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# BIOADHESIVE MICROSPHERES: A REVIEW ON PREPARATION AND IN-VITRO CHARACTERIZATION

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## ABSTRACT

Microspheres have been widely accepted as a means to achieve an oral and parenteral controlled release drug delivery system. Mucoadhesion is a topic of great interest in drug delivery system. Several researchers have worked on different polymers, using different methods for the preparation of microspheres. In recent years mucoadhesive microspheres have been developed for oral, buccal, rectal, ocular, vaginal routes. The present review focuses on the methods of preparation of microspheres and the in-vitro characterization details of the prepared microspheric formulations along with their applications.

**Keywords:** Microsphere, mucoadhesion, method of preparation, in-vitro evaluation.

## INTRODUCTION

Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics. However, the success of these novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with the absorbing membranes, which can be achieved by coupling bioadhesion characteristics to microspheres and developing novel delivery systems referred to as "bioadhesive microspheres". Mucoadhesion prolongs the residence time of the dosage form at the site of application and thus results in improving and enhancing the bioavailability of the drug. [1,2]

Mucoadhesive drug delivery system are delivery system utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time. Hence, uptake and consequently bioavailability of the drug is increased by reduced frequency of dosing resulting in better patient compliance. [3,4]

The term "mucoadhesion" was coined for the adhesion of the polymers with the surface of the mucosal layer. [5] Bioadhesion is a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate such as adhesion between polymer and a biological membrane in case of polymer attached to the mucin layer of mucosal tissue.[6]

The present review gives an insight on the various methods of preparation of microspheres, their in-vitro characterization as well as the applications of microencapsulation.

## METHODS OF PREPARATION

### Preparation of Microspheres by Thermal cross-linking

Citric acid, as a cross-linking agent was added to 30 ml of an aqueous acetic acid solution of chitosan (2.5% w/v) maintaining a constant molar ratio between chitosan and citric acid ($6.90 \times 10^{-3}$ mol chitosan: 1 mol citric acid). The chitosan cross-linker solution was cooled to 0°C and then added to 25 ml of corn oil previously maintained at 0°C, with stirring for 2 minutes. This emulsion was then added to 175 ml of corn oil maintained at 120°C, and cross-linking was performed in a glass beaker under vigorous stirring (1000 rpm) for 40 minutes. The microspheres obtained were filtered and then washed with diethyl ether, dried, and sieved. [7]

Microspheres of ambroxol hydrochloride were prepared by solvent evaporation technique using chitosan as a matrix-forming agent and cross-linked using formaldehyde and heat treatment. Morphological and physicochemical properties of microspheres were then investigated by scanning electron microscopy (SEM), X-ray diffractometry (XRD), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FTIR) spectroscopy. The cross-linking of chitosan takes place at the free amino group because of formation of imine bond as evidenced by FTIR. The DSC, XRD, and FTIR analysis showed that chitosan microspheres cross linked by heating were superior in properties and performance as compared to the microspheres cross-linked using formaldehyde. [8]

Rifampicin containing microspheres were designed by using a biodegradable and biocompatible polymer, gelatin B, using a thermal gelation method. The microspheres were cross-linked with natural cross-linker, sucrose, to avoid the toxicities due to the synthetic di- and poly-aldehydes. This formulation was found to be controlled release for drug in the gastro-intestinal tract. [9]

**Preparation of Microspheres by Glutaraldehyde crosslinking**

A 2.5% (w/v) chitosan solution in aqueous acetic acid was prepared. This dispersed phase was added to continuous phase (125 ml) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (w/v) Span 85 to form a water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a 3- blade propeller stirrer (Remi Equipments, Mumbai, India). A drop-by-drop solution of a measured quantity (2.5 ml each) of aqueous glutaraldehyde (25%v/v) was added at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed, first with petroleum ether (60°C-80°C) and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde, respectively. The microspheres were then finally dried in vacuum desiccators. [10]

Chitosan microspheres containing phenobarbitone were successfully prepared by glutaraldehyde cross-linking of an aqueous acetic acid dispersion of chitosan in light liquid paraffin containing sorbitan mono-oleate as a stabilizing agent. [11]

Zidovudine-Chitosan microspheres were prepared by a suspension cross-linking method. The chitosan was dissolved in 2% acetic acid solution and this solution was dispersed in the light liquid paraffin. Span-80 was used as an emulsifier and glutaraldehyde as cross-linking agent. [12]

**Preparation of microspheres by Tripolyphosphate (TPP)**

Chitosan solution of 2.5% w/v concentration was prepared. Microspheres were formed by dropping the bubble-free dispersion of chitosan through a disposable syringe (10 ml) onto a gently agitated (magnetic stirrer) 5% or 10% w/v TPP solution. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water, and then they were air dried. [2,13,14]

**Preparation of Microspheres by Ionic gelation Technique**

The alginate solutions comprising 2.5% to 4% w/v sodium alginate were prepared by initially dissolving the polymer in deionized water using gentle heat, being stirred magnetically. On complete solution, an accurately weight quantity of furosemide was added to each solution to afford homogeneous dispersions. The dispersions were sonicated for 30 min to remove any air bubbles that may have been formed during the stirring process. The sodium alginate-drug dispersions (25 ml) were added drop wise via a 20-gauge hypodermic needle fitted with a 10 ml syringe into 50 ml of 5% w/v of cross-linking agents, being stirred at 200 rpm for 10 min. The cross-linking agents were used $CaCl_2$, $Al_2(SO_4)_3$ and $BaCl_2$. The droplets from the dispersions instantaneously gelled into discrete furosemide-alginate matrices upon contact with the solution of cross-linking agents. The formed alginate microspheres were further allowed to stir in the solution of cross-linking agents for an additional 1 h. On expiration of this period the solution of cross-linking agents was decanted and the microspheres were washed with 3 × 50 ml volumes of deionized water. The microspheres were thereafter dried at 80° C for 2 h in a hot-air oven. [15]

**Preparation of Microspheres by emulsification phase separation method**

Dispersed phase consisting of 40 ml of 2% v/v aqueous acetic acid containing 2.5% w/v chitosan was added to the continuous phase consisting of hexane (250 ml) and Span 85 (0.5% w/v) to form a w/o emulsion. After 20 minutes of mechanical stirring, 15 ml of 1N sodium hydroxide solution was added at the rate of 5 ml per min at 15-minute intervals. Stirring speed of 2200 rpm was continued for 2.5 hours. The microspheres were separated by filtration and subsequently washed with petroleum ether, followed by distilled water and then air dried. [16]

**Preparation of Ethylcellulose Microspheres**

Microspheres of ethyl cellulose and salbutamol sulfate were prepared by the w/o/o double emulsion solvent diffusion technique. Weighed amount of both were dissolved in 5 ml of a mixture of acetonitrile and dichloromethane (1:1). The initial w/o emulsion was stirred at 500 rpm for 3-5 min. the w/o primary emulsion was then slowly added to light liquid paraffin containing 0.5% span 80 as oil-soluble surfactant with constant stirring for 2.5 h. Measured volume of n-hexane was added to harden the formed microspheres and the stirring was further continued for 30-60 min. the prepared microspheres were collected and washed several times with n-hexane and further dried at room temperature. [17]

The formulation and evaluation of microspheres of stavudine by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion method using ethyl cellulose and ethyl cellulose in combination with polyvinyl pyrrolidone was performed. A mixed solvent system consisting of acetonitrile and dichloromethane in an 1: 1 ratio and light liquid paraffin was chosen as primary and secondary oil phase, respectively. Span 80 was used as surfactant for stabilizing the secondary oil phase. [18]

**Spray Drying**

In spray drying 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 leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100 μm. Micro particles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of process is feasibility of operation under aseptic conditions. This process is rapid and leads to the formation of porous microparticles. [19]

**Solvent Evaporation**

The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. Drug or core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material is disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in the coating polymer solution, matrix – type microcapsules are formed. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous. The microspheres of metformin, were made by solvent evaporation method. The effect of process variables including drug/polymer ratio, stirring rate and polymer type on mean microsphere size, drug entrapment efficiency, yield, drug content, micromeritic properties and drug release was studied. [20]

**Wet Inversion Technique**

Chitosan solution in acetic acid was dropped in to an aqueous solution of counter ion sodium tripolyposphate through a nozzle. Microspheres formed were allowed to stand for 1 hr and cross linked with 5% ethylene glycol diglysidyl ether. Microspheres were then washed and freeze dried. Changing the pH of the coagulation medium could modify the pore structure of CS microspheres. [21]

**Complex Coacervation**

CS microparticles can also prepare by complex coacervation, Sodium alginate, sodium CMC and sodium polyacrylic acid can be used for complex coacervation with CS to form microspheres. These microparticles are formed by interionic interaction between oppositely charged polymers solutions and KCl and $CaCl_2$ solutions. The obtained capsules were hardened in the counter ion solution before washing and drying. [22,23]

**Hot Melt Microencapsulation**

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 µm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether.

The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. polyanhydrides. Microspheres with diameter of 1-1000 µm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed. [24]

**IN-VITRO EVALUATION OF MICROSPHERES**

**FT-IR Studies**

Drug polymer interaction studies are carried out by using Fourier Transformed Infrared (FTIR) spectrophotometer to find out any possible interaction between the drug and the polymers used in the formulations. FTIR spectra of pure drug, physical mixtures, formulations and blank microspheres were obtained in KBr pellets in the ranges, 4000- 400 $cm^{-1}$.

**Morphology and particle size**

The shape and surface characteristics were studied using scanning electron microscope (SEM). The micro particles were coated uniformly with gold-palladium by using Sputter coater (POLARON SC-76430) after fixing the sample in individual stabs. The parameters of SEM were an acceleration voltage of 20kV, a chamber pressure of 0.6 mm Hg and a magnification of X800. [25]

The size distribution in terms of average diameter of the microspheres was determined using optical microscopy method. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope fitted with an ocular micrometer and stage micrometer.[26]

**Micromeritic Properties of Microspheres**

**a. Angle of repose (θ)**

Angle of repose is the maximum angle possible between the surface of a pile of microspheres and the horizontal plane. Angle of repose of different formulation of microspheres, which measures the resistance to particle flow, was measured according to fixed funnel standing method by the following formula:

$$\theta = \tan^{-1}\frac{h}{r}$$

Where, θ is angle of repose, r is radius and h is height of the pile. The rougher and more irregular the surface of the particles, the higher will be the angle of repose. [26]

**b. Bulk density**

Bulk density is defined as the mass of microspheres divided by the bulk volume. It was measured by tapping method. [27] The bulk and tapped densities were measured by taking the sample in 10 ml of graduated cylinder contained and tapping the cylinder mechanically. Initial volume and tapped volume was noted down and bulk density and tapped density was calculated. The experiments were performed in triplicate.

***c.* Carr's Index (% C)**

Flow properties of microparticles were determined in terms of compressibility index (Ci) or Carr index value was computed according to the following equation:

$$\%\,C = \frac{Dt - Db}{Dt} \times 100$$

where, Dt is tapped and Db is bulk density, respectively.

**d. Hausner's ratio (H)**

Flow properties of microparticles were determined in terms of Hausner's ratio by comparing the tapped density to the bulk density using the following equation:

$$H = \frac{Dt}{Db}$$

**Table 1: Angle of repose, Carr's index and Hausner's ratio as an indication of powder flow properties**

| Angle of Repose (°) | Carr's index (%) | Hausner's ratio | Type of flow |
|---|---|---|---|
| > 20 | 5-15 | - | Excellent |
| 20-30 | 12-16 | < 1.25 | Good |
| 30-40 | 18-21 | - | Fair to passable |
| - | 23-35 | > 1.25 | Poor |
| - | 33-38 | 1.25-1.5 | Very poor |
| > 40 | > 40 | - | Extremely poor |

**Entrapment Efficiency, Drug loading and % Yield of microspheres**

The entrapment efficiency of the microspheres or the percent entrapment can be determined by keeping the microspheres into the buffer solution and allowing lysing. The lysate obtained is filtered or centrifuged and then subjected for determination of active constituents as per monograph required. [28] The entrapment efficiency, drug loading and % yield of microspheres were calculated by using following formulas: [20]

Entrapment efficiency (%) = (Calculated drug content/ Theoretical drug content) X 100

Drug loading (%) = (Weight of Drug/ Weight of Microspheres) X 100

Yield (%) = (Weight of Microspheres/ Total expected weight of drug and polymer) X 100

**Swelling index**

Swelling index was determined by measuring the extent of swelling of the microspheres in the given buffer. The water sorption capacity of the microspheres was determined by allowing exactly weighed amount of dry microspheres to swell in distilled water or given buffer at room temperature for at least 10 hr. The wet weight of the swollen microspheres was determined by first blotting the microspheres with filter paper to remove surface water and then weighing them immediately. The swelling index (S) of the microspheres was then calculated using following formula: [25]

$$S = (We - Wo) / Wo \times 100,$$

Where, We = weight of the gel microspheres at equilibrium swelling,

Wo = initial weight of the microspheres.

**In-vitro mucoadhesion studies**

The mucoadhesion property of the microspheres was evaluated using an in vitro adhesion test known as the wash-off method. The mucoadhesion of the prepared microspheres was evaluated employing the method described by Lehr et al. (1990) with slight modification. The mucoadhesiveness of these microspheres were compared with that of a nonbioadhesive material, ethylene vinyl acetate (EVA) microsphere. Freshly excised pieces of intestinal mucosa (2 x 2 cm.) from sheep were mounted onto glass slides (3 x 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. [29]

About 100 microspheres were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a U.S.P. tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and down-movement in the test fluid at 37º C contained in a 1 litre vessel of the machine. At the end of one hour, and at hourly intervals up to 12 hr, the machine was stopped and the number of microspheres still adhering to the tissue was counted. The test is performed at both simulated gastric fluid and simulated intestinal fluid, respectively. It is assumed that as the adhesion strength increases, the adhesion number also increases. The adhesion number ($N_a$) is defined as the ratio between the number of particles (N) remaining after the application of a certain detachment force and the number of particles ($N_o$) originally present on the test surface. The adhesion number is often expressed as a percentage. [30]

$$Na = N/N_0 \text{ X } 100$$

**In-vitro Dissolution Studies**

Only those formulations which give promising in-vitro results are further processed for in-vivo studies. The standard IP/BP/USP dissolution apparatus is used to study in-vitro release profile in the dissolution media that is similar to the fluid present at the absorption site as per monograph, using rotating basket or paddle type dissolution apparatus. [28] The time interval, composition of fluids, agitation and mesh size of the screen are the usual variants in the methods.

Quantities of microspheres equivalent to 100 mg was placed in the cylinder shaped baskets, which were covered at the sides and the bottom with nylon cloth to retain the microspheres.

The basket was rotated at 50 rpm in the fluid contained in the cylindrical containers. The rotational movement of basket was for simulating in-vivo agitation. Volume of the fluid contained was 900 ml, which was maintained throughout the period of testing to provide sink conditions. The temperature was maintained at 37±0.2ºC. 10 ml sample was withdrawn after every one hour interval and replaced with the same volume of test fluid medium. Withdrawn sample was filtered and estimated for absorbance using UV spectrophotometer. Finally, the drug content in all the fluids was estimated from the calibration curve of drug to determine the pattern of release. The dissolution studies were carried out in triplicate and mean values was plotted as percentage drug released was plotted against time interval.

**Stability Study**

The period of stability of pharmaceutical preparation is considered the time from the date of manufacture of the formulation until its chemical and biological activity is not less than 90% of the labeled potency and its physical properties have not changed appreciably. Because of the multiplicity of ingredients in most of the pharmaceutical formulations, there exists the possibility of interactions taking place, as well as each ingredient having different degradative characteristics.

The ideal situation would be to study the degradation pattern of each ingredient in the mixture individually. This is, of course, difficult to evaluate the stability of any component of a pharmaceutical preparation by determining some property of the degradation as a function of time. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions, re-test periods and shelf lives to be established.

The drug-loaded microspheres were stored at various storage conditions (room temperature, 37ºC and 45ºC/75% RH in airtight sealed vials. The drug content of the microspheres was determined spectroscopically at regular time intervals and the drug release profiles were studied at 0 and 60 days. [31]

**APPLICATIONS OF MICROSPHERES**

Some of the applications of microspheres are described as follows: [31]

1. In controlled and sustained release dosage forms.

2. Microspheres can be used to prepare enteric-coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.
3. It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microspheres.
4. The separations of incompatible substances, for example, pharmaceutical eutectics have been achieved by encapsulation. This is a case where direct contact of materials brings about liquid formation. The stability enhancement of incompatible aspirin-chlorpheniramine maleate mixture is accomplished by microencapsulating both of them before mixing.
5. Microspheres can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.
6. Microspheres have also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after microencapsulation.
7. The hygroscopic properties of many core materials may be reduced by microspheres.
8. Many drugs have been microencapsulated to reduce gastric irritation.
9. Therapeutic magnetic microspheres are used to deliver chemotherapeutic agent to liver tumor.
10. Drugs like proteins and peptides can also be targeted through this system. Mucoadhesive microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action.
11. Radioactive microspheres are used for imaging of liver, spleen, bone marrow, lung, etc. and even imaging of thrombus in deep vein thrombosis can be done.

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