.06	1.06
5	0.74	0.61	0.94	0.96	0.91	1.08	1.1
6	0.76	0.71	0.99	0.97	0.94	1.08	1.12

Table 4: *In vitro* mucoadhesion test data of glipizide mucoadhesive microcapsules

Batches	Percentage of microcapsules adhering to tissue at different time interval (hr)					
	0	**0.5**	**2**	**4**	**6**	**8**
F1	50	92	76	50	32	12
F2	50	90	70	51	28	10
F3	50	96	80	72	50	41
F4	50	94	76	66	40	36
F5	50	100	78	70	41	38
F6	50	94	86	60	52	42
F7	50	96	88	62	53	45

Table 5: *In vitro* dissolution data of glipizide mucoadhesive microcapsules.

Time in hours	Cumulative percent drug release						
	F1	F2	F3	F4	F5	F6	F7
0.25	6.97	6.54	8.38	7.25	7.83	6.83	7.11
0.5	11.53	8.84	12.95	12.38	13.25	11.25	11.69
0.75	15.56	13.70	16.85	15.99	17.27	15.27	15.53
1	18.47	17.88	19.91	18.20	19.19	18.19	18.92
2	23.24	22.36	26.53	24.10	23.38	23.38	24.09
3	39.59	27.58	31.48	29.46	32.59	29.59	29.66
4	46.83	32.40	37.59	45.42	46.26	36.26	35.47
5	51.56	48.52	42.89	52.13	53.68	42.68	41.40
6	59.23	51.84	50.06	59.57	61.85	48.85	48.13
7	65.35	66.59	56.84	66.35	68.77	55.77	55.69
8	68.76	70.38	60.96	70.05	72.03	60.03	59.36
9	74.31	75.74	67.52	75.75	78.89	63.89	63.14
10	81.75	83.12	71.56	80.49	82.19	69.19	68.45
11	83.25	90.11	74.63	84.54	88.54	71.54	71.51
12	84.60	96.44	77.72	86.50	94.33	74.33	73.40

Table 6: Model fitting data of glipizide mucoadhesive microcapsules

Batches	Zero order		Korsemeyer's equation peppas		
	Regression coefficient(R)	k	Slope(n)	Regression coefficient(R)	k
F1	0.9812	5.3729	0.5885	0.9913	12.9671
F2	0.9799	5.9419	0.6032	0.9968	13.7108
F3	0.9873	5.9091	0.6388	0.9922	12.6733
F4	0.9892	6.1808	0.5574	0.9948	15.5727
F5	0.9847	5.9078	0.5905	0.9958	14.1824
F6	0.9824	5.7769	0.5986	0.9971	13.5558
F7	0.9824	6.2313	0.5987	0.9982	13.2342

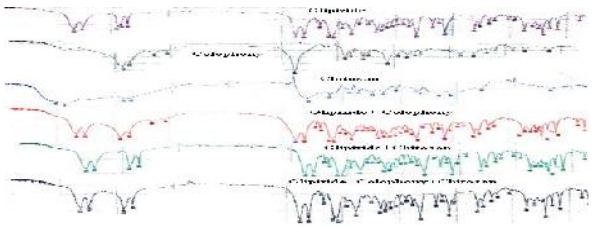

Fig 1: Comparitive FTIR spectra of glipizide, colophony, chitosan and mixtures.

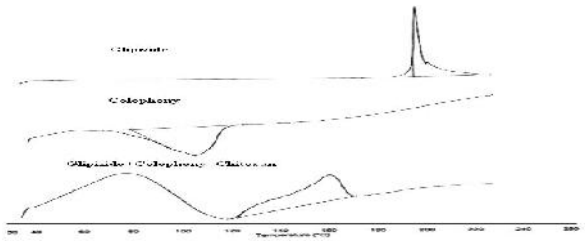

Fig 2: DSC Thermograms of glipizide, colophony and mixture.

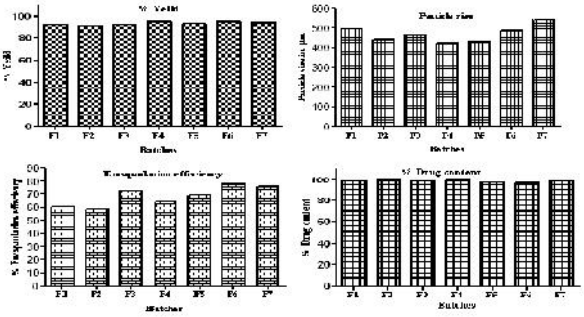

Fig 3: evaluation profiles of glipizide mucoadhesive microcapsules.

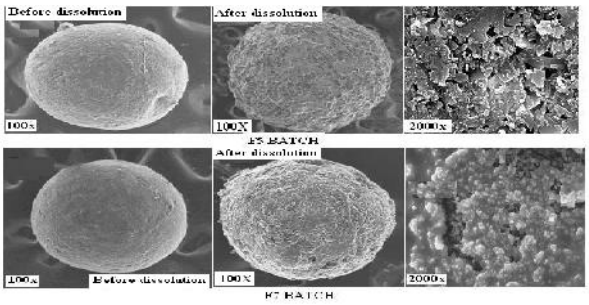

Fig 4: scanning electron microscope images of F5 and F7 formulations.

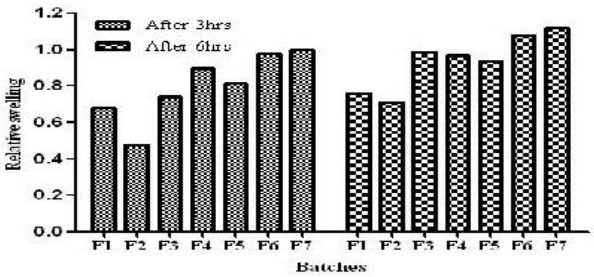

Fig 5: Swelling studies profiles of glipizide mucoadhesive microspheres.

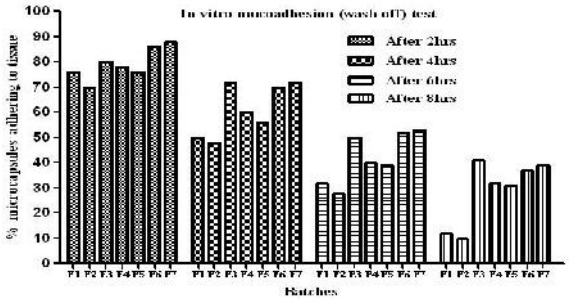

Fig 6: *In vitro* mucoadhesion profile of glipizide mucoadhesive microcapsules.

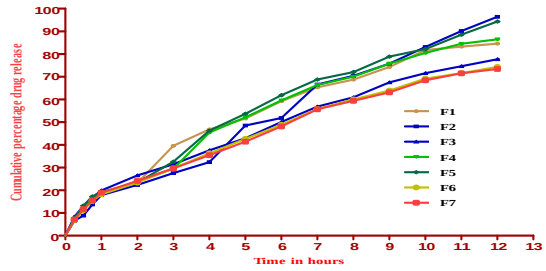

Fig 7: *In vitro* dissolution profiles of glipizide mucoadhesive microcapsules.

The mucoadhesive microcapsules of glipizide were prepared by emulsification solvent evaporation technique using chitosan/colophony and evaluation parameter data was given in table 2 and figures 3. The percentage yield was found to be in the range of 91.32±0.73 to 95.61±2.11 indicate small drug loss during preparation and the specified method was found to be reproducible. The drug content was in the range of 97.04±2.31to 99.82±1.23 for F1 to F7 formulations, low SD values indicate uniform distribution of the drug within the various batch of the microcapsules prepared. The encapsulation effeciency was in the range of 60.21±1.12 to 78.21±1.58, as the concentration of chitosan increases the encapsulation effeciency increases, which may be attributed

to stable gel complex in presence of abiatic acid present in colophony .further increase in concentrations of chitosan resulted in decreased encapsulation effeciency. Chitosan and drug concentration influenced negatively on encapsulation effeciency because at higher concentrations, colophony leads to the formation of aggregates upon additon of chitosan. The particle size was found to be in the range of 423.32 µm to 542.32 µm, the results showed that increase in particle size in colophony, chitosan and drug concentrations. Increase in both polymers-colophony and chitosan lead to interaction between corboxyl groups of colophony and the amino groups of chitosan, leading to increased size. Increase in ratios of colophony: chitosan from 1:3, there is sharp increase in particle size.

The external morphology of microspheres was studied by scanning electron microscope (SEM). The microcapsules were found to be uniform, discrete, and fairly spherical in shape, while the surface roughness was slightly increased with the incorporation of drug. The nature of prepared microcapsules indicated the microcapsules to be monolithic and multinucleate type, figure 4.

The results of swelling stuides suggest the decreasing trend with increase in concentration of colophony at different time intervals and increase in concentration of chitosan results in increase in swelling at different time intervals it is mainl due to more swelling properties of chitosan table 3 and figure 5.

Microcapsules with a polymer coat consisting of colophony and chitosan exhibited good mucoadhesive properties in *in-vitro* wash off test compared to microcapsules prepared with colophony/chitosan as a coating polymer alone table 4 and figure 6.The wash off was slow in the case of microcapsules containing colophony:chitosan microcapsules compared to colophony/chitosan microcapsules alone. The slow wash off observed for colophony could be due to presence of carbonyl and other functional group present in abietic acid which was further increased with chitosan as copolymer inducing synergetic effect. This may be attributed hydrogen bonding established and there by an increased adhesive strength.

***In vitro* release:** The presence of chitosan/colophony as mucoadhesive polymer in the microcapsules resulted in controlled drug release. This can be attributed to poor swelling of chitosan as well as low errosion of colophony. Increase in chitosan/ colphony results in reduced porosity, hence gives different drug release profiles. Increase in concentrations of colophony the drug release was faster due to low ionic interaction and faster solubilization of microcapsules. At high concentrations of chitosan the drug release was retarded for longer duration of time.

The cumulative percentage drug release was found to be 84.60, 96.44, 77.72, 86.50, 94.33, 74.33 and 73.40 respectively over a period of 12 h for F1 to F7 formulations. Further the dissolution data were subjected for model fitting by using dissolution software DISSO V3. The results shows that the release of the drug from all the formulations followed zero order kinetics with 'r' values of 0.9812, 0.9799, 0.9873, 0.9892, 0.9847, 0.9824 and 0.9854 and the best fit model was found to be Korsemeyer-Peppas with 'n' values of 0.5885, 0.6032, 0.6388, 0.5574, 0.5905, 0.5986 and 0.5887for F1, F2, F3, F4, F5,F6 and F7 respectively. In all the formulations the release exponent 'n' was more than 0.5 indicating the release was non-fickian mechanism and is diffusion controlled. The results were given tables 5, 6 and figure 7.

4. CONCLUSIONS

Glipizide muchoadhesive microcapsules were succefully prepared using natural polymers viz., chitosan and colophony by emulsification solvent evaporation method. From the results it was concluded that colophony/chitosan are selected as promising mucoadhesive carriers for controlling drug delivery of glipizide.

5. ACKNOWLEDGEMENT

The authors are greatful to principal and staff of V.L.College of pharmacy for provideing facilities during the project. We also acknowledge M/s Lupin laboratories, Pune for provideing gift sample of pure drug Glipizide.

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Conflict of Interest: None

Source of Funding: Nil