

Review Article

Albumin Microspheres: New Approach for Sustained Drug DeliveryPUSHPENDRA BHUSHAN MISHRA*¹, VIMAL ARORA¹, HARPAL SINGH¹, NANDINI VASHISTHA, SAURAV KUMAR¹¹Department of pharmaceutics, CT Institute of Pharmaceutical Sciences, Shahpur, Jalandhar, Punjab-144020, India

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ABSTRACT

Sustained drug delivery systems are designed to deliver drug, at predetermined rates for predefined periods of time, and have been used to overcome the shortcoming of conventional drug formulations. And in the recent approach for the sustained drug delivery has been the development of microspheres using albumin as a polymer to sustain the release of the drug. This review deals with the methods used and the results obtained by the use of albumin (bovine serum albumin and egg albumin) as a polymer in the formulation in various research works. The in-vitro studies in various research works give a very promising statement that albumin can be used as a polymer in the formulation at a large scale without much of the complications.

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INTRODUCTION

Sustained drug delivery systems have been designed in such a manner that they deliver the drug, at predetermined rates for predefined periods of time, and hence can be used to overcome the shortcoming of conventional drug formulations. In the recent past there has been tremendous advancement in the formulation technology of modified release dosage form with sustained release oral dosage form being the most widely accepted approach as compared to conventional immediate release formulations^{1,2}. The recent approach for sustain release system has been the development of the microsphere using albumin over various other polymers available.

Microsphere is a term used for small spherical particles, with diameters in the micrometer range (typically 1 μ m to 1000 μ m (1mm)) and thus microspheres are sometimes referred to as micro-particles. Microspheres can be manufactured from various natural and synthetic materials. Various natural and synthetic polymers being used are³:

Natural: Agarose, Carrageenan, Chitosan, Starch, Albumin, Collagen and Gelatin, Poly dextran, Poly starch.

Synthetic polymers: Lactides, Glycolides & their co polymers, Poly anhydrides, Poly alkylcyano acrylates, Poly methyl methacrylate (PMMA), Acrolein, Epoxy polymers.

From the various polymers discussed above, it is really important to select a polymer which can be used in the formulation without much of problem during preparation and can produce a longer duration of action without any significant amount of side effect.

So the selection of albumin as a polymer was done and the selection was done on the basis of some facts like its natural availability, biodegradation into natural products⁴, lack of toxicity, and its non-antigenicity⁵ with good aqueous solubility. As well as it also has a property of protein binding and physical entrapment. It also supports passive as well as facilitated release of various type of incorporated drugs from the polymer matrix⁶. So the concept for the formulation of albumin based microspheres to increase the bioavailability of the drugs to get a sustained release of drug, resulting in decrease in the dosing frequency came into existence.

The albumin microspheres were first described by Kramer in 1974 and since then the albumin microspheres have been extensively investigated in controlled release systems as vehicles for the delivery of therapeutic agents to local sites. The first biodegradable and biocompatible albumin

*Author for Correspondence:

Email: mr.pushpendramishra@gmail.com

microspheres were formulated in the 1970s (Kramer 1974). Albumin microspheres are metabolized in the body, and the size of particles, degree of stabilization, and site of metabolism are the main factors influencing the extent of metabolism. The drug release from the microspheres can be widely modulated by the extent and nature of cross-linking, size, the position of the drug, and its incorporation level in the microspheres. Colloidal forms of albumin have been considered as potential carriers of drugs for their site-specific localization or their local application to anatomically discrete sites.

In the recent past the albumin has been used as a carrier for targeting drugs to various specific sites. Albumin based delivery systems can be used to target drugs to the inflamed joint. It has been reported that there is an improvement in the targeting efficiency of the drug to arthritic regions when the drugs coupled with Albumin are intravenously administered⁷. The circulation half-lives of the drugs have also been reported to increase dramatically when the drugs are conjugated with albumin⁸. Increasing the circulation half-life of the formulation by reducing its uptake by the reticuloendothelial system has been shown to improve the targeting efficiency of the formulation to the arthritic paws of rats. There are several reports on the use of long circulating liposomes to target the drugs to the arthritic joints⁹.

Advantages of albumin microspheres

Albumin microspheres have several advantages related to them as compared to others, some of them are:

- Constant and prolonged therapeutic effect.
- reduction in the dosing frequency and thereby improve the patient compliance
- biocompatibility
- reduction in the adverse drug reactions
- relatively higher stability
- controlled and targeted release

Research work carried out using albumin as polymer

Thakkar et al. (2005) carried out the preparation of Celecoxib-loaded albumin microspheres. The microspheres are prepared by using emulsification chemical cross linking method with use of natural polymer bovine serum albumin. The microspheres prepared were characterized on the basis of entrapment

efficiency, particle size, and in vitro drug release. The Surface morphology of the microspheres was done by scanning electron microscopy. In vitro release studies indicated that the microspheres are sustained released and they release of the drug for ~6 days. The blood kinetic studies showed that Celecoxib loaded albumin microspheres exhibited prolonged circulation than the Celecoxib solution¹⁰.

Eroglu et al. (2000) carried out the preparation of bovine serum albumin microspheres containing Dexamethasone Sodium Phosphate and the in vitro evaluation of the same. The microspheres were prepared by emulsion polymerization technique. An aqueous solution of glutaraldehyde (25% w/v) was used as the cross linking agent in two different amounts. The studies showed that the release time is increased as the amount of glutaraldehyde is increased¹¹.

Gulsu et al. (2012) carried out the preparation and characterization of ketoprofen loaded albumin microspheres using emulsion polymerization technique. The microspheres were evaluated on various parameters. The effect of polymer concentration, stirring rate, crossing agent used and crosslinking time was also observed. It was observed that with the increase in polymer concentration the mean particle size increased. The stirring rate and crosslinking used also have an impact on the properties of the microspheres, whether it is particle size or the release characteristic¹².

Tuncayi et al. (2000) developed albumin microspheres of diclofenac sodium and also carried out the in-vitro and in-vivo evaluation of the same. The microspheres were prepared using emulsion polymerization technique. The results of the studies conducted showed that albumin can be used as a carrier matrix to provide an extended duration of active substance. From the studies, it was also concluded that concentrations of stabilizing agent and the duration of stabilization are also an important factors which determine the release characteristics¹³.

Jayaprakash et al. (2009) prepared and evaluated the biodegradable microspheres of methotrexate. The objective of this study was to prepare methotrexate microspheres of bovine serum albumin with sustained release properties. The microspheres were prepared in the different ratios of the drug and polymer by using emulsion crosslinking method. The

microspheres prepared were subjected to various physicochemical evaluations and to *in vitro* dissolution studies. The studies revealed that the microspheres of 1:6 ratio were the best and the drug release from microspheres was constant and prolonged¹⁴.

Rathod et al. (2010) developed egg albumin microspheres containing pilocarpine nitrate by heat stabilization method. The prepared microspheres were intended for ocular delivery and were prepared with various concentrations of polymers. Microspheric gels were prepared by triturating drug-loaded microspheres with Carbopol-940 gels. Biological response of microspheric gels was measured by reduction in intraocular pressure. Bioavailability parameters of all the prepared formulations were studied and comparisons were made. From the studies conducted it was also observed that the polymer concentration, stirring speed, crosslinking agent and crosslinking time have an effect on the properties of the microspheres formed. Finally it was concluded that the bioavailability of pilocarpine nitrate was increased by incorporation of drug into microspheres and subsequently in gels¹⁵.

Mathew et al. (2007) carried out the formulation and evaluation of Ketorolac Tromethamine loaded albumin microspheres for potential intramuscular administration. Albumin microspheres were prepared by emulsion crosslinking method. The microspheres prepared were characterized on the basis of entrapment efficiency, particle size, and *in vitro* drug release. From the experimental results obtained it may be concluded that the developed albumin microspheres could be useful for once-a-day IM administration of Ketorolac Tromethamine¹⁶.

Chinna Gangadhar et al. (2010) developed Indomethacin Microspheres using natural (egg albumin) and synthetic (eudragit) polymers as Controlled Release Dosage Forms. Microspheres were prepared by solvent evaporation method using an acetone/liquid paraffin system and coacervation by phase separation method using petroleum ether and coconut oil as dispersion and continuous phase systems. The droplet stabilizing was done by using magnesium stearate. And microspheres are hardened by using n-hexane. The microspheres prepared were then evaluated for their micromeritic properties, drug content and encapsulation efficiency. The characterization of microspheres was done by Fourier transform infrared

spectroscopy (FT-IR), and scanning electron microscopy (SEM). The *in vitro* release studies was performed by buffer change method to mimic Gastro-Intestinal Tract (GIT) environment in pH 1.2, and then at pH 7.4, phosphate buffer. Finally it was concluded that the encapsulation efficacy of egg albumin was more as compared to eudragit and ethyl cellulose¹⁷.

Singh et al. (2012) developed Ivabradine HCl (IBH) microspheres using egg albumin. IBH loaded egg albumin microspheres were prepared by heat denaturation technique. Various evaluation parameters were assessed, with a view to obtain sustained release of drug. The prepared IBH microspheres were then subjected to FTIR, SEM, particle size and size distribution, % yield, % drug loading, entrapment efficiency, *in vitro* dissolution studies, release kinetics and DSC. The *in-vitro* performance of IBH microspheres showed that sustained release was dependent upon the polymer concentration. The dissolution data's of all the formulations made were obtained and on comparing the dissolution data of all the formulation, the best release was obtained from the formulation with a drug: albumin ratio of 1:2¹⁸.

Arora et al. (2013) carried out the preparation and evaluation of egg albumin microspheres of Itraconazole. The microspheres were prepared by using heat denaturation technique. The prepared microspheres were evaluated on various parameters like the size, morphology, micromeritic properties, percent drug entrapment, *in-vitro* dissolution studies and the scanning electron microscopy (SEM). The *in vitro* dissolution studies showed that the drug release for the batch EA4 (drug: polymer ratio - 1:4) was 87.02 ± 5.89 after 10 hrs. It was observed that the release of drug from the microsphere decreased with the increase in polymer concentration¹⁹.

Jain et al. (2011) developed a microsphere of Ketorolac tromethamine using albumin. The microsphere was prepared by emulsion cross linking technique using different ratios (drug: albumin). The prepared microspheres were subjected to various physicochemical evaluation and *in vitro* release studies. After evaluation of the microspheres it was finally concluded that the microspheres of 1:5 (drug: albumin) ratio have the most constant and prolonged release, thus the increased efficacy²⁰.

Tabassi et al. (2003) carried out preparation and characterization of albumin microspheres

encapsulated with Propranolol Hydrochloride. The microspheres were prepared by emulsion heat stabilization technique. The prepared microspheres were studied for particle size distribution, drug loading, release characteristics, bio adhesion and in-vitro controlled diffusion across the rat intestine. The studies conducted showed that the prepared microspheres possessed good bio adhesion properties. It had been observed that the microspheres prepared showed bioadhesion of 70% on the surface mucosa of rat jejunum. The drug release from albumin microspheres was mainly controlled by diffusion and showed a biphasic pattern with a high initial release (burst effect), followed by a more gradual terminal release. Finally it was concluded that a sustained release form of the drug can be obtained by utilizing microsphere system with better bio adhesion²¹.

Basavaraj et al. (2012) developed gastro retentive microspheres of ketoprofen using egg albumin and ethyl cellulose as the polymer. Microspheres were prepared by using heat denaturation technique and emulsion solvent evaporation technique respectively. The prepared microspheres were evaluated for micromeritic properties, particle size, percentage yield, in-vitro buoyancy, entrapment efficiency, drug polymer compatibility, scanning electron microscopy and in-vitro drug release studies. A release of 96.78% for egg albumin microspheres was obtained as compared to 88.31% of ethyl cellulose microsphere, meanwhile it was also observed that the release rate decreased with the increase in the polymer concentration²².

Praveen et al. (2011) worked on the formulation and evaluation of aceclofenac loaded bovine serum albumin microspheres using suspension cross linking method. The prepared microspheres were evaluated on various parameters like the size, morphology, micromeritic properties, percent drug entrapment, in-vitro dissolution studies and the scanning electron microscopy. The scanning electron microscopy shows that the microspheres prepared had a smooth surface meanwhile a release of 83.16% was observed²³.

Dubey et al. (2002) developed egg albumin microspheres of 5-Flourouracil. The microspheres were prepared by chemical cross linking technique. Various evaluation parameters were assessed, along with various factors which can play an important role in the

formulation and the amount of drug entrapped. As a result of all the evaluations done on the formulations, it was found that the heating temperature as well as the heating time greatly effects the mean particle size, drug entrapment efficacy of the albumin microspheres. From all the evaluations done and data collected it was finally concluded that the maximum entrapment was obtained at a temperature of 90°C for a time of 5 min²⁴.

Burgees et. al. (1987) prepared albumin microspheres by using two different stabilization processes: chemical denaturation and heat denaturation. The In vitro drug (prednisolone) release studies were conducted on different microsphere preparations and the results were correlated to the stability of the microspheres. It was observed that heat denaturation had a significant effect on the in vitro release rates; the more denatured the albumin, the slower the drug release rate, whereas on the other side chemical denaturation, using glutaraldehyde, did not have a marked effect on drug release from the microspheres. From the studies it was concluded that the two of the major limitations of albumin microsphere systems, i.e. poor drug entrapment and premature release have been overcome using prednisolone loaded heat denatured microspheres²⁵.

Parashar et. al. (2010) worked on the formulation and evaluation of the biodegradable microspheres of tinidazole using bovine serum albumin. The microspheres were prepared by emulsion cross linking method. Different ratios of polymer was used in the preparation. The microspheres were evaluated for particle size, Melting point, TLC, entrapment efficiency and in vitro release studies. The studies showed that the batch with drug: polymer ratio (1:4) was the best as it gave a constant and prolonged release of the drug²⁶.

Techniques used for the preparation of microspheres

1. Solvent evaporation technique

a. Single emulsion technique

This method is widely used for the preparation of the microparticulate carriers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique.

The natural polymers are firstly dissolved or dispersed in an aqueous medium followed by

dispersion in non-aqueous medium like oil. The next step involves the cross linking of the dispersed globules. The cross linking can be achieved either by the means of heat or by using chemical cross linkers. The chemical cross linking agents which are being commonly used are glutaraldehyde, formaldehyde, di acid chloride etc. It is important to note that heat denaturation is not suitable for thermo labile substances. While using chemical cross linking agents one should be careful as it suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation. After the crosslinking step, the globules formed are then subjected to centrifugation, washing and separation^{15, 27}(Fig. 1)

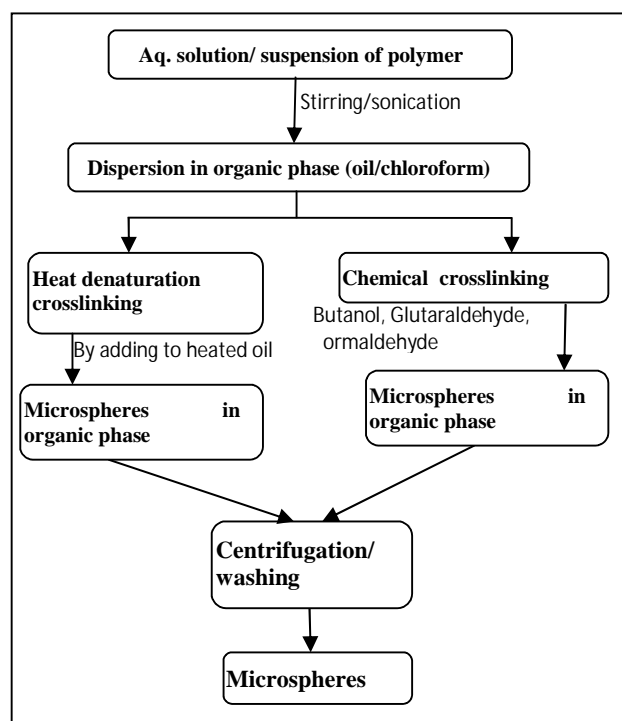


Figure 1: Single emulsion technique

b. 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. It is best suited for water soluble drugs, peptides, proteins and the vaccines. This method can be used for both the natural as well as synthetic polymers.

The aqueous protein solution is firstly prepared, which is then dispersed in a lipophilic organic continuous phase. The protein solution may contain the active constituents. The continuous phase is generally consist of the polymer solution

which will eventually encapsulate the protein contained in the dispersed aqueous phase. The primary emulsion is then subjected to the homogenization or the sonication process before being added to the aqueous solution of the polymer. This results in the formation of a double emulsion. The resultant emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction^{16, 28} (Fig. 2).

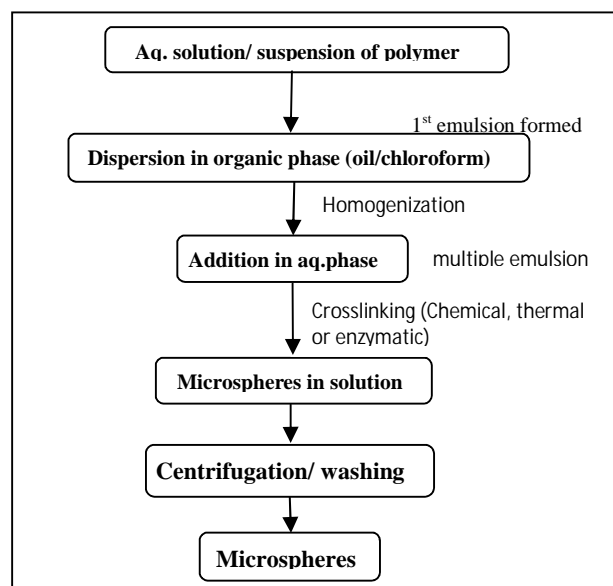


Figure 2: Double emulsion technique

2. Phase separation coacervation

This process is based on the principle of decreasing the solubility of the polymer in organic phase which in turn will affect the formation of polymer rich phase called the coacervates.

In this method, the drug particles are firstly made to be dispersed in a solution of the polymer and then an incompatible polymer is added to the system which makes polymer to phase separate and engulf the drug particles. The addition of the non-solvents results in the solidification of the polymer.

The process variables play a very important role, since the rate of formation of the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. Stirring of the suspension using a suitable speed stirrer is done in order to avoid agglomeration because it has been observed that as the process of microspheres formation begins the formed globules tend to form agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles

since there is no defined state of equilibrium attainment^{17, 29} (Fig. 3).

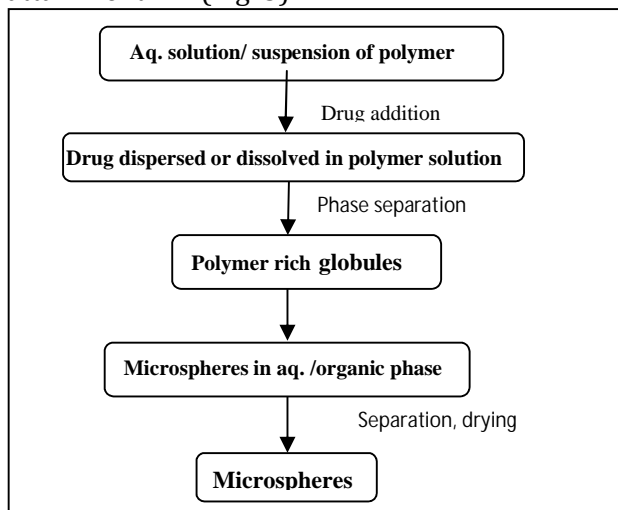


Figure 3: Phase separation coacervation

3. Spray drying and spray congealing

These 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 cooling of the solution, the two processes are named spray drying and spray congealing respectively.

In this method the polymer is firstly dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. and then the drug in the solid form is dispersed in the polymer solution under high speed homogenization. The resulting dispersion is then atomized in a stream of hot air. The atomization process leads to the formation of small droplets or fine mist, from which the solvent evaporates instantaneously leading to the formation of the microspheres in the size range 1-100 μm . The separation of micro particles is done by means of the cyclone separator whereas the traces of solvents are removed by vacuum drying. The major advantages of this process are the feasibility of the operation under aseptic conditions. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate and sulphathiazole are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous microparticles³⁰ (Fig. 4)

4. Polymerization method

a. Normal polymerization

This involves various techniques like bulk, suspension precipitation, emulsion & micellar

polymerization processes. In *bulk polymerization*, a monomer along with initiator is heated to initiate the polymerization. Initiator is then added to accelerate the rate of reaction. Drug is added during the process of polymerization. The polymer so obtained is fragmented to microspheres.

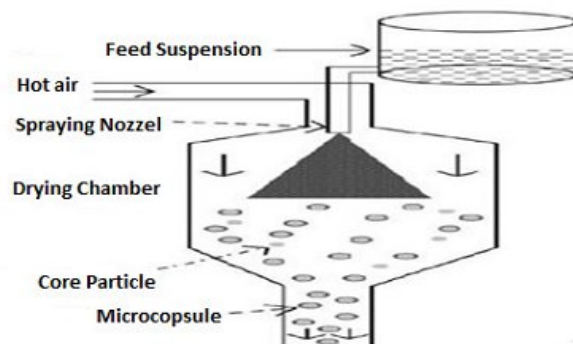


Figure 4: Spray drying and congealing

Suspension polymerization is also called as bead/pearl polymerization. It is carried out by heating the monomer or mixture of monomers with active principles (drug) as droplets of dispersion in a continuous phase. The droplets may also contain an initiator & other additives³¹. The *emulsion polymerization* differs from the suspension polymerization due to the presence of initiator in the aqueous phase, which later diffuses to the surface of the micelles or the emulsion globules. The suspension & emulsion polymerization can be carried out at lower temperature, since continuous external phase is normally water through which heat can easily dissipate^{11, 12}.

b. Interfacial polymerization

It involves the reaction of different monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelopes the dispersed phase. In this two reacting monomers are employed, one of which is dissolved in the continuous phase while the other being dispersed in the continuous phase. Monomer present in either phases diffuse rapidly & polymerize rapidly at the interface. If the polymer is soluble in the droplet it will lead to the formation of monolithic type of the carrier on the other hand if the polymer is insoluble in the monomer droplet, the formed carrier is of capsular (reservoir) type. The degree of polymerization can be controlled by the reactivity of monomer chosen, their concentration, the composition of the vehicle of either phases & by the temperature of the

system. The particle size can be controlled by controlling the droplets or globule size of the disperse phase. The polymerization reaction can be controlled by maintaining the concentration of the monomers, which can be achieved by the addition of an excess of the continuous phase³² (Fig. 5).

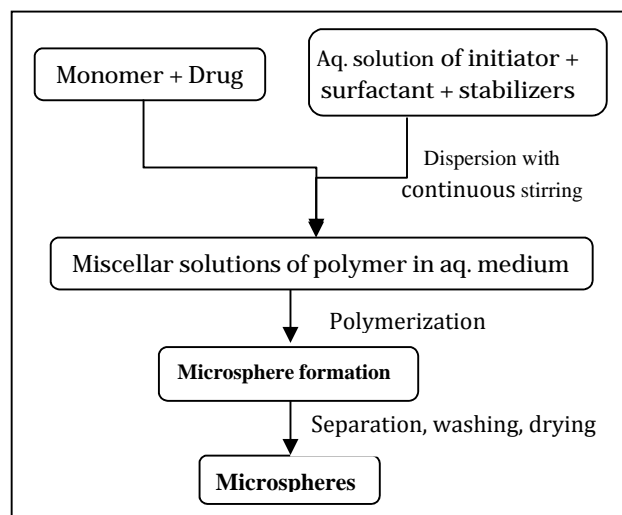


Figure 5: Polymerization Method

Characterization of albumin microspheres

The characterization of the microspheres involve the use of various evaluation techniques to determine the properties of the microspheres formed. The evaluations include the study of various aspects like average particle size, shape and morphology of microspheres, micromeritic properties of microspheres (bulk density, tapped density, hausner's ratio, Carr's index, angle of repose), drug entrapment efficiency and percent drug release.

Attenuated total reflectance Fourier Transform- 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 ATR FTIR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions

Micromeritic properties of microspheres³³

The prepared microspheres were evaluated for micromeritic properties like bulk density, tapped

density, Carr's index, Hauser's ratio and angle of repose.

Apparent bulk density

Bulk density was determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder.

$$\Delta U = M/V_U$$

Where,

M = Mass of microspheres (in gms.)

V_U = volume of microspheres (initial untapped volume)

Tapped density

Tapped density can be determined by mechanical tapping of a measuring cylinder containing the powder sample. After observing the initial volume, the cylinder is mechanically tapped, and the volume readings are taken until little further volume change is observed. The mechanical tapping is achieved by raising the cylinder and then allowing it to drop under its own weight for a specified distance.

$$b = m/v_b$$

Where,

m = mass of microspheres (in gms.)

V_b = volume of microspheres (final tapped volume)

Carr's Index

The Carr index (Carr's Compressibility Index) is an indication of the compressibility of a powder. It is named after the pharmacologist Charles Jelleff Carr (1910–2005). The Carr's Index is calculated by the formula

$$\text{Carr's Index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100$$

Hausner's ratio

The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. It is named after the engineer Henry H. Hausner (1900–1995). The Hausner ratio is calculated by the formula

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

Angle of repose

Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles. The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a

cone-like pile of material formed while falling from the cone.

$$\theta = \text{Tan}^{-1}(h/r)$$

Where, θ = Angle of Repose

h= height of the heap

r= radius of the heap formed

Particle size analysis of microspheres

The particle size analysis was done using optical microscope using calibrated ocular micrometer. The mean particle size was calculated by measuring 100 particles. The average particle size was determined using Edmondson's equation³⁴.

$$D = \frac{\sum nd}{\sum n}$$

Where,

D = average particle size (in μm)

n = number of microspheres

d = mean of the size range

Drug entrapment efficiency of microspheres³⁵

For the drug entrapment efficiency of microspheres, 50 mg of microspheres were accurately weighed and dissolved in 50 ml of methanol in a volumetric flask to get a solution containing one mg drug per ml. The resulting solution was filtered through whatman filter paper and then suitably diluted to check for the absorbance on the UV spectrophotometer. The absorbance was measured at 262 nm using UV spectrophotometer.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical weight of the drug}} \times 100$$

In vitro dissolution studies of microspheres:³⁶

The in vitro release of drug from the micro particles can be performed according to USP XXII type I dissolution apparatus at suitable pH conditions. The temperature should be maintained at $37 \pm 0.5^\circ\text{C}$ and the rotation speed of 100 rpm. Then 5 ml of sample should be withdrawn at various time intervals and replaced with an equal volume of fresh dissolution media. The samples withdrawn are then analyzed at a specific wavelength using UV spectrophotometer.

Scanning electron microscopy (SEM) of microspheres

Scanning electron microscopy has been widely used to determine the particle size distribution,

texture and the surface morphology of the microspheres.

CONCLUSION

From the researches carried out it has been concluded that, albumin is an important polymer because of the natural origin, good aqueous solubility, non-antigenicity and biodegradability. And it can be used to provide good entrapment efficiency along with the better controlled release. The in-vitro dissolution studies show that the release of the drug from the microspheres is for a longer duration of time which is greater as compared to the conventional dosage form. The results obtained in all the research work shows that the use of the hydrophilic polymer (egg albumin) sustained the release of the drug in the body which would help in the reduction of the dose for administration as well as the frequency of the administration.

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