MUCO ADHESIVE MICROSPHERES OF AN ORAL ANTI-DIABETIC DRUG GLIPIZIDE

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Summary

Glipizide is an oral anti hyperglycaemic drug, used in the management of non-insulin dependent diabetes mellitus (NIDDM) which is having low bioavailability (50%). The bioavailability can be improved by altering the pharmacokinetic profile of the drug by controlling the release of the drug from the formulation. Hence, the present study Chitosan (Natural Polymer) mucoadhesive microspheres were prepared to prolong the release of the drug into systemic circulation. Mucoadhesive microspheres were prepared with emulsification phase seperation method varying the Chitosan/Drug ratio (1:1, 3:1, 6:1). The drug polymer compatibility studies were carried out using FT-IR. The study revealed that no interaction between drug and polymer. The prepared mucoadhesive microspheres were evaluated for Drug entrapment efficiency, particle size, swelling index, in-vitro wash off test and in-vitro release. The result indicates that characteristics of prepared mucoadhesive microspheres by using Chitosan/Drug ratio of 3:1 were conducive to the formation of sustained release drug delivery system. The volume of cross-linking agent stirring speed was varied from 10 – 70ml and spherical free flowing shaped microspheres obtained except 10 and 20ml of glutaraldehyde. The prepared mucoadhesive microspheres were evaluated for Drug entrapment efficiency and In vitro wash off test (1% mucoadhesion after 1 hour).

Key words: Mucoadhesive microspheres, Glipizide, Chitosan, Glutaraldehyde

Introduction

A primary object of using muco adhesive 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].

Muco adhesive microsphere 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 residentce time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the durg 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].

To overcome the relatively short GI time and improve localization for oral controlled or sustained release drug delivery system. The polymers which adhere to the mucin/epithelial surface are effective and lead to significant improvement in oral drug delivery based on this three broad categories.

1. Polymer that becomes sticky when placed in water and owes their bioadhesion to sickness.
2. Polymers that adhere through non-specific, on-covalent interactions which are primarily electrostatic in nature.
3. Polymer that binds to specific receptor site on the cell valcrate[4-5]. Microspheres of biodegradable, and non-biodegradable drug carries when administered parenterally, the carrier toxicity over a long period of time. An important requirement of polymers is that degradation products should be non-toxic because such products eventually enter systemic circulation or result in tissue deposition[4-5]. Biodegradable carriers which degrade in the body to degradation products do not pose the problem of carrier toxicity and biodegradable microspheres can be prepared from certain synthetic as well as natural polymers.

Mechanism of drug release theoretically the release of drug from biodegradable microspheres classified (Baker 1987). But in actual practice the mechanism is more complex and an interplay of different mechanisms may operate.

I. Degradation controlled monolithic system.
II. Diffusion controlled monolithic system.
III. Diffusion controlled reservoir system.
IV. Erodible poly agent system.

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]

Chitoson (obtain by deacetylation of Chitin) is a natural and cationic polymer that has been proposed for use in microsphere systems by a number of authors and carbopol 974, Hydroxy Propyl Methyl Cellulose Carboxy and Methyl Cellulose is a synthetic good muco-adhesive and biodegradable polymers.

Thus the development of controlled-release dosage forms would clearly be advantageous. Moreover, the site of absorption of Sulfonyl urea's 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 by using natural mucoadhesive polymer (Chitosam) by using Glipizide as a drug and Microspheres were characterized by in-vitro tests.

Selection of polymers

Polymers used as matrices for durg delivery can be classified under three basic types; water soluble polymers biodegradable polymers and non-biodegradable polymers. Both natural and synthetic polymers are used as matrix materials in the preparation of biodegradable microspheres. Biopolymers used for durg delivery purpose are:

1. Natural Polymers:
   Animal proteins:- Albumin, Collagen, Gelatin, Fibrinogen, Casein, Fibrin.
   Animal polysaccharides:- Chitin, Chitosan, Hyaluronic acid.
   Plant polysaccharides:- Starch, Dextrin, Dextran, Alganic acid.
2. Synthetic Plymers:
   Animal proteins:- Poly (Lactic/Glycolic acid).
   Animal polysaccharides:- Poly (Beta hydroxyl butyric acid)
   Poly (E-Caprolactone, Poly anhydrides.
   Plant polysaccharides:- Poly (Ortho esters), Poly alkyl cyano acrylate.

Mucoadhesive are the attachment of a natural (or) synthetic polymer to a biological substrate. A mucoadhesive controlled release device can improve the effectiveness of a drug by helping to maintain the drug concentration between the effective and toxic levels, inhibiting the dilution of the drug in the body fluids and allowing targeting and localization of a drug at a specific site.

The work was carried on both using Natural and synthetic polymers. Chitosan was selected as natural polymer and work was carried. Regarding synthetic polymers carbopol 974, Hydroxy Propyl Methyl Cellulose and Carboxy Methyl Cellulose the literature review's and basic trails is going on so yet synthetic polymers are not selected.

Chitosan is a linear polysaccharide composed of randomly distributed Beta (1-4) linked D-Glucosamine (deacetylated unit) and n-acetyl-D Glucosamine (Acetylated unit) and it is an cationic polymer having very good mucoadhesive and biodegradable properties[30].

Materials and Method

Glipizide (Oral-anti diabetic drug) was obtained as gift sample from Madras Pharmaceuticals, Chennai. Chitosan (Natual mucoadhesive polymer) was obtained from Fourt's India Limited, Chennai. Dioctyl Sodium Sulfosuccinate (DOSS). Acetic acid, Petroleum ether, Liquid paraffin, Methylen Chlored, Glutaraldehyde, Formaldehyde, Phosphate buffer, Ratstomach mucosa was obtained from Brown's College of Pharmacy, Kammam, A.P., Carbopol 974, Hydroxy Propyl Methyl Cellulose and Carboxy Methyl Cellulose (Synthetic polymers) was obtained as gift sample from Madras Pharmaceuticals, Chennai.

UV Spectrophotometer, Scanning Electron Microscopy, USP XXIV, Basket apparatus (Dissolution), HPLC, Image analyser, Sieve analyser, Optical Microscope, Propeller stirrer (1000 rpm), USP Tablet disintegration apparatus.

Preparation of Microspheres

Microspheres were prepared by simple emulsification phase separation technique by using chitosan (natural polymer) and different volume of cross linking agent (Glutaraldehyde) is added as per method described in Tanoo et al.

Chitosan was dissolved in 150ml of 1% v/v aqueous acetic acid solution. Drug was dispersed in the polymer solution. The polymer to drug ratio were varied in batches (1:1, 3:1 and 6:1) by using low, high, and medium stirring speed,. The resultant mixture will be extruded through a syringe (No.20) in 1lit of liquid paraffin (Heavy and light 1:1 ratio). Containing 0.2% Dioctyl sodium sulfosuccinate and stirring was performed using propeller stirrer at different stirring speed. After 15 min cross linking agent glutaraldehyde was added and a stirring was continued (25%v/v aqueous solution) of the amount of cross linking agents and cross linking times were varied (10ml, 20ml, 40ml, 60ml, and 70ml). They are finally washed with water to remove excess of cross linking agent. The microsphere were then dried at room temperature (at 25^0C & 60% RH for 24 hours).

Evaluation of Microspheres

Assay

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 were 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 analysed 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

The drug entrapment efficiency for batches A1-A9 & B1-B20 are reported in Table I & II.

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. The particle sizes of different batches A1-A9 are reported in Table I.

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]. The swelling index for microspheres for batches A1-A9 is reported in Table I.

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. The results of in vitro wash of test of batches A1-A9 and B1-B20 were shown in Table I & II respectively.

Drug release study

The drug release study will performed using USP XXIV basket apparatus[22]. At 37^0C+0.5^0C and at 50 rpm using 900ml of phosphate buffer (pH7.4) as a dissolution medium as per test prescribed for glipizide extended release tablets. 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 t_{80} was calculated using the weibull-equation[27]. The average values of the t80 of batches A1-A9 are mentioned in table I.

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 stud 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].

Result and Discussion

The mucoadhesive microspheres of an oral anti-diabetic drug glipizide were prepared by simple emulsification phase separation technique. Chitosan was selected as a natural polymer for the preparation because of its biodegradable and mucoadhesive properties. Chitosan has good solubility in acetic acid. 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.

Table-I

Batch code	Polymer Drug ratio	Stirring speed (rpm)	In vitro wash off test (1% mucoadhesion after 1hr)	Drug Entrapment Efficiency (%)	Swelling Index	Particle size	t_{80} (minutes)
A1	A	A	52	54	0.888	60.6	234
A2	A	B	46	52	0.824	58.2	230
A3	A	C	43	49	0.812	50.2	218
A4	B	A	78	72	1.182	67.1	202
A5	B	B	69	70	1.123	64.0	229
A6	B	C	62	66	1.082	60.8	248
A7	C	A	80	77	1.412	98.0	492
A8	C	B	73	73	1.298	89.8	465
A9	C	C	67	70	1.242	74.4	376

Polymer : Drug ratio

A – 1 : 1 – 500 rpm Low stirring speed.

B – 3 : 1 – 1000 rpm Medium stirring speed.

C – 6 : 1 – 1500 rpm High stirring speed.

Polymer concentration is important factors, mention in Lee and based on Viscosity of polymers solution. Three different concentration 0.5%, 1% & 2% v/v were selected. From this 1% concentration show a maximum sphericity was observed so we select 1% w/v of polymer in 1% v/v acetic acid solution and 1:1 Heavy and light paraffin was used as dispersion medium and 0.2% v/v of DOSS is added as anionic surfactant to dispersion medium was found to be essential to minimize aggregation of microspheres.

The volume of cross linking agent stringing speed was varied from 10-70 ml and spherical free flowing shaped microspheres obtained except 10 and 20 ml of glutaraldehyde. Hence spherical free flowing microspheres are obtained.

The percentage of mucoadhesion and drug entrapment efficiency showed significant effect on batch A9-A20 showed in table I. Microspheres batches B1-B4 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 B5-B8 prepared by using 20ml of glutaraldehyde showed good mucoadhesion properties and Drug entrapment efficiency.

Batches B9-B12 was prepared by using 40ml of glutaraldehyde showed spherical free flowing microspheres and also showed good mucoadhesion and 63% of drug entrapment efficiency. Batches B13-B16 was showed 68% of drug entrapment efficiency and also showed 75% mucoadhesion. The microspheres of batches B17-B20 was showed spherical free flowing microspheres and showed 72% of drug entrapment efficiency and decrease in mucoadhesion take place. The cross linking agent increase means the mucoadhesiveness is decreases and cross-linking time did not show a significant effect on the percentage of drug entrapment efficiency.

From table II shows that stirring speed has a negative effect on t_{80} because as the particle size and percentage of drug release also depend on the polymer to drug ratio.

Table – II

Batch code	Volume of Glutaraldehyde (ml)	Cross linking time (h)	In vitro wash off test (1% mucoadhesion after 1h)	Drug Entrapment Efficiency (%)	Sphericity of Microspheres
B1	10	1	86	38	Very Irregular
B2	10	2	80	40	
B3	10	3	75	42	
B4	10	4	79	44	
B5	20	1	82	51	Slightly Irregular
B6	20	2	76	55	
B7	20	3	69	57	
B8	20	4	63	60	
B9	40	1	73	57	Spherical From Following
B10	40	2	67	59	
B11	40	3	60	61	
B12	40	4	59	62	
B13	60	1	75	63	
B14	60	2	66	64	
B15	60	3	61	66	
B16	60	4	55	68	
B17	70	1	59	70	
B18	70	2	52	72	
B19	70	3	45	71	
B20	70	4	39	71	

All batches were prepared by Polymer to Drug of 3 : 1

Conclusion

From this we conclude that 40ml – 60ml of glutaraldehyde was an optimum amount and the concentration of glutaraldehyde increase means the mucoadhesiveness is decreases and there was no significant effect in time. Stirring speed has negative effect on t_{80}. Further the work is carrying on the synthetic polymer.

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