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Research Article

**PREPARATION AND EVALUATION OF CHITOSAN - GLICLAZIDE
MICROPARTICULATE DRUG DELIVERY SYSTEMS BY AN
EMULSIFICATION- DESOLVATION- CROSSLINKING TECHNIQUE****P.Veera Lakshmi¹, K.P.R Chowdary², A.Prameela Rani³, K. Ravi Shankar⁴**

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Abstract:

Recently much emphasis is being laid on the development of microparticulate DDS in preference to single unit systems because of their potential benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. The objective of the present study is to prepare and evaluate microparticulate drug delivery systems of Gliclazide using chitosan, a mucoadhesive polymer for oral controlled release. A new technique namely emulsification-desolvation-crosslinking method was tried for the preparation of chitosan microparticles. The Chitosan- Gliclazide microparticles prepared were evaluated for various physical and drug release characteristics.

Spherical Chitosan- Gliclazide microparticles could be prepared by the emulsification-desolvation-crosslinking method. The method is industrially feasible as it involves emulsification and removal of the solvent, which can be controlled precisely. The emulsification-desolvation-crosslinking method was reproducible with regard to size and size distribution of the microparticles. About 68-75 % of microparticles in each batch were in the size range 35/50 mesh (398.5 μ m). Encapsulation efficiency was in the range 97.1-99.5 % in the preparation of microparticles. Gliclazide release from the chitosan microparticles was slow and spread over longer periods of time. The drug release depended on the proportion of core: coat in the microparticles. A good linear relationship ($R^2 = 0.874$) between percent coat and release rate (K_0) was observed. The relationship could be expressed by the linear equation, $y = 11.849 - 0.3035x$ where x is percent coat and y is release rate (K_0). Gliclazide release from the chitosan microparticles prepared was by diffusion mechanism. Fickian diffusion was observed in the case of microparticles which gave relatively rapidly release (F1 and F2) and the release was by non-Fickian diffusion in the case of microparticles which gave slow release of gliclazide (F3 and F4). Microparticles (F3) prepared using a core: coat ratio of 8:2 gave slow and controlled release of Gliclazide over 12 hours similar to that of commercial gliclazide SR tablets. A microparticle (F3) is considered as a promising microparticulate DDS for oral controlled release of Gliclazide over 12 hours for b.i.d administration.

Key words: Microparticulate drug delivery systems, Chitosan, Gliclazide, Emulsification-desolvation-crosslinking method, Oral controlled release.

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INTRODUCTION:

The design of microparticulate drug delivery systems is an efficient technique to provide the sustained and controlled delivery of drugs over long periods of time. Microparticulate drug delivery systems [1] consist of small particles of solids or small droplets of liquids surrounded by walls of natural & synthetic polymer films of varying thickness and degree of permeability acting as a release rate controlling substance and have a diameter upto the range of 0.1µm-200µm. Microparticulate dosage forms [2] are pharmaceutical formulations in which the active substance is present as a number of small independent subunits. To deliver the recommended total dose, these subunits are filled into capsules, encapsulated or compressed into a tablet. Microparticulate drug delivery systems contain discrete particles that make up a multiple-unit system. They provide many advantages over single-unit systems because of their small size. Multiparticulates are less dependent on gastric empty time, resulting in less inter and intra-subject variability in gastrointestinal transit time. They are also better distributed and less likely to cause local irritation [3]. Recently much emphasis is being laid on the development of microparticulate dosage forms in preference to single unit systems because of their potential benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying [4].

Design of microparticulate drug delivery systems requires a suitable polymer to serve the intended purpose. Several polymers such as benzyl cellulose, cellulose nitrate, cellulose acetate, epoxy resin, ethyl cellulose, polyethylene, polymethyl methacrylate, polystyrene, polyvinyl acetate, Eudragit S-100, starch acetate have been used in the design of microparticulate drug delivery systems [5,6]. In the present investigation Chitosan, a mucoadhesive polymer was tried for the preparation of microparticulate drug delivery systems of gliclazide for oral controlled release.

Chitosan (poly(b-(1-4)-2-amino-2-deoxy-d-glucose)), the high molecular weight cationic polysaccharide derived from chitin, has become increasingly important in the pharmaceutical field due to its good biocompatibility, biodegradability and low toxicity.[7,8] Owing to its good mucoadhesive properties,[9,10] chitosan has been employed in mucosal site-specific systems.[11-14] Moreover, chitosan has been shown to be a potential penetration enhancer for the transmucosal (intestinal, nasal,

buccal and vaginal) absorption of hydrophilic drugs with a high molecular weight.[15-18] Chitosan has been proposed as a useful excipient for sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds.[19-22] Chitosan has been used in the design of different types of drug carriers for various administration routes such as oral, ocular, buccal, nasal, transdermal, parenteral and vaginal. Chitosan dosage forms can be engineered into different shapes and geometries such as nanoparticles, microparticles, hydrogels, films, fibers, sponges, inserts and rods.[23-29] Cationic chitosan can form gels with non-ionic multivalent anionic counterions such as polyphosphate[30,31] and sodium alginate[32] by ionic crosslinking. Tripolyphosphate is a non-toxic polyanion that can interact with chitosan via electrostatic forces to form ionic crosslinked networks because of its quick gelling ability. In several reports chitosan is used along with sodium alginate for the preparation of microcapsules and microparticles [33-42]. In the present study chitosan alone was used to prepare gliclazide microparticulate drug delivery systems. An emulsification-desolvation-crosslinking method was tried for the preparation of chitosan microparticles.

The objective of the present study is to prepare and evaluate microparticulate drug delivery systems of Gliclazide using Chitosan for oral controlled release. Gliclazide is a potential second generation, short-acting sulfonylurea oral hypoglycaemic agent widely used for the treatment of non-insulin-dependent diabetes mellitus [43]. In general, rapid gastrointestinal absorption is required for oral hypoglycaemic drugs in order to prevent a sudden increase in blood glucose level after food intake in patients with diabetes mellitus. However, the absorption rate of gliclazide from the gastrointestinal tract is slow and varied among subjects. Slow absorption of a drug usually originates from either poor dissolution of the drug from the formulation or poor permeability of the drug across the gastrointestinal membrane [44]. The dose of Gliclazide is 40-80 mg as conventional tablets and 60 mg as sustained release tablets. The conventional tablets are to be taken 2-3 times a day to maintain normal plasma glucose levels. Sustained release formulations offer better patient compliance by reducing the frequency of dosage administrations and also provide continuous effect. Emulsification-Desolvation-Crosslinking method was tried for the preparation of Chitosan-gliclazide microparticles.

MATERIALS AND METHODS:

Materials:

Gliclazide was a gift sample from M/s Micro Labs, Pondicherry, Chitosan, 75-85 percent deacetylated was obtained from commercial sources. Sodium tri polyphosphate (Sigma), Acetic acid (Qualigens), Chloroform (Qualigens) and Soyabean oil were used. All other materials used were of pharmacopoeial grade.

Methods:

Estimation of Gliclazide:

An UV Spectrophotometric method based on the measurement of absorbance at 227 nm in phosphate buffer of pH 7.4 was used for the estimation of gliclazide. The method was validated for linearity, accuracy, precision and interference by the excipients. The method obeyed Beer's law in the concentration range of 1 – 10 µg/ ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variance (RSD) were found to be 0.80% and 1.2% respectively. No interference by the excipients used in the study was observed.

Preparation of Chitosan--Gliclazide

Microparticulate DDS:

An emulsification -desolvation -crosslinking method was tried for the preparation of chitosan-gliclazide microparticles. Chitosan solution (2%w/v) was prepared by dissolving 2g of chitosan in 100 ml of 1% v/v acetic acid solution by sonication for 30 minutes to form a homogeneous solution. Core material, gliclazide (0.8 g) was added to 10 ml of polymer (chitosan) solution, which contain 0.2 g of chitosan and dispersed thoroughly. This provides a core: coat ratio of 8:2. The chitosan - drug dispersion was added in a thin stream to 300 ml of soyabean oil containing 10 percent chloroform taken in a 600 ml beaker while stirring at 1000 rpm to emulsify the added dispersion as fine droplets. A Remi medium duty stirrer with speed meter (Model RQT 124) was used for stirring. Stirring was continued for 20 minutes to remove acetic acid from polymer solution into chloroform (desolvation) and to form chitosan-gliclazide microparticles. Tripolyphosphate solution (5 % w/w, pH 5.0) containing 1 % glutaraldehyde (100 ml) was added while stirring as crosslinking agent. Stirring was continued for 30 minutes for crosslinking and hardening of the chitosan microparticles formed. The rigid microparticles formed were collected by decantation and washed repeatedly with petroleum ether to remove the adhering oil. The product was then air

dried to obtain discrete microparticles of chitosan - gliclazide. Different proportions of core: coat namely 19:1 (F1), 9:1 (F2), 8:2 (F3) and 7:3 (F4) were used to prepare microparticles with varying amount of coat polymer.

Estimation of Drug Content and Encapsulation Efficiency:

Four samples of 100mg each were taken from each batch of microparticles prepared and assayed for gliclazide content at 227nm .Encapsulation efficiency was estimated using the equation,

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Estimated drug content (\%)}}{\text{Theoretical drug content (\%)}} \times 100$$

Size Analysis:

For the size distribution analysis, different fractions in a batch were separated by sieving using a range of standard sieves. The amounts retained on different sieves were weighed.

Drug Release Study:

Release of gliclazide from the microparticles of size 30/50 mesh was studied in phosphate buffer of pH 7.4 (900 ml) using an 8 station dissolution rate test apparatus (model Disso-2000, M/s Lab. India) with a paddle stirrer (Apparatus 2) at 50 rpm. A temperature of $37^{\circ} \pm 1$ °C was maintained throughout the experiment. A sample of microparticles equivalent to 60 mg of gliclazide was used in each test. Samples (5 ml) were withdrawn through a filter (0.45 µ) at different time intervals over 12 h and were assayed at 227 nm for gliclazide content. The sample (5 ml) taken at each sampling time was replaced with drug free dissolution fluid and a suitable correction was applied for the amount of drug lost in sampling for the estimation of amount of drug released at various times. Each drug release experiment was conducted in triplicate (n=3).

Analysis of Release Data:

Drug release data were analyzed as per zero order, first order, Higuchi [45] equation and Korsmeyer-Peppas [46] equation models to assess the release kinetics and mechanism.

RESULTS AND DISCUSSION:

Chitosan is sparingly soluble in water; practically insoluble in ethanol (95%) and other organic solvents. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids like acetic acid and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids). Upon

dissolution, amine groups of the polymer become protonated, resulting in a positively charged polysaccharide. In the present study chitosan was dissolved in 1% v/v acetic acid solution.

An emulsification – desolvation- crosslinking method was used for the preparation of microparticles of chitosan-gliclazide. The method involves emulsification of the chitosan solution in 1%v/v acetic acid containing the dispersed drug particles in an immiscible liquid medium (soya bean oil containing 10 % chloroform) as microdroplets, followed by removal of the acetic acid from the polymer solution into chloroform by desolvation and crosslinking with tripolyphosphate and glutaraldehyde solution to form rigid microparticles. The microparticles were collected by decantation and washed repeatedly with petroleum ether to remove the adhering oil. The product was then air dried to obtain discrete microparticles. The microparticles were found to be discrete, spherical and free flowing. The sizes could be separated readily by sieving and a more uniform size range of microparticles could

easily be obtained. The sieve analysis of different batches of microparticles prepared indicated that a large proportion, 68-75%, in each batch were in the size range of 35/50 mesh (398.5 μ m). The reproducibility of the method with regard to size distribution of the microparticles was evaluated by preparing three batches of microparticles under identical conditions in each case. Size analysis indicated that about 68-75% of the microparticles were in the size range of 35/50 mesh in all the batches. Microparticles of this size (398.5 μ m) were selected for further evaluation.

The physical characteristics of the microparticulate DDS prepared are given in Table 1. Low coefficient of variation (cv) in percent drug content (< 2.0 %) indicated uniformity of drug content in each batch of microparticles. The encapsulation efficiency was in the range 97.1 -99.5 %. Drug content of the microparticles was found to be the same in the two sizes, 20/35, 35/50 mesh. A t-test of significance indicated that the difference in the drug content of the two sizes in each case is not significant ($P>0.05$).

Table 1: Physical Characteristics of the Microparticulate DDS Prepared

DDS	Mesh Size	Mean size (μ m)	Core: Coat ratio	Gliclazide content (%) ($\bar{x}\pm$ sd)	Encapsulation efficiency (%)	Percent Coat Polymer
F1	20/35	670	19:1	94.2 \pm 1.2	99.0	5.8
	35/50	398.5	19:1	94.6 \pm 1.8	99.5	5.4
F2	20/35	670	9:1	87.4 \pm 1.3	97.1	12.6
	35/50	398.5	9:1	87.6 \pm 1.1	97.3	12.4
F3	20/35	670	8:2	79.2 \pm 1.9	99.0	20.8
	35/50	398.5	8:2	79.4 \pm 1.6	99.2	20.6
F4	20/35	670	7:3	68.2 \pm 1.6	97.4	31.8
	35/50	398.5	7:3	68.6 \pm 1.5	98.0	31.4

Gliclazide release from the various microparticles of size 30/50 was studied in phosphate buffer pH 7.4. For comparison gliclazide release from one commercial product of gliclazide SR tablets was also studied. The drug release profiles are shown in Fig.1. The release data were analyzed as per Zero order,

First order, Higuchi [45] equation and Korsmeyer-Peppas [46] equation models to assess the release kinetics and mechanism. The kinetic parameters (r^2 values, rate constants and n values) in the analysis of release data as per various kinetic models are given in Table 2.

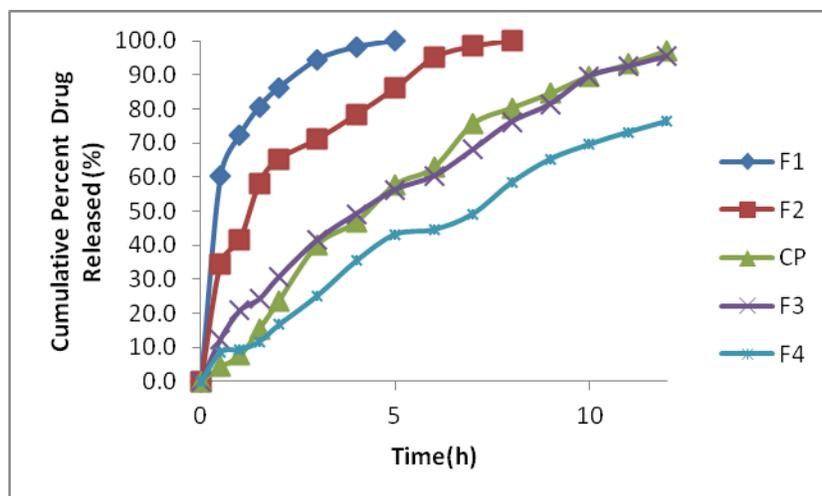


Fig.1: Gliclazide Release Profiles of Various Microparticulate DDS Prepared and Commercial SR Tablets (CP)

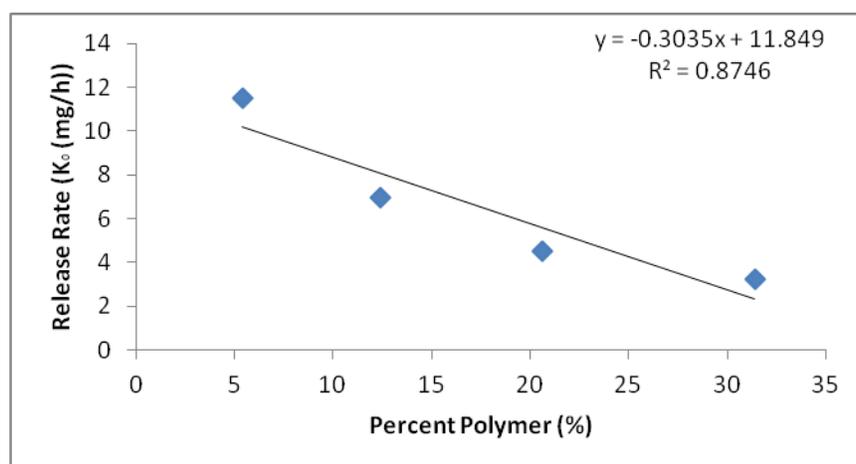


Fig.2: Relationship between Percent Coat and Release Rate (K_0) of Microparticulate DDS (Size 398.5 μ m)

Gliclazide release from all the chitosan microparticles tested was slow and spread over longer periods of time. The release depended on the proportion of core and coat in the microparticles. As the coat proportion was increased the release rate was decreased. A good linear relationship ($R^2 = 0.8448$) between percent coat and release rate (k_0) was observed as shown in Fig 2.

The relationship could be expressed by the linear equation, $y = 11.849 - 0.3035x$ where x is percent coat and y is release rate (K_0).

A comparison of R^2 values in various models revealed that the R^2 value was higher in the case of Korsmeyer Peppas model in all the cases. As such the release data of all the microparticles tested obeyed

Korsmeyer Peppas equation model which indicates that the drug release from the microparticles was by diffusion mechanism. The release exponent (n) in Korsmeyer Peppas equation model was 0.24 and 0.40 in the case of formulation F1 and F2 respectively indicating that the drug release from these microparticles was by Fickian diffusion mechanism. In the case of formulation F3 and F4 the release exponent (n) was 0.64 and 0.79 respectively indicating that the drug release from these microparticles was by non-Fickian (anomalous) diffusion mechanism. In the case of commercial product the release exponent (n) was found to be 1.00 indicating that the drug release from the commercial SR tablets was by zero order diffusion mechanism.

Table 2: Kinetic Parameters (R^2 Values, Rate Constants and n values) in the Analysis of Release Data as per Various Kinetic Models

DDS	Zero order		First order		Higuchi	Korsmeyer Peppas	
	K_0	R^2	R^2	K_1	R^2	n	R^2
F1	11.49	0.6464	0.9855	0.929	0.9003	0.24	0.9929
F2	6.94	0.8380	0.9308	0.506	0.9788	0.40	0.9792
F3	4.53	0.9703	0.9436	0.230	0.9890	0.64	0.9977
F4	3.25	0.9782	0.9793	0.120	0.9825	0.79	0.9881
CP	5.09	0.9602	0.9492	0.262	0.9691	1.00	0.9725

The results of the present study, thus, indicated that chitosan- gliclazide microparticles could be prepared by emulsification –desolvation-crosslinking method. These microparticles could be used for oral control release of Gliclazide. Formulation F3 prepared using a core: coat ratio of 8:2 gave slow and controlled release of Gliclazide over 12 hours similar to that of commercial gliclazide SR tablets. The drug release profiles of formulation F3 and Commercial gliclazide SR tablets were compared by difference factor (f1) and similarity factor (f2). The f1 and f2 values were found to be 2.46 and 63.11 respectively indicating that the drug release profiles of formulation F3 and Commercial SR product are similar. As such formulation F3 is considered as a promising microparticulate DDS for oral controlled release of Gliclazide over 12 hours for b.i.d administration .

CONCLUSIONS:

1. Spherical Chitosan- Gliclazide microparticles could be prepared by the emulsification-desolvation-crosslinking method. The method is industrially feasible as it involves emulsification and removal of the solvent, which can be controlled precisely.
2. The emulsification-desolvation-crosslinking method was reproducible with regard to size and size distribution of the microparticles. About 68-75 % of microparticles in each batch were in the size range 35/50 mesh (398.5 μ m)
3. Encapsulation efficiency was in the range 97.1-99.5 % in the preparation of microparticles.
4. Gliclazide release from the chitosan microparticles was slow and spread over longer periods of time. The drug release depended on the proportion of core:coat in the microparticles.

5. A good linear relationship ($R^2 = 0.874$) between percent coat and release rate (K_0) was observed. The relationship could be expressed by the linear equation, $y = 11.849 - 0.3035x$ where x is percent coat and y is release rate (K_0).
6. Gliclazide release from the chitosan microparticles prepared was by diffusion mechanism.
7. Fickian diffusion was observed in the case of microparticles which gave relatively rapidly release (F1 and F2) and the release was by non-Fickian diffusion in the case of microparticles which gave slow release of gliclazide (F3 and F4).
8. Microparticles (F3) prepared using a core: coat ratio of 8:2 gave slow and controlled release of Gliclazide over 12 hours similar to that of commercial gliclazide SR tablets.
9. Microparticles (F3) is considered as a promising microparticulate DDS for oral controlled release of Gliclazide over 12 hours for b.i.d administration

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