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DESIGN AND CHARACTERIZATION OF MICROPARTICLES CONTAINING CHARANTIN BY IONIC GELATION TECHNIQUE.

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ABSTRACT

The aim of the present study is to prepare and characterize microparticles containing Charantin using chitosan as the polymer. **Methods:** The Charantin loaded Microparticles were prepared by Ionic gelation method. Microparticles of different core: coat ration were prepared and characterize for process yield, entrapment efficiency, particle size, zeta potential, *in vitro* drug release, kinetic studies and stability studies. **Results:** The prepared Microparticles were spherical in shape. The formulation FI4 registered has best formulation among all 5 formulations. The Chitosan microparticles have a particle diameter ranging approximately 71.33µm and a zeta potential 45.0mV. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 12 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which microspheres were stored at various temperatures. **Conclusion:** The best-fit release kinetics was achieved with First order followed by Higuchi plot. The release of Charantin was influenced by the drug to polymer ratio and particle size and was found to be diffusion controlled. According to the data obtained, this Chitosan-based microparticles opens new and interesting perspectives as drug carriers for treating the Diabetus mellitus.

INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of body systems particularly blood vessels, eyes, kidney, heart and nerves. Diabetes mellitus has been classified into two types i.e. insulin dependent diabetes mellitus (Type I) and non-insulin dependent diabetes mellitus (Type II). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells whereas Type II diabetes is characterized by peripheral insulin resistance and impaired.Allopathic drugs are used for the treatment of diabetes have their own side effect & adverse effect like hypoglycemia, nausea, vomiting, hypernatremia,

flatulence, diarrhea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anemia, dyspepsia, dizziness, joint pain. So instead of an allopathic drugs, herbal drugs are the great choice which is having more or less no side effect & adverse effects. Based on the material of origin, Ayurveda medicines are divided into three classes, namely herbal, mineral and animal. Among this, herbal formulation has gained great importance and rising global attention recently. Ayurveda has about 700 type of plants listed in its medicinal systems. The use of such herbals is mentioned in the ancient Ayurvedic literature such as Charaka Samhita and Sushruta Samhita. The discovery of herbals is further complemented with knowledge on the method of isolation, purification, characterization of active ingredients and type of preparation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds roots, barks, stems and etc.) used for preparing medicines. The herbal drugs like Azadirachta indica, Mangifera indica, Ocimumsanctum leaves, Momordica charantia, leaves of Gymnemasylvestre, stems of Tinosporacordifolia, bark of Cinnamomumzeylanicum, rhizome of Curcuma longa and roots of Withaniasomnifera, are showing antidiabetic activity. Charantin is the main phytoconstituent present in fruits of Momordica charantia which is a steroidal glycoside and exist as equal mixture of stigmasterol glucoside and β-sitosterol glucoside. It has got blood sugar lowering property equivalent to insulin. Charantin at dose of 50mg/kg shows antidiabetic activity as like insulin. Generally there are so many conventional treatment for treating diabetes mellitus, but they are having own side effects like poor patient compliances and high cost. One of the way to overcome these problems could be association with the biodegradable polymeric carriers like microspheres. Microspheres have played a vital role in the development of controlled/sustainedrelease drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve controlled drug release. Microspheres can be defined as solid spherical particles ranging from 1 to 1000 µm in size. The microspheres are free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature. Chitosan possesses some ideal properties of a polymeric carrier for microparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan microparticles. Hence, the objective of the work was to formulatechitosan microparticles containing charantin by ionic gelation method and evaluate its physicochemical characteristics such as particle size, surface morphology, zeta potential, drug loading capacity and *in vitro* release characteristics.

Materials and Methods

Charantin used was bought from Shreedha phytochemicals, Jaipur, india and chitosan from India sea foods, Cochin. Glacial acetic acid, tween 80 and sodium tripolyphosphate were obtained from SD fine chemical ltd, Mumbai,India. All other chemicals used were of analytical grade.

Preparation of Microparticles: Chitosan microparticles were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25, v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. Under magnetic stirring at room temperature, 5 ml of 0.84% (w/v) TPP aqueous solution was added dropwise using syringe needle into 10 ml chitosan solution containing 10 mg of Charantin. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was continued for about 30 min. The resultant microparticles suspensions were centrifuged at 12000 × *g* for 30 min using C24 centrifuge. The formation of the particles was

a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) (**Table 1**).

Characterization of prepared microparticles

Fourier transform infra-red spectroscopy (FT-IR) analysis: The IR spectra of the samples were recorded on an FTIR spectrophotometer (Perkin Elmer 1600 series) using KBr pellet (12 mm disc), compressed in a hydraulic press at 10 tons for 30 seconds.

Practical yield: Freeze dried microparticles were collected and weighed to determine practical yield (PY) from the following equation 1.

$$PY(\%)= \frac{\text{Microparticles weight}}{\text{Theoretical mass}} \times 100\%$$

The individual values for three replicates were determined, and their mean values are reported.

Drug content: The drug content in each formulation was determined by weighing microparticles equivalent to 30mg of Charantin and dissolving in 100 ml of 7.4 pH phosphate buffer, followed by stirring. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured spectro-photometrically at 281 nm using 7.4 pH phosphate buffer as blank. The drug content of the prepared microparticles was determined by the formula:

$$\text{Drug content } (\%)= \frac{\text{Weight of drug in microparticles}}{\text{Weight of microparticles}} \times 100\%$$

Entrapment efficiency (EE%): The entrapment efficiency is also known as Association Efficiency. The drug loaded microparticles were centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer (Das *et al.*, 2005).

Efficiency (DEE) was calculated as follows:

$$DEE\ \%= \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100\%$$

Scanning Electron Microscopy: The shape and surface topography of microparticles were examined using Scanning Electron Microscopy (SEM) (JSM-T20. Tokyo, Japan). An appropriate sample of polymeric microparticles was mounted on metal stubs, using double-sided adhesive tapes. Samples were gold coated and observed for morphology, at acceleration voltage of 15KV.

Particle size distribution: The size distributions along the volume mean diameters of the suspending particles were measured by dynamic scattering particle size analyser (Nanotrac Particle Analyzer 150, Microtrac Inc., PA, USA) (Alexis et al., 2008).

***In vitro* release studies:** *In vitro* dissolution of all the formulations was conducted by using USP Dissolutionapparatus II. Microspheres equivalent to 100 mg of Charantin were taken and placed in the Jar of dissolution apparatus. The dissolution media used Ph 7.4 buffer solution. The temperature was maintained at 37 ± 0.5°C and rotation was set at 50 rpm. 10.0 mL ofsample was taken, filtered, and analyzed at 281 nm using spectrophotometerto get percentage drug release versus time profile. Fresh medium was replaced for eachsample taken out of the apparatus to maintain the sink condition.

Kinetic modeling: In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of microparticles were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas plot (log of cumulative % drug release vs. log time). R2 and 'n'values were calculated for the linear curve obtained by regression analysis of the above plots (Table No.2).

Table No.1: Formulation and physicochemical characterization of Charantin Microparticles

Sl.no	Formulation code	Drug & polymer ratio	%entrapment efficiency
01	FI1	1:1	62.61%
02	FI2	1:2	82.56%
03	FI3	1:3	56.50%
04	FI4	1:4	88.80%
05	FI5	1:5	69.72%

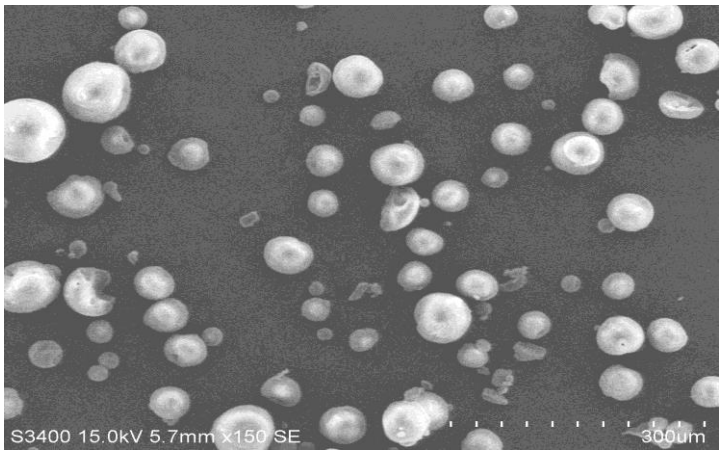

Figure No.1: SEM of formulation FI-4

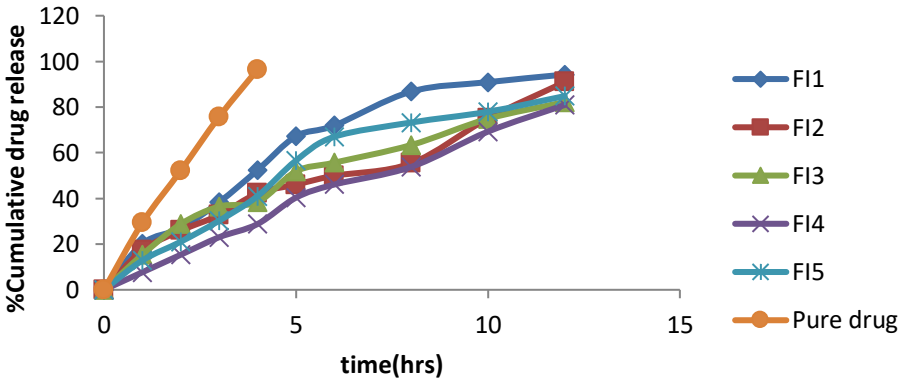

Figure No.2: % cumulative drug release of Charantin Microparticles

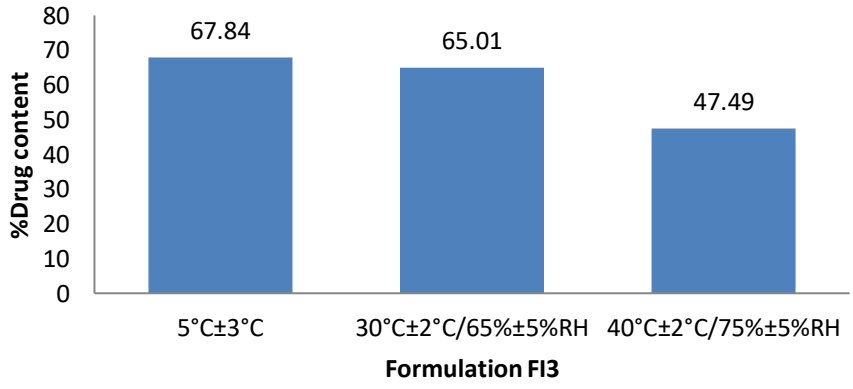

Figure No.3: Stability study: comparison of % drug content of formulation FI-4 at 5±3℃, room temperature and 40℃ ± 2℃/75% RH

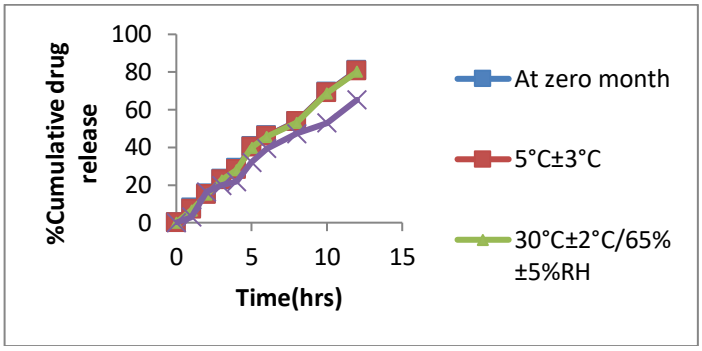

Figure No.4: Stability study: comparison of *in vitro* drug release profile for formulation FI4 at 5± 3℃, room temperature and 40℃ ± 2℃/75% RH after three months storage.

Table No.2: Correlation coefficients according to different kinetic equations

Formulation code	% Cumulative Drug Release	Zero Order	First order	Higuchi	Peppas	'n' values
FI1	94.25	0.9107	0.9885	0.9651	0.659	0.8309
FI2	90.94	0.9662	0.9750	0.9531	0.6541	0.7901
FI3	82.19	0.9453	0.9897	0.9848	0.660	0.7914
FI4	80.94	0.9923	0.9392	0.9318	0.8325	0.5617
FI5	84.81	0.9217	0.9825	0.9497	0.7323	0.7222

Stability study: The stability study was carried out using the batch FI-4. Formulation FI-4 was divided into 3 sets of samples and stored at 5±3℃ in refrigerator, room temperature and 45 ± 2℃, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined by the method as in drug content, In *vitro* release study of formulation FI-4 was also carried out after 90 days of storage.

RESULTS AND DISCUSSION

Microparticles prepared by Ionic gelation technique were found to be discrete and through SEM analysis, the average particle size was found to be 71.33µm and the drug entrapment efficiency of microparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 62.61%, 82.56%, 56.50%, 88.8% and 69.72%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The Cumulative percentage drug released for FI-1, FI-2, FI-3, FI-4 and FI-5 after 12 h were found to be 94.25%, 90.94%, 82.19%, 80.94% and 84.81% respectively. Zeta potential for FI-4 was found to be 45.0 mV and it shows good stability. It was apparent that *in vitro* release of Charantinshowed a very rapid initial burst and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the microparticles. In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi respectively. As indicated by higher R^2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, Non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be greater than 0.50, This indicates that the release approximates Non-fickian diffusion

mechanism. The results of drug content of ideal formulation FI-4after 90 days of stability testing at different storage conditions were shown in Fig. 3. *In vitro* release profiles for the same formulation stored at different storage conditions were also showed in Fig. 4. By observing this data with the previous data of FI-4, it was observed that there was a slight decrease in drugcontent when the formulation was stored at 4˚C and Room temperature, but there was significant decrease in drugcontent when the formulation was stored at 40 ± 2˚C/75% RH because at higher temperature, there might be chances of drug degradation that result in decrease of the drug release

CONCLUSION:

Charantin microparticles were prepared by Ionic gelation method were found to be suitable for controlled release. The microparticles prepared by using Chitosan as a polymer show prolonged release rate with increasing polymer concentration when compared with other formulations, on the basis of drug content, entrapment efficiency, particle size, surface morphology, zeta potential and *in nitro* release data's, the FI-4 was selected as an optimum formulation. The stability studies were carried out for the selected formulation FI-4; the results showed that maximum drug content and closet*invitro* release to previous data was found for FI-4 stored at 4˚C, room temperature and 40℃ ± 2℃/75% RH. Thus the microparticles of Charantin (FI-4) with core: coat ratio 1:4 was found to be spherical, discrete and free flowing and able to sustain/control the drug release effectively.

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