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Mucoadhesive Microspheres as carriers in Drug Delivery: a Review

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Abstract

Microspheres constitute an important part of novel drug delivery system by virtue of their small size and efficient carrier capacity. Due to their short residence time, bioadhesive characteristics can be coupled to microspheres to develop mucoadhesive microspheres. Bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are held together for a prolonged time period by means of interfacial forces. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 µm range in diameter having a core of drug and entirely outer layers of polymer as coating material. Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, controlled and sustained release of drug from dosage form and specific targeting of drugs to the absorption site. The present study aims to provide an overview of various aspects of mucoadhesive microsphere based on various polymers, methodology of preparation of mucoadhesive microspheres, method of evaluation and their applications in drug delivery.

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Introduction

Drug action can be improved by developing new drug delivery system, such as the mucoadhesive microsphere drug delivery system. These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the action site leading to a bioavailability increase and both local and systemic effects [1]. The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic

circulation of body. However oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 μm range in diameter having a core of drug and entirely outer layers of polymer as coating material. However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling bioadhesion characteristics to microspheres and developing "mucoadhesive microspheres". Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site [2].

Mucoadhesion and microspheres

Mucoadhesion or bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are held together for a prolonged time period by means of interfacial forces. In biological systems, bioadhesion can be classified into 3 types.

- Type 1, adhesion between two biological phases, for example, platelet aggregation and wound healing.
- Type 2, adhesion of a biological phase to an artificial substrate, for example tissue, cell adhesion to culture dishes and biofilm formation on prosthetic devices and inserts.
- Type 3, adhesion of an artificial substance to a biological substrate, for example, adhesion of synthetic hydrogels to soft tissues

For drug delivery purpose, the term "bioadhesion" implies attachment of a drug carrier system to a specific biological location. The biological surface can be epithelial tissue or the mucus coat on the surface of a tissue. If adhesive attachment is to a mucous coat, the phenomenon is referred to as "Mucoadhesion". Mucoadhesion is defined as the interaction between a mucin surface and a synthetic or natural polymer [3]. Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations such as "microspheres" along with the active pharmaceutical ingredient (API).

Microspheres are defined as spherical particles having size less than 200μm and made up of polymer matrix in which therapeutic substance is dispersed throughout the matrix at the molecular or macroscopic level. The rationale of developing mucoadhesive microsphere drug delivery system lies behind the fact that the formulation will be 'held' on a biological surface for localized drug delivery. The API will be released close to the site of action with a consequent enhancement of bioavailability [4].

Mucoadhesive microspheres include microparticles and microcapsules (having a core of drug) of 1-1000μm in diameter and consisting either entirely of a Mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages e.g. efficient absorption and bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact with the mucous layer, specific targeting of drugs to the absorption site. Bioadhesive microspheres can be tailored to adhere to any

mucosal tissue including those found in eye, nasal cavity.

Advantages of mucoadhesive microspheres drug delivery system

(1) As a result of adhesion and intimate contact, the formulation stays longer at the delivery site improving API bioavailability using lower API concentrations for disease treatment.

(2) The use of specific bioadhesive molecules allows for possible targeting of particular sites or tissues, for example the gastrointestinal (GI) tract.

(3) Increased residence time combined with controlled API release may lead to lower administration frequency.

(4) Offers an excellent route, for the systemic delivery of drugs with high first-pass metabolism, there by offering a greater bioavailability [5].

(5) Additionally significant cost reductions may be achieved and dose-related side effects may be reduced due to API localization at the disease site [6].

(6) Better patient compliance and convenience due to less frequent drug administration.

(7) Uniform and wide distribution of drug throughout the gastrointestinal tract which improves the drug absorption.

(8) Prolonged and sustained release of drug.

(9) Maintenance of therapeutic plasma drug concentration.

(10) Better processability (improving solubility, dispersibility, flowability).

(11) Increased safety margin of high potency drugs due to better control of plasma levels.

(12) Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects [7].

(13) Drugs which are unstable in the acidic environment are destroyed by enzymatic or alkaline environment of intestine can be administered by this route e.g. buccal, sublingual, vagina [8].

Polymers used in the formulation of mucoadhesive microspheres

Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, joined by cross-linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place. Mucoadhesive polymers that adhere to the mucin–epithelial surface can be conveniently divided into three broad classes [9].

1. Polymers that become sticky when placed in water and owe their mucoadhesion to stickiness.
2. Polymers that adhere through nonspecific, noncovalent interactions that is primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
3. Polymers that bind to specific receptor site.

All three polymers types can be used for drug delivery [6].

Characteristics of an ideal mucoadhesive polymer

1. The polymer and its degradation products should be nontoxic and should be nonabsorbable from the GI tract.
2. It should be nonirritant to the mucus membrane.
3. It should preferably form a strong noncovalent bond with the mucin–epithelial cell surfaces.
4. It should adhere quickly to most tissue and should possess some site specificity.
5. It should allow easy incorporation of the drug and should offer no hindrance to its release.
6. The polymers must not decompose on storage or during the shelf life of the dosage form.
7. The cost of the polymer should not be high so that the prepared dosage form remains competitive [9].

Traditional non-specific first-generation mucoadhesive polymers

First-generation mucoadhesive polymers may be divided into three main subsets, namely:

(1) Anionic polymers

(2) Cationic polymers

(3) Non-ionic polymers.

Of these, anionic and cationic polymers have been shown to exhibit the greatest mucoadhesive strength. Consequently, such charged polymeric systems will now be examined in more depth.

1. Anionic polymers

Anionic polymers are the most widely employed mucoadhesive polymers within pharmaceutical formulation due to their high mucoadhesive functionality and low toxicity. Such polymers are characterized by the presence of carboxyl and sulphate functional groups that give rise to a net overall negative charge at pH values exceeding the pKa of the polymer. Typical examples include polyacrylic acid (PAA) and its weakly cross-linked derivatives and sodium carboxymethylcellulose (NaCMC). PAA and NaCMC possess excellent mucoadhesive characteristics due to the formation of strong hydrogen bonding interactions with mucin. Polycarbophil (Noveon) and carbomer (Carbopol), PAA derivatives have been studied extensively as mucoadhesive platforms for drug delivery to the GI tract. Polycarbophil is insoluble in aqueous media but has a high swelling capacity under neutral pH conditions, permitting high levels of entanglement within the mucus layer. Polycarbophil is also reported to increase its mass 100 times in aqueous media at neutral pH. Additionally the non-ionized carboxylic acid groups bind to the mucosal surfaces via hydrogen bonding interactions. PAA polymers are available in a wide range of molecular weights, form transparent, easily modified gel networks, are non-irritant, non-toxic and are considered safe for oral use by the FDA. Furthermore, gel formation in such platforms is well understood, occurring as a result of electrostatic repulsion between anionic groups. One clear distinction between carbomer and polycarbophil is the level of cross-linking and the cross-linking agent itself. Carbomers are cross-linked with allyl sucrose or allylpentaerythritol, whereas polycarbophil polymers are cross-linked with divinyl glycol. Both compounds have the same acrylic backbone but vary in their cross-link density that is often tailored to suit pharmaceutical and/or cosmetic performance.

2. Cationic polymers

Of the cationic polymer systems, undoubtedly chitosan is the most extensively investigated within the current scientific literature. Chitosan is a cationic polysaccharide, produced by the deacetylation of chitin, the most abundant polysaccharide in the world, next to cellulose. The intriguing properties of chitosan have been known for many years with many examples of its use in agriculture, industry and medicine. Agriculturally, chitosan has been utilized as an antipathogenic, and from an industrial standpoint investigated as a metal-recovering agent. Chitosan has been noted for its film-forming properties and has used extensively in cosmetics. Furthermore, chitosan has been employed as a dye binder for textiles, a strengthening additive in paper and as a hypolipidic material in diets. Among presently explored mucoadhesive polymers, chitosan is gaining increasing importance due to its good biocompatibility, biodegradability and due to their favourable toxicological properties. Whereas PAAs bind to mucus via hydrogen bonds chitosan has been reported to bind via ionic interactions between primary amino functional groups and the sialic acid and sulphonic acid substructures of mucus. Additionally, the hydroxyl and amino groups may interact with mucus via hydrogen bonding. The linearity of chitosan molecules also ensures sufficient chain flexibility for interpenetration. Whilst chitosan may provide improved drug delivery via a mucoadhesive mechanism, it has also been shown to

enhance drug absorption via the paracellular route through neutralization of fixed anionic sites within the tight junctions between mucosal cells. As previously discussed, chitosan is derived via the deacetylation of the naturally occurring, insoluble precursor chitin. Depending on the origin, chitin will generally become soluble in an aqueous acidic media when the degree of deacetylation exceeds 50%. This increase in solubility in an aqueous media is as a result of the protonation of the –NH2 function on the C-2 position of the D-glucosamine repeat unit. The major benefit of using chitosan within pharmaceutical applications has been the ease with which various chemical groups may be added, in particular to the C-2 position allowing for the formation of novel polymers with added functionality. Using such modifications, the properties of chitosan may be tailored to suit the requirements of specific pharmaceutical–technological challenges.

Novel second-generation mucoadhesive polymers: -

The major disadvantage in using traditional non-specific mucoadhesive systems (first generation) is that adhesion may occur at sites other than those intended. Unlike first-generation non-specific platforms, certain second-generation polymer platforms are less susceptible to mucus turnover rates, with some species binding directly to mucosal surfaces; more accurately termed "cytoadhesives". Furthermore as surface carbohydrate and protein composition at potential target sites vary regionally, more accurate drug delivery may be achievable.

1. Lectins

Lectins are naturally occurring proteins that play a fundamental role in biological recognition phenomena involving cells and proteins. For example, some bacteria use lectins to attach themselves to the cells of the host organism during infection. Enhancement of mucosal delivery may be obtained through the use of appropriate cytoadhesives that can bind to mucosal surfaces. The most widely investigated of such systems in this respect are lectins. Lectins belong to a group of structurally diverse proteins and glycoproteins that can bind reversibly to specific carbohydrate residues. After initial mucosal cell-binding, lectins can either remain on the cell surface or in the case of receptor-mediated adhesion possibly become internalized via a process of endocytosis. Such systems could offer duality of function in that lectin based platforms could not only allow targeted specific attachment but additionally offer a method of controlled drug delivery of macromolecular pharmaceuticals via active cell-mediated drug uptake. Whilst lectins offer significant advantages in comparison to first-generation platforms, it is worth noting that such polymers suffer at least in part from premature inactivation by shed off mucus. This phenomenon has been reported to be advantageous, given that the mucus layer provides an initial yet fully reversible binding site followed by distribution of lectin-mediated drug delivery systems to the cell layer. Although lectins offer significant advantages in relation to site targeting, many are toxic or immunogenic, and the effects of repeated lectin exposure are largely unknown.

2. Thiolated polymers

Thiolated polymers (thiomers) are a type of second-generation mucoadhesive derived from hydrophilic polymers such as polyacrylates, chitosan or deacetylated gellan gum. Examples re Chitosan–iminothiolane (250-fold improved mucoadhesive properties), Polyacrylic acid–cysteine (100-fold improved mucoadhesive properties), Polyacrylic acid–homocysteine (Approximately 20-fold improved mucoadhesive properties), Chitosan–thioglycolic acid (Tenfold improved mucoadhesive properties), Chitosan–thioethylamidine (Ninefold improved mucoadhesive properties) and Alginate–cysteine (Fourfold improved mucoadhesive

properties) etc. The presence of thiol groups allows the formation of covalent bonds with cysteine-rich sub domains of the mucus gel layer, leading to increased residence time and improved bioavailability. In this respect thiomers mimic the natural mechanism of secreted mucus glycoproteins that are also covalently anchored in the mucus layer by the formation of disulphide bonds. Whilst first-generation mucoadhesive platforms are facilitated via non-covalent secondary interactions, the covalent bonding mechanisms involved in second- generation systems lead to interactions that are less susceptible to changes in ionic strength and/or the pH. Moreover the presence of disulphide bonds may significantly alter the mechanism of drug release from the delivery system due to increased rigidity and cross-linking. In such platforms a diffusion-controlled drug release mechanism is more typical, whereas in first-generation polymers anomalous transport of API into bulk solution is more common [6].

Applications of microspheres

Some of the applications of microspheres are described in detail as following: -

1. Controlled and sustained release dosage forms.

2. Microsphere 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. Microsphere does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microsphere.

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. Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.

6. Microsphere has 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 microsphere.

8. Many drugs have been microencapsulated to reduce gastric irritation [10].

9. Microsphere method has also been proposed to prepare intrauterine contraceptive device.

10. Therapeutic magnetic microspheres are used to deliver chemotherapeutic agent to liver tumour. 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.

12. 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 [11].

Methods of preparation of mucoadhesive microspheres

Incorporation of solid, liquid or gases into one or more polymeric coatings can be done by micro encapsulation technique. The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co-precipitation etc. The various methods of preparations are:-

Phase separation coacervation technique

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Polylactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. 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 since as the process of microspheres formation begins the formed polymerize globules start to stick and form the 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 [10].

Emulsion cross linking method

In this method drug is dissolved in aqueous gelatin solution which is previously heated for 1 hr at 40 ^{o}C. The solution is added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35 ^{o}C, results in w/o emulsion then further stirring is done for 10 min at 15 ^{o}C Thus the produced microspheres are washed respectively three times with acetone and isopropyl alcohol which then air dried and dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 hrs for cross linking and then treated with 100mL of 10mm glyciene solution containing 0.1%w/v of tween 80 at 37 ^{o}C for 10 min to block unreacted glutaraldehyde. Examples for this technique is Gelatin A microspheres.

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. A 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 in soluble 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 comparison of mucoadhesive microspheres of hyaluronic acid, Chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelatin prepared by complex coacervation were made [2].

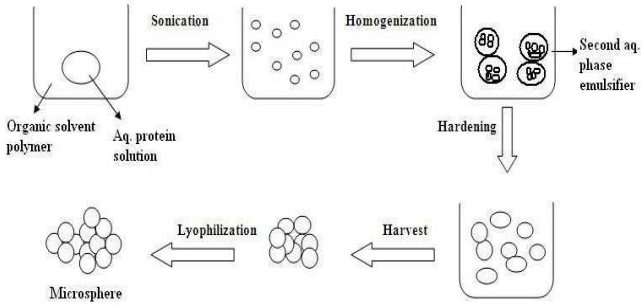

Figure 1: Solvent evaporation method for preparation of microspheres

Ionic gelation

Alginate/chitosan particulate system for diclofenac sodium release was prepared using this technique. In this method drug is added to aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it is added dropwise to a solution containing Ca2+ /Al3+. Microspheres which are formed were kept in original solution for 24 hr for internal gellification followed by filtration for separation. The complete release is obtained at pH 6.4-7.2 but the drug will not release in acidic pH [11].

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 1-100µm. 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 this leads to the formation of porous micro particles.

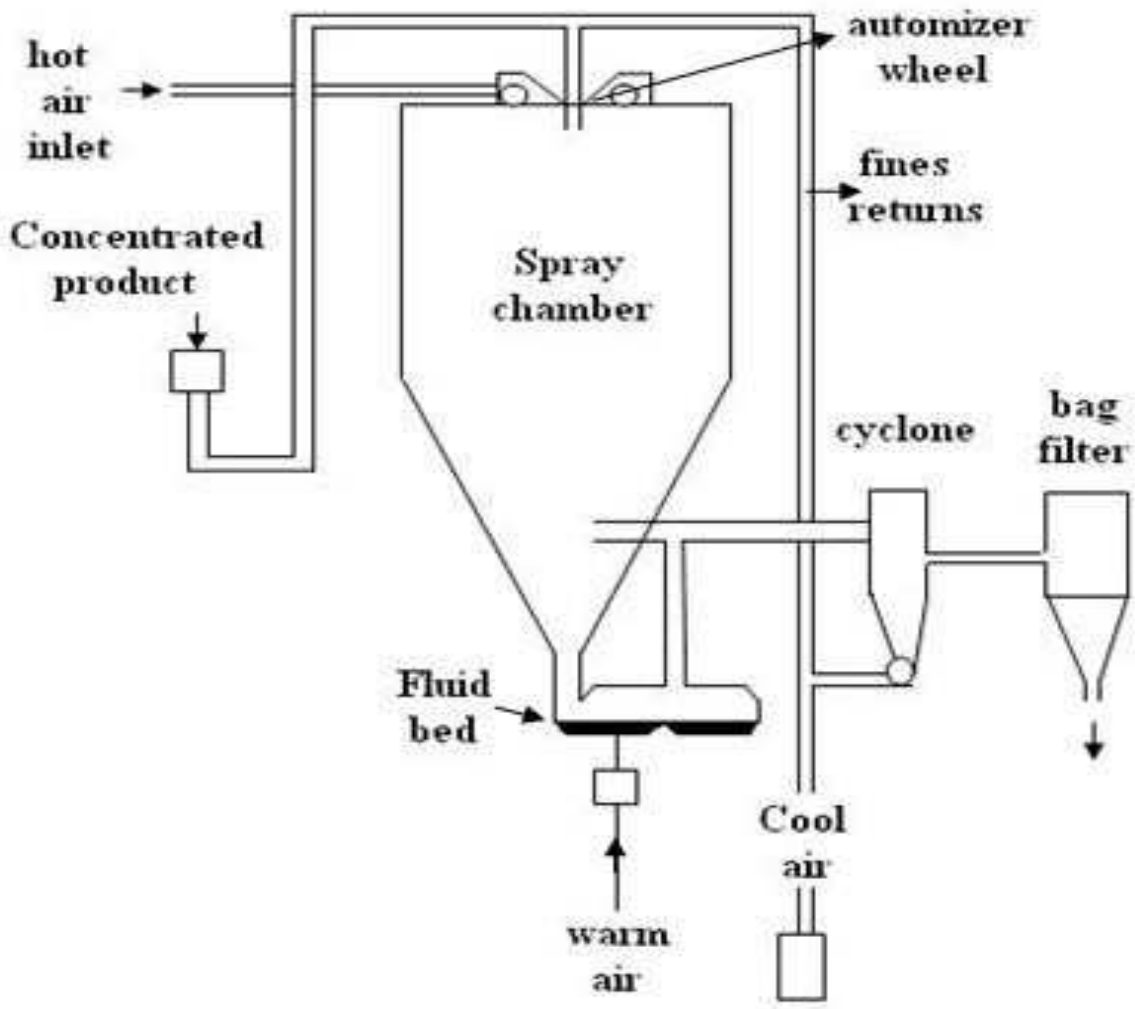

Figure 2: Spray drying method for preparation of microspheres

Multiple emulsion polymerization technique:-

Multiple emulsion method involves formation of (o/w) Primary emulsion (non aqueous drug solution in polymer solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of cross linking agent (glutaraldehyde) and evaporation of organic solvent. This method of preparation is ideal for incorporating poorly aqueous soluble drug, thus enhancing its bioavailability. The microspheres prepared by multiple emulsion technique make the poorly aqueous soluble drug such as ketorolac tromethamine more bioavailable [10]

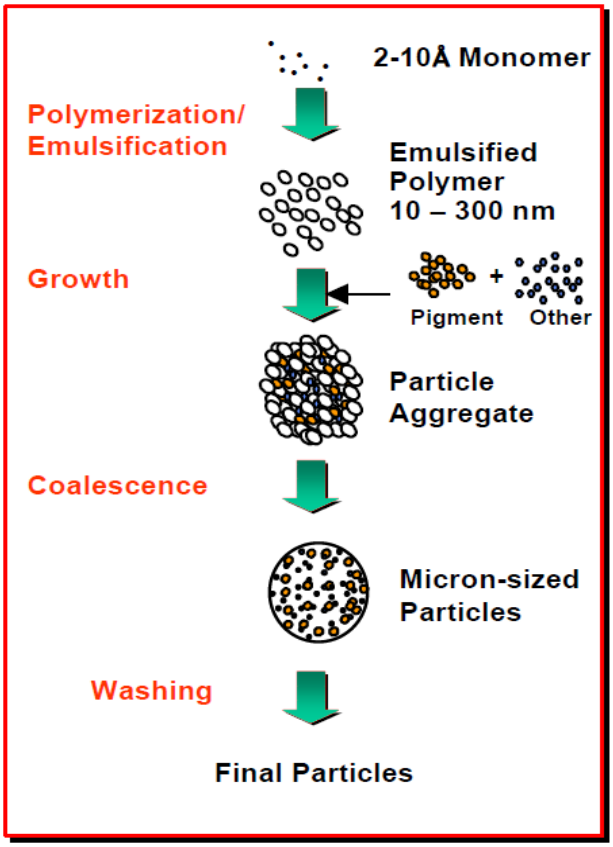

Figure 3: Microsphere preparation by multiple emulsion method.

Orifice-Ionic Gelation Method

Sodium alginate and the mucoadhesive polymer are dispersed in purified water (50 ml) to form a homogeneous polymer mixture. Drug is added to the polymer matrix and mixed thoroughly to form a smooth viscous dispersion. Resulting dispersion is then sprayed into calcium chloride (10% w/v) solution by continuous stirring. Produced droplets are retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce rigid spherical microspheres. The resulting microspheres are collected by decantation, and the product thus separated is washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then dried at 45°C for 12 hrs.

Characterization/ evaluation of mucoadhesive microspheres

1. Interaction study by TLC/ FTIR.

IR spectroscopic studies:-

The IR spectra of the free drug and the microspheres are recorded. The identical peaks corresponding to the functional groups features confirm that neither the polymer nor the method of preparation has affected the drug stability.

Thin layer chromatographic studies:-

The drug stability in the prepared microspheres can also be tested by the TLC method. The Rf values of the prepared microspheres can be compared with the Rf value of the pure drug. The values indicate the drug stability.

UV-FTTR (Fourier transform infra red):-

The drug polymer interaction and also degradation of drug while processing for microencapsulation can be determined by FTIR. In this method the pellets of drug and potassium bromide are prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra are scanned in the wave number range of 4000- 600 cm-1. FTIR study is carried on pure drug, physical mixture, formulations and empty microspheres [10].

2. Particle size distribution of prepared microspheres.

The size of the prepared microspheres can be measured by the optical microscopy method using a

calibrated stage micrometer for randomly selected samples of all the formulations.

Optical microscopy:-

This method is used to determine particle size of microspheres by using optical microscope (Meizer OPTIK) The measurement is done under 45x (10x eye piece and 45x objective) and100 particles are calculated.

3. Surface topography by Scanning Electron Microscopy (SEM).

SEM of the microspheres shows the surface morphology of the microspheres like their shape and size.

Scanning electron microscopy (SEM):-

Surface morphology of microspheres is determined by the method SEM. In this method microspheres are mounted directly on the SEM sample slub with the help of double sided sticking tape and coated with gold film under reduced pressure. Scanning Electron photomicrographs of drug-loaded microspheres are taken. A small amount of microspheres s spread on gold stub. Afterwards, the stub containing the sample is placed in the Scanning electron microscopy (SEM). A Scanning electron photomicrograph is taken at an acceleration voltage of 20KV and chamber pressure of 0.6 mm Hg [11].

4. Particle size analysis.

The particle sizes and particles size distributions are further analyzed by using dynamic light scattering technique, Microspheres are dispersed into 100 ml of water and sonicated for 1 min to remove agglomerations. The mean volume diameter (Vd) is recorded and polydispersity is determined by the SPAN factor. A high value of SPAN indicates a wide distribution in size and a high polydispersity.

5. Swelling index.

This technique is used for Characterization of sodium alginate microspheres. Different solution (100mL) are taken such as (distilled water, buffer solution of pH (1.2, 4.5, 7.4) are taken and alginate microspheres (100mg) are placed in a wire basket and kept on the above solution and swelling is allowed at 37 ^{o}C and changes in weight variation between initial weight of microspheres and weight due to swelling is measured by taking weight periodically and soaking with filter paper [11].

The swelling index of the microsphere is calculated by using the formula:-

Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 100 [2].

6. Entrapment Efficiency.

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

7. Stability studies.

By placing the microspheres in screw capped glass container and stored them at following conditions:-

1. Ambient humid condition
2. Room temperature (27+/-2 0C)
3. Oven temperature (40+/-2 0C)
4. Refrigerator (5 0C -80C).

It is carried out of a 60 days and the drug content of the microsphere is analyzed [11].

8. 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 density of the microsphere carrier is determined.

9. Bulk density.

The microspheres fabricated are weighed and transferred to a 10-ml glass graduated cylinder. The cylinder is tapped using an autotrap until the microsphere bed volume is stabilized. The bulk density is estimated by the ratio of microsphere weight to the final volume of the tapped microsphere bed.

10. 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 [10].

11. In vitro drug release studies.

In-vitro release studies can be performed according to USP XXII type 2 dissolution apparatus at suitable pH conditions. The temperature should be maintained at 37±0.5°C and the rotation speed of 100 rpm. Then 5 ml of sample should be withdrawn at various time intervals and replenished with an equal volume of fresh dissolution media. The drug content in the sample can be analyzed spectrophotometrically at specific wavelength (nm) [2].

12. In vitro mucoadhesion test.

The mucoadhesive property of the optimized microspheres prepared by different methods is evaluated by an in vitro mucoadhesion testing method known as the wash-off method. A rat stomach mucosa is tied onto the glass slide using a thread. In this method microspheres are spread onto wet rinsed tissue specimen and the prepared slide is hung onto one of the grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is switched on and the tissue specimen is given up and down movements for 2 h in the beaker of the disintegration test apparatus, which contained the stimulated gastric fluid (pH 1.2). The microspheres remaining at the surface of gastric mucosa are then collected, and the percentage of the remaining microspheres is calculated. The experiment is performed in triplicate. The percentage mucoadhesion is calculated by the following formula:

Percent mucoadhesion = (Weight of adhered microsphere /Weight of applied microspheres) ×100 [12].

13. In situ Bioadhesivity Studies.

Bioadhesivity testing is done by a novel in situ method. A freshly cut 5-6cm long piece of small intestine of rat is obtained and cleaned by washing with isotonic saline. The piece is cut open and the mucosal surface is exposed. Known weights of microspheres are added evenly on the mucosal surface. The intestinal piece is maintained at 80% (RH) relative humidity for 30mts in a desiccator. The piece is taken out and phosphate buffer pH 6 is allowed to flow over the intestinal piece for about 2 mts at a rate of 20ml/min. The perfusate is collected and dried to get the particles not adhered. The percent of bioadhesion is estimated by the ratio of amount applied to adhere micro matrices [13].

Future challenges

Future challenges of microspheres look bright particularly in the area of medicinal field because of its wide spectrum of application in molecular biology, e.g. microsphere based genotyping platform is used to detect six single nucleotide polymorphism, yittrium-90 microspheres is used to prevent tumour after liver transplantation and it's advanced way in delivery of vaccines and proteins.

Table 1: List of drugs which are given as microspheres

S. No	Drug	Category	Polymer	Reference
1	Metformin Hcl	Antidiabetic	Sodium alginate	14
2	Amoxicillin trihydrate	Antibiotic	Ethyl Cellulose	15
3	Ibuprofen	Analgesic	Sodium alginate	16
4	Pioglitazone Hcl	Antidiabetic	Carbopol 934	17
5	Trimetazidine Hcl	Antianginal	Chitosan	18
6	Furosemide	Diuretic	Sodium alginate Carbopol	19
7	Insulin	Antidiabetic	Sodium alginate, Chitosan	20
8	Furazolidine	Antiulcer	Eudragit RS100, Carbopol 974P, HPMC	21
9	Aceclofenac	Analgesic	Sodium alginate HPMC, Chitosan, Carbopol.	22
10	Acyclovir	Antiviral	Sodium alginate	23
11	Atenolol Propranolol	β Blockers	Polyacrylic acid, Polyvinyl pyrrolidine	24
12	Rantidine Hcl	Antacid	Sodium alginate	25
13	Glipizide	Oral Hypoglycemic	Chitosan	26
14	Captopril	ACE Inhibitor	Sodium alginate, HPMC, Chitosan, Carbopol 934P, Cellulose acetate phthalate	7
15	Ketoprofen	Analgesic	Sodium alginate, Chitosan, Pectin, Xanthum gum	28
16	Salbutamol sulphate	Bronchodilator	Carbopol, HPMC	29
17	Torsemide	Diuretic	Sodium alginate , HPMC	30
18	Ketorolac	Antiinflammatory and Analgesic	Eudragit RS100, Eudragit RL100	31
19	Acetazolamide	Diuretic	Eudragit RS, Eudragit RL	32
20	Metronidazole	Antiamoebic	Guargum, Sodium alginate	33
21	Famotidine	Antiulcer	Sodium CMC, Sodium alginate	34
22	Monteleukast sodium	Antiallergic	HPMC, Eudragit, Carbopol	35
23	Salbutamol sulphate	Bronchodilator	HPMC, Carbopol	36

CONCLUSION

Mucoadhesive microspheres drug delivery system have been gaining a lot of interest of various researchers and scholars, because of their advantages of controlled and sustained release action, and versatility as a drug carrier. Mucoadhesive microspheres will ensure the maintenance of effective plasma concentration over prolonged period of time by extending the release of drug. These carrier systems will also increase the residence time of the drug in the gastrointestinal tract. Mucoadhesive drug delivery is a promising area for systemic delivery of orally inefficient drugs as well as an attractive alternative for noninvasive delivery of potent peptide and perhaps protein drug molecules**.**

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