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Developmentand components of chitosan primarily based microspheres of glibenclamide

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Abstract:

Microspheres is novel drug delivery system for improving therapeutic action of drug, increasing prolong action, lowering dose frequency of dosage form and to improve patient complies. Microspheres are reducing oral administration side effect such as gastric irritation in stomach. Glibenclamide microspheres were developed by ionotropic gelation method using sodium alginate and chitosan. Calcium chloride was used as a cross linking agent. Prepared microspheres were evaluated for entrapment efficiency, microsphere size, morphology, FTIR, DSC, in-vitro drug release and drug release kinetics. Prepared Glibenclamide microspheres were found discrete, free flowing and spherical. The mean particle size ranged from 349-540 μm and percentage yield ranged between 70 to 98.92%. The size of microsphere was increased by increasing concentration sodium alginate and calcium chloride while the entrapment efficiency was increased with increasing concentration of chitosan. XRD studies confirmed the crystalline nature of Glibenclamide. SEM studies showed that the microspheres are spherical and with rough surface. The in-vitro drug release study was carried out in phosphate buffer pH 7.4. Percent drug release was decreased with increase in concentration of sodium alginate and calcium chloride. Decreased drug release rate was obtained in case of F3 formulation containing sodium alginate and chitosan at 3:1 ratio and 5% calcium chloride as a cross-linking agent. The present study conclusively that Glibenclamide microsphere could be prepared successfully and formulation F3 was shows satisfactory result.

The prepare Glibenclamide microspheres to maintain an effective of drug concentration in serum for long period of time and reducing gastric irritation.

Keywords:

Glibenclamide, Chitosan, HPLC, FTIR, UV spectrum

1. Introduction:

Drug delivery by entrapping the drug into a carrier such as microspheres, nanoparticles and liposome's which changes the release and absorption characteristics of the drug. The microparticulate delivery systems are considered as a reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microspheres are sometimes referred to as microparticles. They are made of polymeric, waxy or other protective materials, i.e. biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin; the synthetic polymers include polylactic acid and polyglycolic acid. Microspheres are small and have large surface to volume ratio. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity.[1]

Microparticles are of two types:

A well designed new approach of carrier technology offers an intelligent approach for

1. Microcapsules: The entrapped substance is completely surrounded by a distinct capsule wall.
2. Microspheres: The entrapped substance is dispersed throughout the microsphere matrix. Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. However, the success of these microspheres is limited due to the short residence time at the site of absorption. It would therefore advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier characteristics. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs.The concept of microsphere as a drug delivery system especiallyoffering a sustained and controlled action of drug to desired area of effect. The drug should be delivered to specific target sites at a rate and concentration that permit optimal therapeutic efficacy while reducing side effect to minimum. Microspheres are characteristically free flowing powders consisting of biodegradable in nature and ideally having a particle size less than 200

μm.[1-2]

2. Methods of preparation of microsphere:

2.1. Single emulsion technique:

The micro particulate carriers of natural polymers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, acid chloride etc. Heat denaturation is not suitable for thermolabilesubstances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation.[7, 8]

2.2. Double emulsion technique:

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like leutinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/ extraction. 7, 8

2.3. Polymerization techniques:

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization

II. Interfacial polymerization.

Both are carried out in liquid phase.

2.4. Phase separation and coacervation:

Phase separation method is specially designed for preparing the reservoir type of the system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however, some of the preparations are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids. In matrix type device, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the coacervates. The Coacervation can be brought about by addition of the third component to the system which results in the formation of the two phases, one rich in the polymer, while the other one. In this technique, the polymer is first dissolved in a suitable solvent and then making its aqueous solution disperses drug. The process is carried out under continuous stirring to control the size of the microparticles. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer as the process of microsphere formation begins the polymerize globules start to stick and form the agglomerates.[7, 8]

2.5. Spray drying and congealing:

Spray drying and spray congealing methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or the cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization lead to the formation of small droplets or the fine mist from which the solvent evaporates leading to the formation of microspheres in a size range 1-100µm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying.[7, 8]

2.6. Solvent extraction:

This method is used for the preparation of microparticles, involves the removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic

solvent such as isopropanol; organic phase is removed by extraction with water. The process decreases the hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer. [7, 8]

2.7. Quassi emulsion solvent diffusion:

A novel quasi-emulsion solvent diffusion method to prepare the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Microsponges can be prepared by a quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol (PVA). The internal phase is consisting of drug, ethyl alcohol and polymer is added at an amount of 20% of the polymer in order to facilitate the plasticity. At first, the internal phase is prepared at 60ºC and added to the external phase at room temperature. After emulsification, the mixture is continuously stirred for 2 hours. Then the mixture can be filtered to separate the microsponges. The product is then washed and dried by vacuum oven at 40ºC for 24 hours. Example: - Ibuprofen.[8- 9]

3. Components:

A number of different substance both biodegradable as well as non-biodegradable have been investigated for the preparation of microsphere Microspheres used usually are polymers. They are classified into two types:

1. Synthetic Polymers
2. Natural polymers, [8]

3.1. Polymers:

Table. 1.1: Types of polymer

Types of polymer	*Types of natural Polymer*	*Examples*
1. Natural polymer	a) Proteins	Albumin, Gelatin, and Collagen
	b) Carbohydrates	Agarose, Carrageenan, Chitosan, Starch
	c) Chemically modified Carbohydrates	Poly dextran, Poly starch.
2. Synthetic polymer	Types of synthetic Polymer	Examples

	a. Non-biodegradable	1.) Poly methyl methacrylate (PMMA) 2.) Acrolein 3.) Epoxy polymer
	b. Biodegradable polymers	1) Lactides 2) Glycolides& their co polymers, 3) Poly alkyl cyano acrylates

- Core material

1. Drug or active constituent
2. Additive like diluents
3. Stabilizers
4. Release rate enhancers or retardants

- Coating material

1. Inert polymer
2. Plasticizer
3. Coloring agent
4. Gelatin, gum Arabica, methyl cellulose, beeswax, carnauba wax.

- Vehicle

1. Aqueous
2. NonAqueous

4. Characterization:

The characterization of the microparticulate carrier is an important phenomenon, which helps to design a suitable carrier for the proteins, drug or antigen delivery. These microspheres have different microstructures. These microstructures determine the release and the stability of the carrier. , 6, 13

4.1. Particle size and shape:

The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM). Both can be used to determine the shape and outer structure of microparticles. LM provides a control over coating parameters

in case of double walled microspheres. The microspheres structures can be visualized before and after coating and the change can be measured microscopically. SEM provides higher resolution in contrast to the LM17. SEM allows investigations of the microspheres surfaces and after particles are cross-sectioned, it can also be used for the investigation of double walled systems. Conflocal fluorescence microscopy is used for the structure characterization of multiple walled microspheres. Laser light scattering and multi size coulter counter other than instrumental methods, which can be used for the characterization of size, shape and morphology of the microsphere.

4.2. Electron spectroscopy for chemical analysis:

The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA). ESCA provides a means for the determination of the atomic composition of the surface. The spectra obtained using ECSA can be used to determine the surfacial degradation of the biodegradable microspheres.

4.3. Attenuated total reflectance fouriertransfom- infrared spectroscopy:

FT-IR is used to determine the degradation of the polymeric matrix of the carrier system. The surface of the microspheres is investigated measuring alternated total reflectance (ATR). The IR beam passing through the ATR cell reflected many times through the sample to provide IR spectra mainly of surface material. The ATRFTIR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions.

4.4. Density determination:

The density of the microspheres can be measured by using a multi volume pychnometer. Accurately weighed sample in a cup is placed into the multi volume pychnometer. Helium is introduced at a constant pressure in the chamber and allowed to expand. This expansion results in a decrease in pressure within the chamber. Two consecutive readings of reduction in pressure at different initial pressure are noted. From two pressure readings the volume and hence the density of the microsphere carrier is determined.

4.5. Isoelectric point:

The micro electrophoresis is an apparatus used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined. The mean velocity at different pH values ranging from 3-10 is calculated by measuring the time of particle movement over a distance of 1 mm. By using this data the electrical mobility of the particle

can be determined. The electrophoretic mobility can be related to s^{14}Curface contained charge, ionisable behaviour or ion absorption nature of the microspheres.

4.6. Surface carboxylic acid residue:

The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugates is prepared by the reaction of ^{14}C-glycine ethyl ester hydro chloride with the microspheres. The glycine residue is linked using the water soluble condensing 1- ethyl-3- (3-dimethyl amino propyl) carbidiimide (EDAC). The radioactivity of the conjugate is then measured using liquid scintillation counter. Thus the carboxylic acid residue can be compared and correlated. The free carboxylic acid residue can be measured for hydrophobic or hydrophilic or any other derivatized type of the microspheres.

4.7. Surface amino acid residue:

Surface associated amino acid residue is determined by the radioactive ^{14}C-acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly. EDAC is used to condense the amino group and the ^{14}C –acetic acid carboxylic acid residue. The method used for determining the free amino or the free carboxylic acid residues are based on indirect estimation, by measuring the radioactivity of the ^{14}C having acetic acid or the glycine conjugate. The accuracy of the method however, depends on the time allowed for conjugation of the radioactive moiety and the reactivity of free functional group.

4.8. Capture efficiency:

The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyses.

The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation: % Entrapment = Actual content/Theoretical content x 100

4.9. Angle of contact:

The angle of contact is measured to determine the wetting property of a micro particulate carrier. It determines the nature of microspheres in terms of hydrophilicity or hydrophobicity. This thermodynamic property is specific to solid and affected by the presence of the adsorbed component. The angle of contact is measured at the solid/air/water interface. The advancing

And receding angle of contact are measured by placing a droplet in a circular cell mounted above objective of inverted microscope. Contact angle is measured at 200C within a minute of deposition of microspheres.

4.10. In vitro methods:

There is a need for experimental methods which allow the release characteristics and permeability of a drug through membrane to be determined. For this purpose, a number of in vitro and in vivo techniques have been reported. In vitro drug release studies have been employed as a quality control procedure in pharmaceutical production, in product development etc. Sensitive and reproducible release data derived from physicochemically and hydrodynamically defined conditions are necessary. The influence of technologically defined conditions and difficulty in simulating in vivo conditions has led to development of a number of in vitro releasemethods for buccal formulations; however no standard in vitro method has yet been developed. Different workers have used apparatus of varying designs and under varying conditions, depending on the shape and application of the dosage form developed.

4.10.1. Beaker method:

The dosage form in this method is made to adhere at the bottom of the beaker containing the medium and stirred uniformly using overhead stirrer. Volume of the medium used in the literature for the studies varies from 50-500 ml and the stirrer speed form 60- 300 rpm.

4.10.2. Interface diffusion system:

This method is developed by Dearden& Tomlinson. It consists of four compartments. The compartment A represents the oral cavity, and initially contained an appropriate concentration of drug in a buffer. The compartment B representing the buccal membrane, contained 1-octanol, and compartment C representing body fluids, contained 0.2 M HCl. The compartment D representing protein binding also contained 1-octanol. Before use, the aqueous phase and 1-octanol were saturated with each other. Samples were withdrawn and returned to compartment A with a syringe.

4.10.3. Modified kesharyChien cell:

A specialized apparatus was designed in the laboratory. It comprised of a KesharyChien cell containing distilled water (50ml) at 370 C as dissolution medium. TMDDS (Trans Membrane Drug Delivery System) was placed in a glass tube fitted with a 10# sieve at the bottom which reciprocated in the medium at 30 strokes per min.

4.10.4. Dissolution apparatus:

Standard USP or BP dissolution apparatus have been used to study *in vitro* release profiles using both rotating elements, paddle25, 26, 27 and basket.Dissolution medium used for the study varied from 100- 500 ml and speed of rotation from 50- 100 rpm.

4.10.5. Other methods:

Few other methods involving plexi glass sample blocks placed in flasks30, agar gel method31, Valia-Chein cell USP n2 III dissolution apparatus32,33, etc have also been reported. Although a number of methods have been reported, the ideal method would be one where sink condition is maintained and dissolution time *in vitro* simulates dissolution time *in vivo.*

4.11. In vivomethods:

Methods for studying the permeability of intact mucosa comprise of techniques that exploit the biological response of the organism locally or systemically and those that involve direct local measurement of uptake or accumulation of penetrates at the surface. Some of the earliest and simple studies of mucosal permeability utilized the systemic pharmacological effects produced by drugs after application to the oral mucosa. However the most widely used methods include in vivostudies using animal models, buccal absorption tests, and perfusion chambers for studying drug permeability.

4.11.1. Animal models:

Animal models are used mainly for the screening of the series of compounds, investigating the mechanisms and usefulness of permeation enhancers or evaluating a set of formulations. A number of animal models have been reported in the literature, however, very few in vivo (animal). Animal models such as the dog, rats, rabbits, cat, hamster, pig, and sheep have been reported. In general, the procedure involves anesthetizing the animal followed by administration of the dosage form. In case of rats, the oesophagus is ligated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed.

4.11.2. Buccal absorption test:

The buccal absorption test was developed by Beckett &Triggs in 1967. It is a simple and reliable method for measuring the extent of drug loss of the human oral cavity for single and multi-component mixtures of drugs. The test has been successfully used to investigate the relative importance of drug structure, contact time, initial drug concentration and pH of the

solution while the drug is held in the oral cavity.

4.12. In vitro-in vivo correlations:

Correlations between in vitro dissolution rates and the rate and extent of availability as determined by blood concentration and or urinary excretion of drug or metabolites are referred to as "*in vitro-in vivo* correlations". Such correlations allow one to develop product specifications with bioavailability.

4.12.1. Percent of drug dissolved In vitro vs peak plasma concentration:

One of the ways of checking the in vitro and in vivo correlation is to measure the percent of the drug released from different dosage forms and also to estimate the peak plasma concentrations achieved by them and then to check the correlation between them. It is expected that a poorly formulated dosage form releases amount of drug than a well formulated dosage form, and, hence the amount of drug available for absorption is less for poorly formulated dosage form than from a well formulated dosage form.

4.12.2. Percent of drug dissolved vs percent of drug absorbed:

If the dissolution rate is the limiting step in the absorption of the drug, and is absorbed completely after dissolution, a linear correlation may be obtained by comparing the percent of the drug absorbed to the percent of the drug dissolved. If the rate limiting step in the bioavailability of the drug is the rate of absorption of the drug, a change in the dissolution rate may not be reflected in a change in the rate and the extent of drug absorption from the dosage form.

4.12.3. Dissolution rate vs absorption rate:

The absorption rate is usually more difficult to determine than the absorption time. Since the absorption rate and absorption time of a drug are inversely correlated, the absorption time may be used in correlating the dissolution data to the absorption data. In the analysis of in vitro and in vivo drug correlation, rapid drug absorption may be distinguished from the slower drug absorption by observation of the absorption time for the dosage form. The quicker the absorption of the drug the less is the absorption time required for the absorption of the certain amount of the drug. The time required for the absorption of the same amount of drug from the dosage form is correlated.

4.12.4. Percent of drug dissolved vs percent of the dose excreted in urine:

The percent of a drug dissolved and the percent of drug absorbed are linearly correlated. There

exists a correlation between the amount of drug in body and the amount of drug excreted in the urine. Therefore, a linear relation may be established between the percent of the drug dissolved and the percent of the dose excreted in the urine. [6]

4.12.5. Percent of drug dissolved vs serum drug concentration:

For drugs whose absorption from GIT is dissolution rate limited, a linear correlation may be established between the percent of drug dissolved at specified times and the serum drug concentrations at corresponding times.

Table. 1.2: List of marketed microsphere drug product [7]

Drug	***Commercial Name***	***Company***
Risperidone	RISPERDAL®,CONSTA®	Janseen®/Alkermes, Inc
Naltrexone	Vivitrol®	Alkermes
Leuprolide	Lupron Depot® EnantoneDepot® Trenantone® EnantoneGyn	TAP Takeda Takeda Takeda
Octreotide	Sandostatin®	LAR Novartis
Somatropin	Nutropin® Depot	Genentech/Alkermes
Triptorelin	Trelstar™ Decapeptyl® SR Depot	Pfizer Ferring
Buserelin	Suprecur® MP	Sanofi-Aventis
Lanreotide	Somatuline® LA	Ipsen-Beafour
Bromocriptine	Parlodel LAR ™	Novartis
Minocycline	Arestin®	

5. Method of preparation of chitosan microspheres:

5.1. Interaction with anions Ionotropic gelation:

Ionotropic gelation can be divided into following categories

1. Low molecular weight counter ions e.g. pyrophosphate, tripolyphosphate.
2. High molecular weight counter ions e.g. octyl sulphate, lauryl sulphate.

5.1.1. Wet phase inversion:

In this method of preparation, chitosan solution in acetic acid was dropped into an aqueous solution of a counter ion sodium tripolyphosphate through a nozzle. Microspheres formed were allowed to stand for 1 hrs, washed and crosslinked with 5% ethylene glycol diglycidyl ether. Finally, the microspheres were washed and freeze-dried to form porous chitosan microshperes.Changing the pH of the coagulation medium could modify the pore structure of the chitosan microsphere.

5.1.2. Emulsification and ionotropic gelation:

In this method the dispersed phase, which consists of an aqueous solution of chitosan, is added to a non-aqueous continuous phase (iso-octane and emulsifier) to form w/o emulsion. Possibility of three kinds of anions (tripolyphosphate, citrate and sulphate) to interact with chitosan by turbidimetric titration. The results indicated that there are electrostatic interactions between the above anions and chitosan in a certain region of solution pH (1.0–7.5) for sulphate/chitosan, 4.5–7.5 for citrate/chitosan and 1.9–7.5 for tripolyphosphate/chitosan), that is related to the natural characteristics of the anions. Out of the pH region where anions interacted with chitosan, no microspheres were formed. However, even in the pH region where anions interacted with chitosan, only irregular microparticles were obtained in the case of the conventional emulsification and ionotropic gelation method, while spherical microspheres with diameters in the range of tens of microns were obtained when a modified process was employed. The key point of the modified process was the introduction of gelatin and allowing the ionic crosslinking process of chitosan/gelatin w/o emulsions to take place under coagulation conditions at a low temperature. The surface of sodium sulphate crosslinked chitosan/gelatine and sodium citrate crosslinked chitosan/gelatin microspheres was very smooth, but large gaps were observed on the surface of tripolyphosphate/Chitosan microspheres. The increase of stirring speed led to a decrease in diameter and a narrowing in size distribution.

5.1.3. Floating hollow chitosan microspheres by ionic interaction with sodium dioctylsulfosuccinate:

Floating microcapsules containing melatonin were prepared by the ionic interaction of chitosan and a negatively charged surfactant, sodium dioctylsulfosuccinate. The characteristics of the floating microcapsules generated were compared with the conventional non-floating microspheres prepared from chitosan and sodium tripolyphosphate. The use of dioctylsulfosuccinate solution in coagulation of chitosan produced well-formed

microcapsules with round hollow core and 31.2–59.74% drug incorporation efficiencies. Chitosan concentration and drug–polymer ratio had a remarkable effect on drug entrapment in dioctylsulfosuccinate/chitosan microcapsules.The dissolution profiles of most of microcapsules showed near zero order kinetics in simulated gastric fluid (SGF, pH 1.2). Moreover, release of the drug from these microcapsules was greatly retarded lasting for several hours (t50 in SGF was 1.75–6.7 hrs, depending on processing factors). Drug release from non-floating microspheres was almost instant. Most of the hollow microcapsules tended to float over simulated biofluids for more than 12 hrs. Swelling studies conducted on various drug-free formulations indicated that dioctylsulfosuccinate/chitosan microcapsules showed less swelling than tripolyphosphate/Chitosan microspheres. Therefore, it was concluded that the floating hollow microcapsules could form an interesting gastroretentive controlled drug delivery system.

5.1.4. Coacervation:

In this process, the polymer is solubilized to form a solution. This is followed by addition of a solute, which forms insoluble polymer derivative and precipitates the polymer. This process avoids the use of toxic organic solvents and glutaraldehyde used in the other methods of preparation of chitosan microspheres. Prepared prednisolone sodium phosphate loaded chitosan microspheres using sodium sulphate as a precipitant. Addition of sodium sulphate to the solution of chitosan in acetic acid resulted in decreased solubility of chitosan, leading to precipitation of chitosan as a poorly soluble derivative.

5.1.5. Complex-coacervation:

Chitosan microparticles can also be prepared by complex coacervation. Sodium alginate, sodium carboxymethylcellulose, carregeenan and sodium polyacrylic acid can be used for complex coacervation with chitosan to form microspheres. These microparticles are formed by interionic interaction between oppositely charged polymers.[6-13]

5.2. Crosslinking with other chemical:

Crosslinking agents such as glutaraldehyde, formaldehyde and genipin have been used for preparation of chitosan microspheres. Detailed procedures of the methods are given in the following sections.

5.2.1. Emulsion crosslinking method:

In this process chitosan solution (in acetic acid) is added to liquid paraffin containing a surfactant resulting in formation of w/o emulsion. A crosslinking agent of varying amount is

added depending upon the crosslinking density required. The microspheres formed are filtered, washed with suitable solvents and dried.

5.2.2. Multiple emulsion method:

Water insoluble drugs are simply dispersed in Chitosan solution and entrapped by emulsion crosslinking process. In case, where the drug gets partitioned more into the oily phase, multiple emulsionsare the way to increase the entrapment efficiency. This method involves formation of (o/w) primary emulsion (non-aqueous drug solution in chitosan solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of glutaraldehyde (crosslinking agent) and evaporation of organic solvent. Chitosan microspheres prepared by multiple emulsion method, loaded with hydrophobic drug (ketoprofen) were found to have good morphological character and satisfactory production yield when prepared using this method Precipitation–chemical crosslinking

This process involves the precipitation of the polymer followed by chemical crosslinking. Precipitation can be done by sodium sulphate followed by chemical crosslinking using glutaraldehyde or formaldehyde.

5.2.3. Crosslinking with a naturally occurring agent:

They are injectable-chitosan based delivery system with low cytotoxicity. The chitosan microspheres with small particle size, low crystallinity and good sphericity were prepared by a spray-drying method followed by crosslinking with a naturally occurring crosslinking agent (genipin). The results of the study demonstrated that the genipincrosslinked chitosan microspheres had a superior biocompatibility and a slower degradation rate than the glutaraldehyde-crosslinked chitosan microspheres. Itwas concluded that the genipin-crosslinked Chitosanmicrospheres may be a suitable polymeric carrier forlong-acting injectable drug delivery.

5.3. Miscellaneous methods:

In addition to the use of above counterions, a number of microencapsulation processes have been reported for the preparation of chitosan microspheres

5.3.1. Thermal crosslinking:

Chitosan microspheres prepared by thermal crosslinking using citric acid. Chitosan solutions of varying concentrations were prepared maintaining a constant molar ratio between chitosan and citric acid. The above chitosan crosslinker solution was then cooled at 0˚C and added to

Corn oil followed by thermal crosslinking at 120˚C.

5.3.2. Solvent evaporation method:

This method involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous (w/o).

5.3.3. Spray drying:

Chitosan microspheres can also be prepared by spray drying. Chitosan solution is sprayed, air-dried followed by the addition of a crosslinking agent e.g. formaldehyde. Better control of drug release when compared to higher drug loadings.

5.3.4. Interfacial acylation:

Chitosan microspheres were prepared by spray hardening and interfacial acylation methods. Injection using different molecular weight Chitosan (70,000–2,000,000). By the spray hardening method, microspheres with particle sizes between 5 and 30 mm could be obtained. On the other hand, chitosan microspheres with the ability to extend the dissolution period.

5.3.5. Coating by chitosan solution:

In this method, previously formed microparticles are coated with chitosan.

6. Result and discussion:

6.1. Characterization of drug:

The drug selected for research work is glibenclamide as is an oral hypoglycemic agent, which is a drug for the treatment of patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) and given in insulin resistance condition. It is potent but slow acting, marked initial insulinameic action, may work when other drugs fail. Higher incidence of hypoglycaemia, single daily dose possible despite short half-life. Using this system based on controlled and sustained release plasma half-life can be maintained. The drug sample was gifted by Welcurepharmaceutical, Indore (MP).

The drug sample was firstly identified for its various pharmacopoeial tests as well as analysed spectrophotometrically by UV, FTIR and HPLC and the result showed the authencity and purity of drug sample.

6.2. Identification by UV spectrophotometry:

The maximum absorbance of drug was determined by 1700 UV Shimadzu spectrophotometer and was found to be at 300 nm which was matched with the standard given in pharmacopoeia i.e. 300 nm.

6.3. Calibrationcurve:

Standard curve of glibenclamide was prepared using different solvents like 0.01M Methanolic hydrochloric acid and 0.1N sodium hydroxide using methanol as a co-solvent by 1700 UV Shimadzu spectrophotometer. The result showed that the glibenclamide follows the Lambert beer law between the concentration ranges of 10-100 µg/ml.

6.4. Melting point:

Melting point of drug was determined by capillary tube melting point apparatus. The melting point was found to be in the range of 169-173^{0}C which was matched with standard melting point given in pharmacopoeia. Hence confirm the identity of the compound.

6.5. Partition coefficient:

Partition coefficient of the drug was determined in n-octanol: PBS. The value of partition coefficient was found to be 1.98. Higher the value of partition coefficient greater is the lipid solubility and vice versa. As the value of partition coefficient is found high, it shows that the drug is lipophilic in nature.

6.6. Drug-excipient compatibility:

Drug-excipients interaction was determined by FTIR spectrum it was found that the peak of glibenclamide has no interference with the peak of excipients. Hence there is no interaction between the drug sample and the excipients likely to be used in the formulation and hence can be used in the formulation.

6.7. HPLC (High performance liquid chromatography) Analysis:

The HPLC analysis of drug sample was also carried out and the retention time was found to be 3.39 min.

6.8. Solubility:

Quantitative solubility of drug was checked in various solvent using 1700 Shimadzu UV spectrophotometer and found that the drug was slightly soluble in methanol, ethanol, sparingly

Soluble in dichloromethane, insoluble in water and water and dissolve in sodium hydroxide.

From the studies performed it was concluded that Chitosan microspheres of glibenclamide can be prepared optimistically.

7. Formulation and evaluation preparation method:

Chitosan microspheres of glibencliamide was prepared by emulsification crosslinking method using gluteraldehyde as crosslinker. Accurately weighed quantity of chitosan was dissolved in 2% (v/v) aqueous acetic acid. The drug (glibencliamide) was added to the chitosan solution and mixed thoroughly. The dispersed phase was then added drop-wise through a disposable syringe (10 ml) to the continuous phase consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing different amounts of surfactant (span 80) to form a water in oil (w/o) emulsion. Stirring was continued at different speed using a 3 blade propeller stirrer. After 20 min of stirring, a measured quantity of aqueous gluteraldehyde (25% v/v) was added dropwise at regular intervals of 1, 2 and 3 hours respectively and continued for 1 hour after the final addition of gluteraldehyde. The preparation was centrifuged at different rpm, the supernatant was decanted and microspheres obtained as residue were washed 3 times with petroleum ether (60- 800C). After the final wash, microspheres were then air dried at room temperature, collected.

7.1. Process variables:

The preparation of chitosan microspheres of glibenclamide involves various process variables but outof them the following were selected.

(A) Effect of core microspheres concentration and polymer concentration

(B) Effect of crosslinking agent.

(C) Effect of stirring rate

Table. 6.1: Working formula for preparation of different batches of microspheres

ulation code	*Drug polymer ratio*	*Stirring speed(rpm)*	*pan80 conc. (%v/v)*	*iquid.paraffin y+Light (ml)*	*Glutaraldehyde conc. (ml)*
GC1	1:1	1500	0.5	100	1.5%
GC2	1:2	1500	0.5	100	1.5%
GC3	1:3	1500	0.5	100	1.5%

GC4	1:4	1500	0.5	100	1.5%
GC5	1:2	1500	0.5	100	1%
GC6	1:2	1500	0.5	100	0.5%
GC7	1:2	500	0.5	100	1.5%
GC8	1:2	2500	0.5	100	1.5%

7.2. Evaluation:

7.2.1. Shape morphology:

All batches of microspheres were studied for shape and size by optical microscopy at magnification of 1300X.

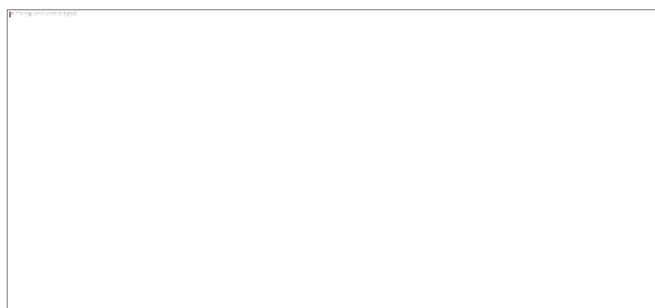

Figure. 6: 1image of microspheres at magnification of 1300X

7.2.2. Percentage yield (% yield):

The prepared chitosan microspheres was collectedand weighed. The measured weight was divided bytotal amount of all non-volatile components, whichwere used for the preparation of microspheres. The % yield was calculated using following formula Percentage yield = Weight of microspheres recovered x 100 Weight (drug + polymer)

Table. 6.2: Average Percentage yield of different formulations

Percentage yield (% yield)				
Ulation code	*AL 1*	*AL 2*	*AL 3*	*Geparticle size*±SD*
GC1	62	63	61	62.00 ± 1.00
GC2	72.5	71.6	73.3	72.46± 0.85
GC3	76.6	76.6	75.8	76.33±0 .46

GC4	86	86	85	85.66±0.57
GC5	85.8	85.9	84.9	85.53 + 0.55
GC6	81.8	81.8	80.8	81.33+ 0.21
GC7	80.0	80.0	81.6	80.80± 0.80
GC8	85	85.1	84.1	84.73±0.55

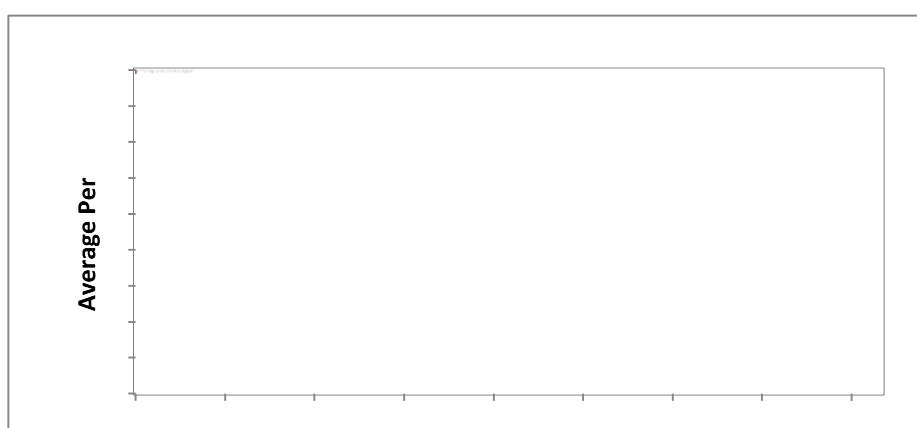

Figure. 6.2: Comparison graph of percentage yield of different formulations

The particle size analysis was done by optical microscope. Chitosan microspheres of glibenclamide (100 mg) was hydrated with PBS (pH 6.8) (10 ml) in a small test tube by manual shaking for 5 min. A drop of the suspension was mounted on the slide and observed under the optical microscope at 100 x magnification. About 300 particles were measured individually with the help of eye piece micrometer, average was taken and their size distribution range, mean diameter was calculated.

Table. 6.3: Particle size analysis of different formulations

S. No.	***Formulation code***	***Geparticle SIZE*±SD***
1.	GC1	6.43±1.354
2.	GC2	6.69±1.50
3.	GC3	8.15±1.25
4.	GC4	9.48±1.74

5.	GC5	7.75±2.00
6.	GC6	7.24±1.54
7.	GC7	8.46 ±2.18
8.	GC8	7.28± 1.76

*Average of three readings

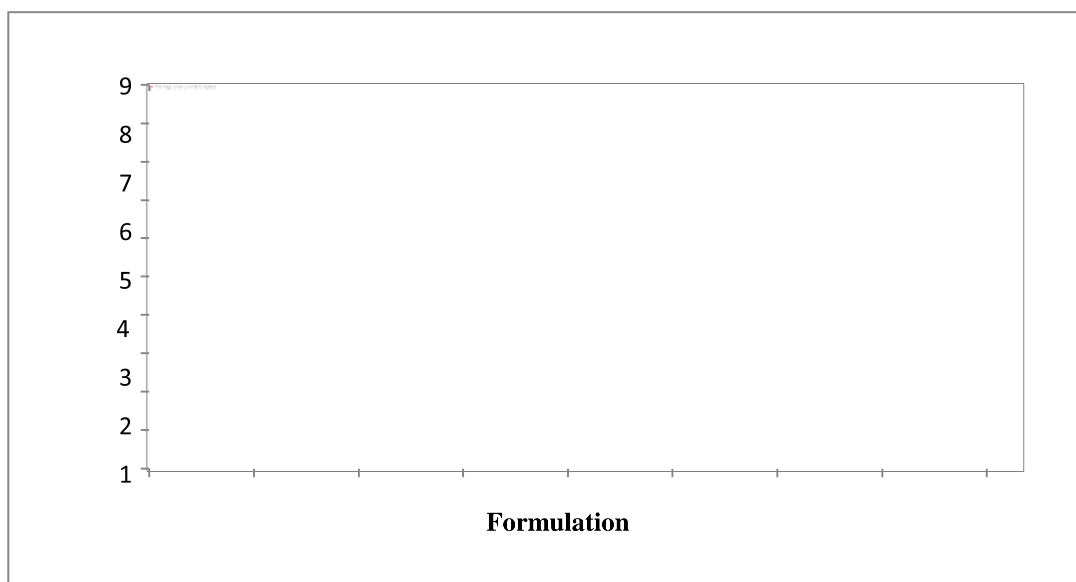

Figure. 6.3: Comparison graph of average particle size of different formulations

7.2.3. Angle of repose:

The flow characteristics are measured by angle of repose.Improper flow is due to frictional forces between theparticles. These forces are quantified by angle of repose. Determination of the repose angle wasobtained for 10 g microspheres (23). For this study,microspheres were poured into a conical flask whichhad a 0.9 cm diameter and was placed 10 cm abovethe surface. Repose angle was calculated accordingto the tangent of the ratio of the height and diameterof the bulk.Itcan calculate by, $\tan \theta = h / r$ or $\theta = \tan^{-1} (h / r)$ where, h =height of pile, r = radius of the base of the pile and θ = angleof repose.

Table. 6.4: Flow property and angle of repose

S. No.	**Angle of repose(˚)**	**Type of flow**	**Type of powder**
1	<25	Excellent	Non-cohesive
2	25-30	Good	Non-cohesive
3	30-40	Passable	cohesive

4	40>	Very poor	Very cohesive

Table. 6.5: Observation of angle of repose of chitosan microspheres of glibenclamide

S. No.	Ulation code	Angle of repose Average+SD	Type of flow
1.	GC1	26.58 ± 2.67	Good
2.	GC2	27.96 ± 1.77	Good
3.	GC3	29.47 ± 2.13	Good
4.	GC4	31.15 ± 1.99	Passable
5.	GC5	27.66 ± 2.37	Good
6.	GC6	27.16 ± 1.50	Good
7.	GC7	26.76 ± 1.50	Good
8.	GC8	29.14 ± 1.26	Good

7.2.4. Drug entrapment efficiency:

Weighed quantity of microspheres were crushed and suspended in methanol to extract the drug from microspheres. After 24 hrs, the filtrate was assayed spectrophotometically at 300 nm for drug content against methanol as blank. Corresponding drug concentrations in the samples were calculated from the calibration plot using a regression equation derived from the standard graph. The drug entrapment efficiency (DEE) was calculated by the equation.

Actual drug content

Encapsulation efficiency =................ 100

Theoretical drug content

Table. 6.6: Entrapment efficiency of chitosan microspheres of glibenclamide

S.No.	Formulation code	%Entrapment efficiency			Average*±SD
1.	GC1	91.76	91.76	91.76	91.79±0.702
2.	GC2	86.50	86.50	86.50	86.50±0.650
3.	GC3	81.50	81.50	81.50	81.88±0.370
4.	GC4	76.20	76.20	76.20	76.20±1.000

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5.	GC5	85.20	85.20	85.20	85.02±0.371
6.	GC6	83.50	83.50	83.50	83.39±0.661
7.	GC7	85.00	85.00	85.00	85.42+0.364
8.	GC8	83.20	83.20	83.20	83.04±0.741

*Average of three readings

*Average of three readings

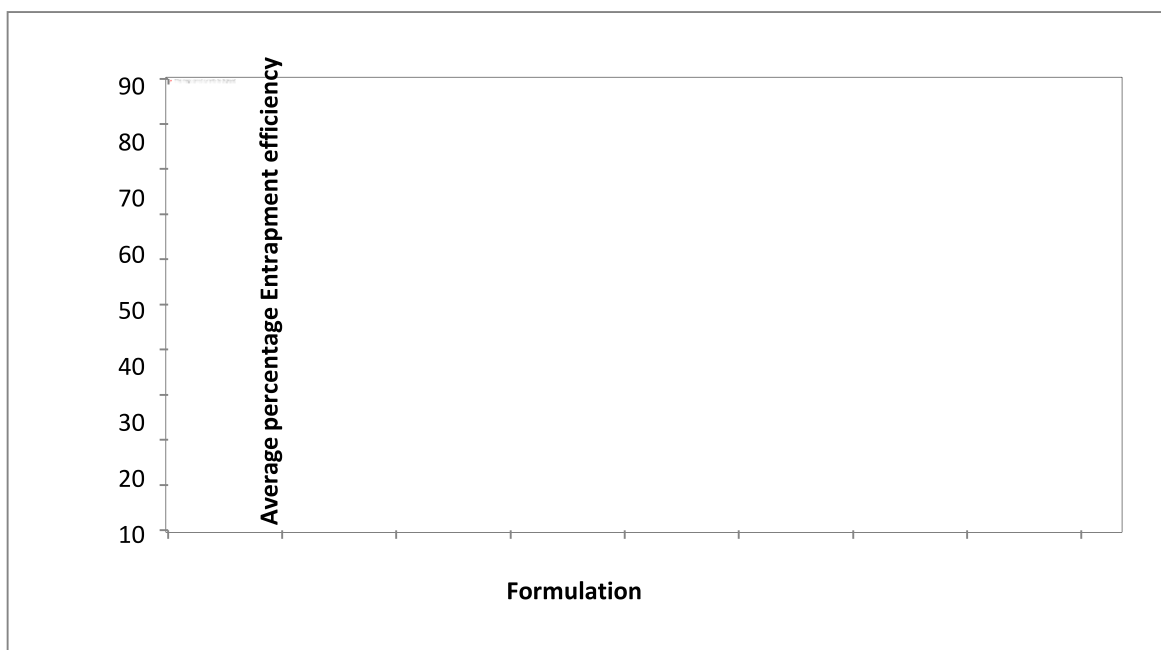

Figure. 6.4: Comparison graph of Entrapment efficiency of different formulations

7.2.5. Swelling index:

The swelling ability of microspheres in physiological media was determined by optical microscopy method. The 100 microspheres were suspended in 5 mL of stimulated gastric fluid USP (pH 1.2). The particle size was monitored by microscopy technique every 1 hour up to 6 hours using an optical microscope. The increased size microsphere of each swollen sample (Wt) was determined by microscope.

Swelling index= (W2 - W1) / W1 x 100 (1)

W1 and W2 represent the final average partical size of chitosan microspheres and the initial average particle size of chitosan microspheres, respectively.

Table. 6.7: Swelling index of chitosan microspheres of Glibenclamide

Swelling index				
S No.	Trial 1	Trial 2	Trial 3	Average*±SD
1.	0.52	0.70	0.49	0.570± 0.113
2.	0.55	0.50	0.62	0.556+0.060
3.	0.86	0.82	0.81	0.83+0.026
4.	0.89	0.81	0.86	0.853+0.040
5.	0.66	0.68	0.71	0.683+0.025
6.	0.80	0.78	0.82	0.800+0.020
7.	0.89	0.83	0.83	0.850+0.346
8.	0.74	0.72	0.73	0.730+0.010

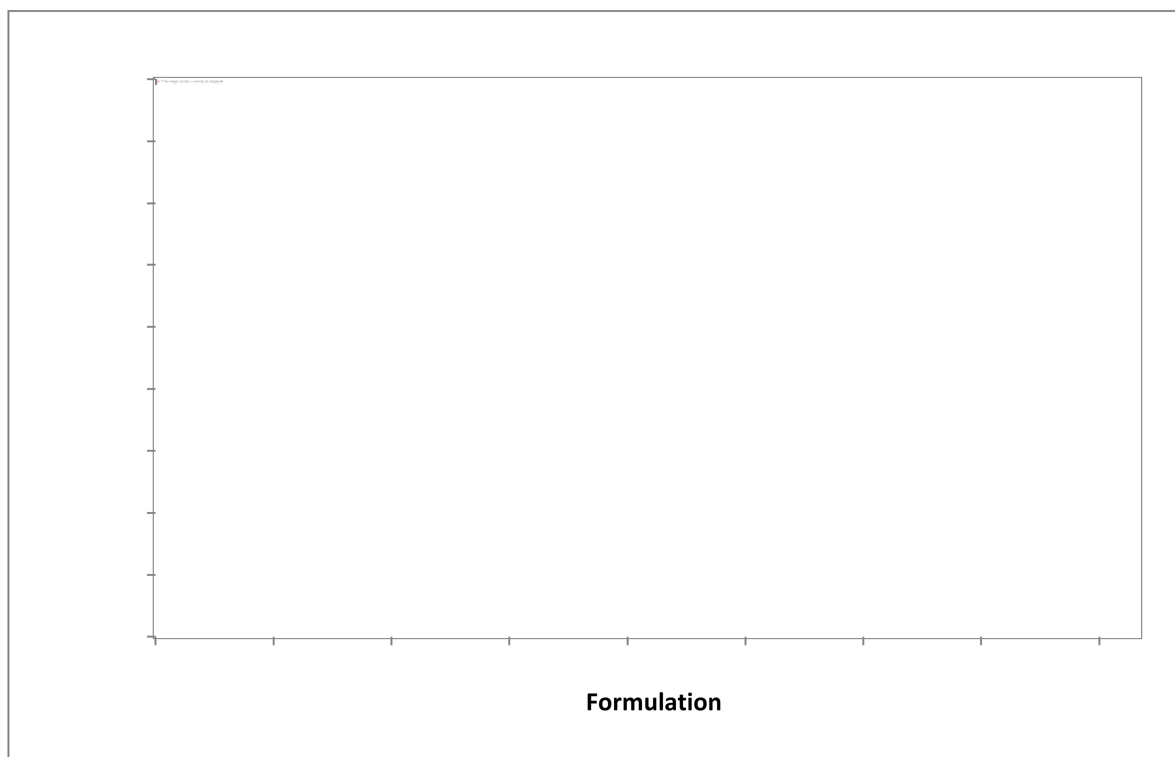

Figure. 6.5: Comparison graph of swelling index of different formulations

7.2.6. In-vitro mucoadhesivity test:

The mucoadhesive property of microspheres was evaluated by in-vitro wash off test for mucoadhesion. A piece of mucosa was mounted onto glass slides. About 50 mg of

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microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of USP disintegration apparatus. By operating the disintegration test machine, the tissue specimen was given a regular up and down movement in 0.1 N HCl/ PBS pH 6.8 at 37°C taken in a 1000ml. At the end of 30 minutes, 1 hour and then at hourly intervals, the apparatus was stopped and the microspheres adhering to the tissue, 0.1NHCl/PBS was centrifuged, dried andthe number of microspheresstill adhering onto the tissue was counted. The mucoadhesiveness of these microspheres was calculated.

No. of microspheres remains

% Mucoadhesion =… *100

No. of applied microspheres

Table. 6.8: % Mucoadhesivity of chitosan microspheres of glibenclamide

% Mucoadhesivity					
S.No	Ulation code	AL 1	AL 2	AL 3	% Mucoadhesivity +SD
1.	GC1	56	55	52	53.32.08
2.	GC2	71	70	69	.0+1.00
3.	GC3	85	82	80	.32.51
4.	GC4	86.7	82	85.1	62.389
5.	GC5	70.3	69.0	73.3	70.86 2.205
6.	GC6	80.3	70.3	76.36	75.65 5.037
7.	GC7	78	70	69.1	72.36 4.899
8.	GC8	73	72.2	71.2	72.10 0.901

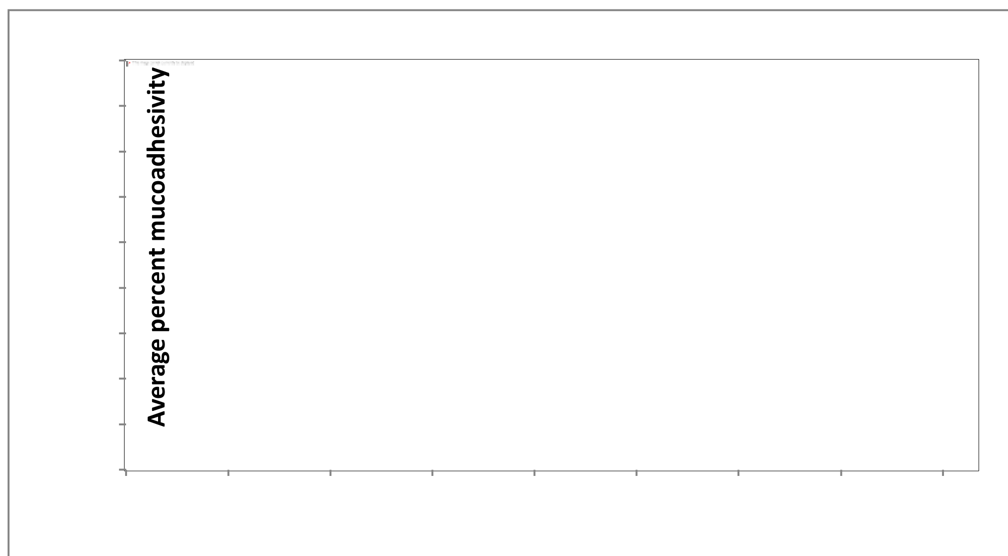


Figure. 6.6: Comparison graph of % Mucoadhesivityof different formulations

7.2.7. In-vitro release study:

The drug release was performed using USP dissolution (basket type) apparatus at 37 ± 0.5°C and at 50 rpm in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) as dissolution medium. Microspheres of 50 mg of glibenclamide were used for the test. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analyzed spectrophotometricaly at 300nm using PBS pH7.4 as a blank. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of test sample.

Table. 6.9: In-vitro drug release study of formulation GC1

Time (hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	00	00
1	7.20	7.20
2	7.90	15.10
3	9.00	24.10
4	10.50	34.60
5	10.56	45.16
6	10.84	56.00
24	11.20	67.20

Table. 6.10: In-vitro drug release study of formulationGC2

Time (hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	00	00
1	10.60	10.60
2	11.25	21.85
3	14.00	35.85
4	15.06	50.91
5	16.00	66.91
6	16.30	83.21
24	16.50	99.62

Table. 6.11 In-vitro drugrelease study of formulationGC3

Time (hrs)	**% Invitro release rate**	**% Cumulative drug release**
0	0	0
1	10.40	10.40
2	12.20	22.60
3	13.02	36.20
4	13.28	49.40
5	14.16	63.50
6	15.20	78.50
24	18.40	96.50

Table no. 6.12: In-vitro drug release study of formulation GC4

Time (hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	0	0
1	12.20	12.2
2	13.05	24.7
3	13.80	37.9
4	14.50	51.7
5	14.51	66.2
6	14.55	80.2
24	15.15	95.3

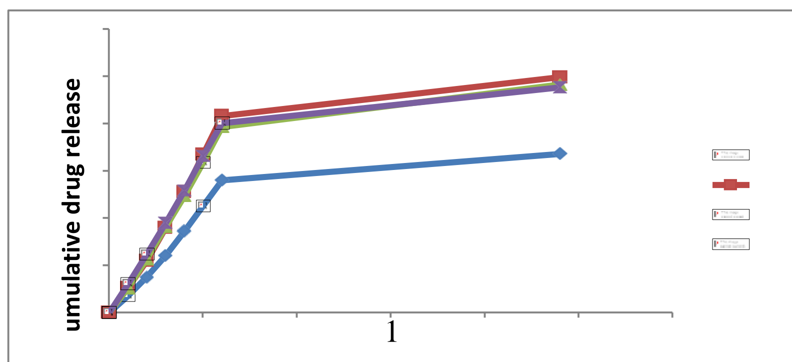


Figure. 6.7: Comparison graph of %cumulative In-vitro drugreleaseof 4 different formulations

Table no. 6.13: In-vitro drug release study of formulation GC5

Time(hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	0.00	0.00
1	6.40	6.40
2	7.40	13.80
3	8.50	22.40
4	10.40	32.70
5	11.30	44.0

6	12.50	56.50

Table no. 6.14: In-vitro drug release study of formulationGC6

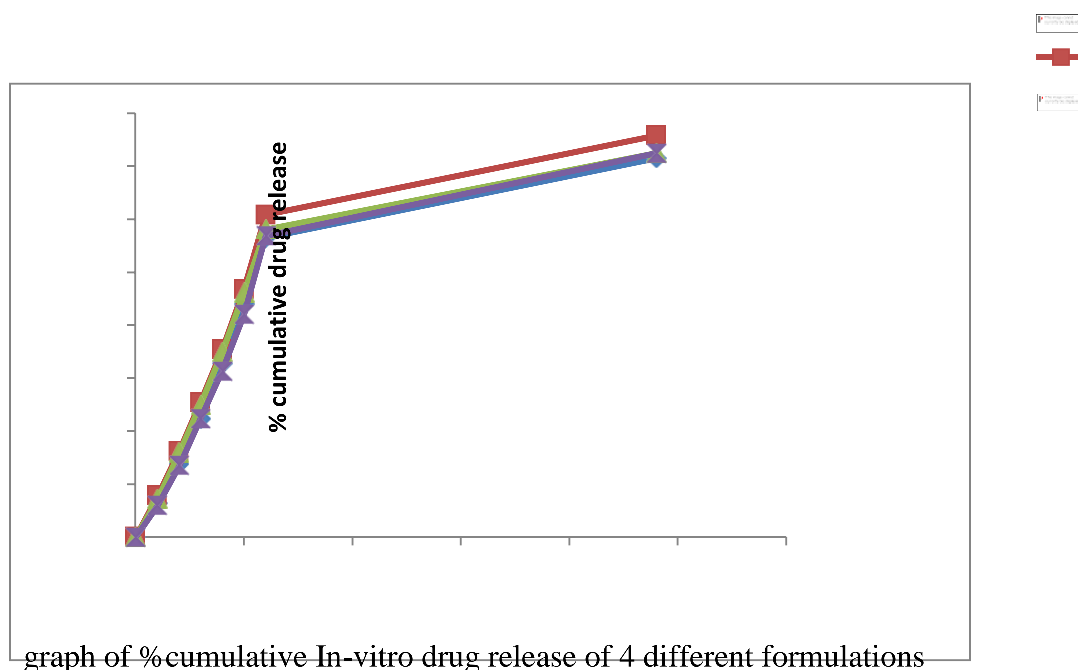
Time(hrs)	% Invitro release rate	% Cumulative drug release
0	00	00
1	7.8	7.8
2	8.4	16.2
3	9.2	25.4
4	10.3	35.4
5	11.1	46.8
6	14.0	60.8
24	15.0	75.8

Table no. 6.15: In-vitro drug release study of formulationGC7

Time (hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	0	0
1	7.20	7.20
2	8.70	15.90
3	9.0	24.90
4	10.20	34.90
5	11.20	46.10
6	12.0	58.10
24	14.40	72.50

Table no. 6.16: In-vitro drug release study of formulation GC8

Time (hrs)	*% Invitro release rate*	*% Cumulative drug release*
0	0	0
1	6	6
2	7.40	13.50
3	8.90	22.30
4	9.30	31.30
5	10.70	42.0
6	14.90	56.90
24	15.30	72.50



graph of %cumulative In-vitro drug release of 4 different formulations

8. Conclusion:

Microspheres are well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. Microsphere as a drug delivery system especially offering a sustained and controlled action of drug to desired area of effect. The drug should be delivered to specific target sites at a rate and concentration that permit optimal therapeutic efficacy while reducing side effect to minimum.

Chitosan is a versatile polymer whose applications range from weight supplement in the

Market to a drug carrier in formulation research. Chitosan has been shown to improve the dissolution rate of poorly soluble drugs and thus can be exploited for bioavailability enhancement of such drugs .This crosslinking has been used extensively for the preparation of chitosan microspheres. It is convenient, safe and offers numerous advantages over conventional ones that includes evading GI incompatibility, variable GI absorption, bypassing first pass metabolism, and enhanced bioavailability, decreased frequency of administration, improved patient compliance, and rapid cessation of drug input and can maintain a suitable plasma concentration.

Diabetes mellitus is a chronic metabolic disorder characterized by high glucose concentration in blood, caused by insulin deficiency, often combined with insulin resistance. Glibenclamide is an oral hypoglycemic agent, which is a drug for the treatment of patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) and given in insulin resistance condition. It is potent but slow acting, marked initial insulinameic action, may work when other drugs fail. Higher incidence of hypoglycemia, single daily dose possible despite short half-life. Using this system based on controlled and sustained release plasma half-life can be maintained. Formulation and evaluation of Glibenclamide loaded chitosan microspheres for controlled release was found to be potential and effective in terms yield, encapsulation efficiency, particle size distribution, and in vitro release characteristics. The investigation of optimum formulation showed controlled drug release.

9. Future aspects:

While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus point of research and might result in the availability of new products with better therapeutic possibilities and substantial benefits for patients. Mucoadhesive microspheres would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations. Thus Chitosan microspheres of glibclamide would become a promising candidate for therapy of diabetes type-II in thefuture.

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