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MUCOADHESIVE MICROSPHERES: A REVIEW

Bhushan S Bhamare*[1], D. M. Shinkar[1], R.B. Saudagar[2]

1]Department of Pharmaceutics, R.G.Sapkal college of Pharmacy, Anjaneri, Nashik

2]Department of Pharmaceutical Chemistry, R.G.Sapkal college of Pharmacy, Anjaneri, Nashik

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For Correspondence:

Bhushan S Bhamare

Department of Pharmaceutics, R.G.Sapkal college of Pharmacy, Anjaneri, Nashik

E-mail:

bhushanbhamare29@gmail.com

ABSTRACT

Mucoadhesion is the concept most widely used in the novel drug delivery systems. Microspheres are one of the novel drug delivery system which possess several applications and are made up of assorted polymers. Microspheres possess potentiality to be employed for targeted and controlled release of drugs and incorporating mucoadhesive property to microspheres will furthermore improve absorption and bioavailability of the drug. The present review deals with the basic concept of mucoadhesive microspheres, polymers used,their preparation methods, applications and finally the various marketed formulations of microspheres. It also shows the therapeutic efficacy of microspheres.

INTRODUCTION

Recently the novel dosage forms which can control the release rate and target the active drug molecule to a particular site have attained a great formulation interest. Microspheres are one of the novel drug delivery system which posses several applications and are made up of assorted polymers[1] Microspheres are small spherical particles (typically 1 μm to 1000 μm), sometimes referred to as microparticles. The microspheres can be made up of either natural or synthetic polymers [2]

Generally microspheres posses' potentiality to be employed for targeted and controlled/extended release of drug, but incorporating mucoadhesive properties to microspheres will furthermore improve absorption and bioavailability of the drugs[3-6] Mucoadhesive microspheres enhance the intimate contact with the mucus layer, and drug targeting to the absorption site by anchoring bacterial adhesions [7] plant lectins[8], antibodies[9] etc. Tailored mucoadhesive microspheres offers the possibilities of localized as well as controlled release of drugs by adherence to any mucosal tissue present in eye, nasal cavity, urinary, and GI tract.

Advantages of MucoadhesiveMicrospheres:-

1. Provide constant and longer therapeutic effect.
2. Reduces the frequency of daily administration and thereby improve the patient compliance.
3. Improve the absorption of drug hence improve the bioavailability of drug and reduce the chances of adverse effects.
4. The morphology of microspheres permits a controllable variability in degradation and drug release.

5. 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.

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

7. Increased residence time combined withcontrolled API release may lead to lower administration frequency.

8. Offers an excellent route, for the systemicdelivery of drugs with high first-pass metabolism, there by offering a greaterbioavailability [10].

9.Additionally significant cost reductions may beachieved and dose-related side effects may be reduced due to API localization at the diseasesite [11].

10. Better patient compliance and convenience dueto less frequent drug administration.

11. Uniform and wide distribution of drugthroughout the gastrointestinal tract whichimproves the drug absorption.

12. Prolonged and sustained release of drug.

13. Maintenance of therapeutic plasma drug concesntration.

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

15. Increased safety margin of high potency drugs due to better control of plasma levels.

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

17. Drugs which are unstable in the acidic environment are destroyed by enzymatic oralkalineenvironment of intestine can beadministered by this route e.g. buccal,sublingual, vagina [13].

Limitation of Mucoadhesive Microspheres:-

Some of the disadvantages were found to be as follows

1. The release from the formulations may get modified.

2. The release rate may vary from a variety of factors like food and the rate of transit though gut, mucinturnover rate etc.

3. Differences in the release rate can be found from one dose to another.

4. Any loss of integrity in release pattern of the dosage form may lead to potential toxicity.

5. These kinds of dosage forms cannot be crushed or chewed.

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 beenachieved by encapsulation. This is a case where direct contact of materials brings about

liquid formation.The stability enhancement of incompatible aspirinchlorpheniraminemaleate 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 noxioussubstances. 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 [14].

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.

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 [15]

Mucoadhesion

Bioadhesion is a phenomenon in which two materials at least one of which is biological in nature are held together by means of interfacial forces. The term “mucoadhesion” define the adhesion of the polymers with the surface of the mucosal layer [16]

Mucus Membranes:-

Mucus membranes are the moist surfaces lining walls of various body cavities such as the gastrointestinal and respiratory tracts.Mucus is secreted by the goblet cells. Mucus is present either as a gel layer adherent to the mucosal surface or in suspended form or as a luminal soluble. The major components of all mucus gels are mucin glycoprotein, water, lipids, and inorganic salts. The mucus serves as a protective barrier and for lubrication also.[17]

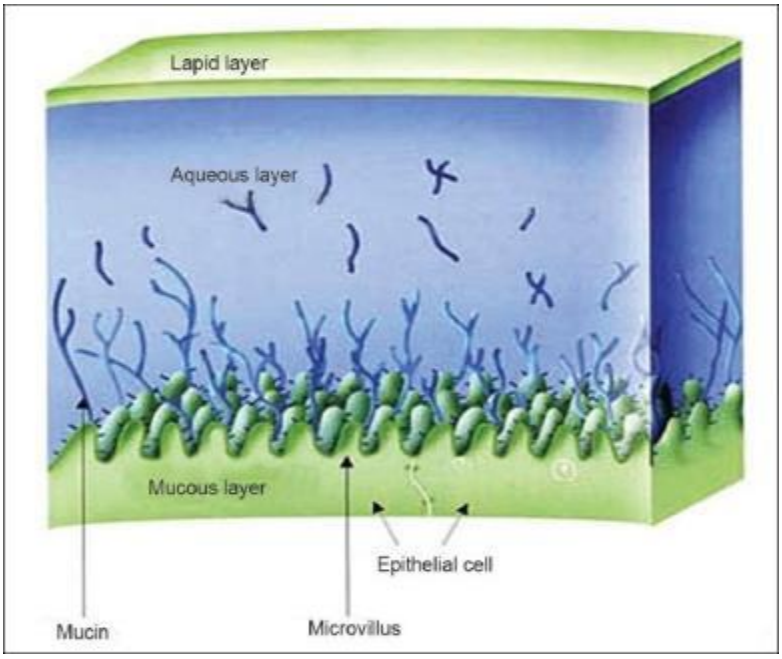

Figure 1: Structure of Mucus Membrane

MECHANISMS OF MUCOADHESION

The mechanisms responsible in the formation ofmucoadhesive bonds are not fully known, yet certain bond formation is involved as a three step process based on the interrelation between mucoadhesive theories and the material properties

Step 1: Polymer wetting and swelling (Wetting theory)

Step 2: Formation of chemical bonds between the entangled chains (Electronic and Adsorption theory)

Step 3: Interpenetration between the polymer chains and the mucosal membrane (Diffusion theory)

STEP 1

The spreading of polymer over the surface of the biological substrate or mucosal membrane causes wetting and swelling of the polymer that too results in developing an intimate contact with the substrate [18]. This swelling of polymers occurs due to its affinity for water. This can bereadily achieved for example by placing a mucoadhesiveformulation such as microspheres within the gastrointestinal tract or vagina. Mucoadhesives are able to adhere to or bond with biological tissues by the help of the surface tension and forces that exist at the site of adsorption or contact [19].

STEP 2

This step involves the formation of weak chemical bonds including primary bonds such as covalent bonds and secondary interactions such as van der Waals and hydrogen bonds

between the entangled polymer chains. Both primary and secondary bonds are exploited the manufacture of mucoadhesive formulations in which strong adhesions between polymers are formed [20].

STEP 3

The glycoprotein's, a high molecular weight polymer forms mainly the surface of mucosal membranes. This step involves the intermingling and entangling of the mucoadhesive polymer chains and the mucosal polymer chains thus forming sempermeable adhesive bonds. The strength of these bonds depends on the degree of penetration between the two polymer groups. The strong adhesive bonds can be formed if one polymer group must be soluble in the other and both polymer types must be of similar chemical structure [21].

Theories of mucoadhesion:-

There are various theories of mucoadhesion which involve different mechanisms for their adhesion. Thus on the basis of all the stated theories the process of mucoadhesion can broadly be classified into two categories: chemical (electron and absorption theory) and physical (wetting, diffusion and cohesive theory) [22-24].

1. **Electronic theory:-**

According to this theory, the difference in electronic structures of polymer and mucus causes electron transfer upon contact of adhesive polymer with a mucus glycoprotein network [25]. As a result this mucoadhesion leads to the formation of electrical doublelayer at the interface as shown in Fig. (**1**) [26]. For example,interaction between positively charged polymer (chitosan) and negatively charged mucosal surface develops the property of adhesive on hydration and provides an intimate contact between the absorbing tissueand dosage form [27].

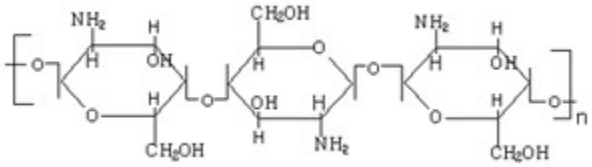

Fig. (2).Diagrammatic representation of electronic theory.

2. **Absorption theory:-**

This theory is based on the presenceof surface forces between the atoms in two surfaces [28].According to this theory, the material adheres after an initialcontact between two surfaces [29]. Two types of chemicalbonds that result in surface adhesion are the primarychemical bonds of covalent nature and secondary chemicalbonds having many

different forces of attraction, such aselectrostatic forces, Vander Walls forces, hydrogen andhydrophobic bonds [27, 30].

3. Diffusion theory:-

This theory is based on the extent ofdiffusivity of polymer chains in the mucus layer [31].According to this theory, a semi permanent adhesive bond iscreated between the polymer chains and the mucus after theirmixing to a sufficient depth as shown in Fig. (**2**) [32]. Thisproperty of penetration of polymer chain to a specific depth tothe mucus depends on the diffusion coefficient and the time ofcontact [33]. However, the diffusion coefficient in turndepends on the value of molecular weight betweencrosslinking. The diffusion coefficient decreases significantlyon increasing the cross linking density [27, 34, 35].

4. Wetting theory:-

This theory depends upon the degree ofcontact angle between the two surfaces in contact. If the contact angle of liquids on the substrate surface is lower,then there is a greater affinity for the liquid to the substratesurface. The component that needs to adhere penetratessurfaceirregularities, hardens and anchors itself to thesurface [27]. This adhesive performance of suchelastoviscous liquids is expressed in terms of wettability,spreadability and critical parameters that can be determinedfrom solid surface contact angle measurements. The mainmechanism defines the energy required to counter thesurface tension at the interface between the two surfacesallowing for a good mucoadhesive spreading and coverageof the biological substrate [37]. When such types of substratesurfaces are brought in contact with each other in thepresence of the liquid, the liquid may act as an adhesivemedium among the substrate surface as shown in Fig. (**3**)[38]. The work of adhesion (*Wa*) is denoted by eqn. (1).*Wa*= YA + YB □YAB (1)where,A& B refers to the biological membrane andmucoadhesion, respectively.

5. Cohesive theory:-

According to this theory, thephenomenon of mucoadhesion is based mainly on theintermolecular interaction amongst like molecules. The workof cohesion (*Wc*) is denoted by eqn. (2).

$$Wc = 2YA \text{ or } YB \quad (2)$$

where,A& B refers to the biological membrane andmucoadhesion, respectively.For a material B to spread on a mucosal surface A,spreading coefficient is given by eqn. (3).

$$SA/B = YA \square YB + YAB) \quad (3)$$

where,SA/B should be positive for a mucoadhesive material toadhere to a mucosal membrane [27].

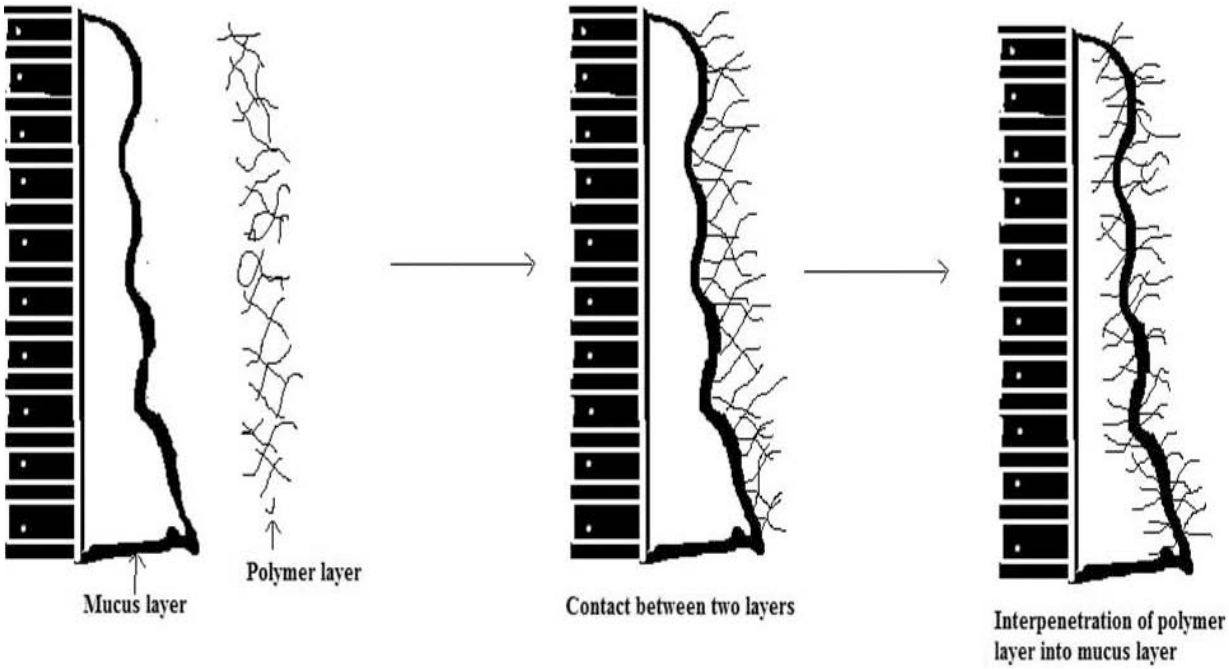

Fig. (3).Representation of diffusion theory

(a) polymer layer and mucus layer before contact;

(b) polymer layer and mucus layer immediately after contact;

(c) polymer layer and mucus layer after contact for a period of time [36]

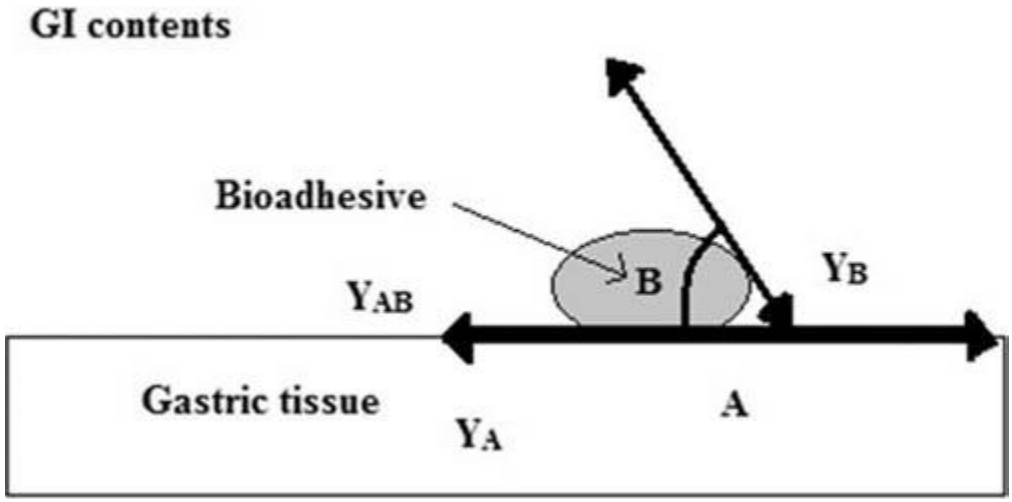

Fig. (4).A representation of the interfacial forces involved inwetting theory.

Fracture theory:

This theory is based on the difficulty of separation of two surfaces after mucoadhesion. It focuses on the ratio of force required for polymer detachment from the mucus to the strength

of their adhesive bond [39]. This theory aims for the determination of fracture strength (*G*) following the separation of two surfaces *via* its relationship to Young's modulus of elasticity (*E*), the fracture energy (ε)and the critical crack length (*L*). It is equivalent to adhesivestrength denoted by eqn. (4) [13, 40].

$$G = (E\varepsilon /L)1/2 \quad (4)$$

where,

E= Young's modulus of elasticity

ε = Fracture energy

L = Critical crack length when two surfaces are separated

Polymers used in mucoadhesive drug delivery system:-[41]

Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, jointed 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.

Hydrophilic polymers:-

The polymers within this category are soluble in water. Matrices developed with these polymers swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolytes extend greater mucoadhesive property when compared with neutral polymers (A. Ludwig, et.al., 2005).Anionic polyelectrolytes, e.g. poly (acrylic acid) and carboxymethylcellulose have been extensively used for designing mucoadhesive delivery systems due to their ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer (G.P. Andrew, et.al., 1995 and S. Rossi, et.al., 2005). Chitosan provides an excellent example of cationic polyelectrolyte, which has been extensively used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties (A. Portero, et.al., 2005). Chitosan undergoes electrostatic interactions with the negatively charged mucinchains thereby exhibiting mucoadhesive property. Structure of Chitosan is shown in Figure 2. The ionic polymers may be used to develop ionic complex with the counter-ionic drug molecules so as to have a drug delivery matrix exhibiting mucoadhesive property. Non-ionic polymers, e.g. poloxamer, hydroxypropyl methyl cellulose, methyl cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone) have also been used for mucoadhesive properties (A. Ludwig, et.al., 2005).

Hydrogels:-

Hydrogels can be defined as three-dimensionally crosslinked polymer chains which have the ability tohold water within its porous structure. The water holding capacity of the hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino and carboxyl groups. Hydrogels prepared by the condensation reaction of poly (acrylic acid) and sucrose indicated an increase in the mucoadhesiveproperty with the increase in the crosslinking density and was attributed to increase in the poly (acrylic acid) chain density per unit area (S.J. Warren, et.al., 1998). Acrylates have been used to develop mucoadhesive delivery systems which have the ability to deliver peptide bioactive agents to the upper small intestine region without any change in the bioactivity of the peptides. Wheat germ agglutinin helped in improving the intestinal residence time of the delivery system by binding with the specific carbohydrate moieties present in the intestinal mucosa.

Thiolated polymers:-

The presence of free thiol groups in the polymeric skeleton helps in the formation of disulphide bonds with that of the cysteine-rich sub-domains present in mucin which can substantially improve the mucoadhesive properties of the polymers e.g. poly (acrylic acid) and chitosan) in addition to the paracellular uptake of the bioactive agents (P.L. Soo, et.al., 2002, R. Saviaeet.al., 2003, C. Allen, et.al., 1999, C.E. Kast, et.al., 2003 and V.M. Leitner, et.al., 2003). Various thiolated polymers include chitosan–iminothiolane, poly (acrylic acid)–cysteine, poly (acrylic acid)–homocysteine, chitosan–thioglycolic acid, chitosan–thioethylamidine, alginate–cysteine, poly (methacrylic acid)–cysteine and sodium carboxymethylcellulose–cysteine (G.P. Andrew, et.al., 1995)

Lectin-based polymers:-

Lectins are proteins which have ability to reversibly bind with specific sugar carbohydrate residues and are found in both animal and plant kingdom (C.M. Lehr, et.al., 2000, E. Haltner, et.al., 1997 and J.D. Smart, 2004). The specific affinity of lectins towards sugar or carbohydrate residues provides them with specific cyto-adhesive property and is being explored to develop targeted delivery systems. Lectins extracted from legumes have been widely explored for targeted delivery systems. Various lectins which have shown specific binding to the mucosa include lectins extracted from *Ulexeuropaeus*I and *Lens culinarius*(J. Hietanen, et.al., 2007).

Ideal characteristics of an mucoadhesive polymer:-

1. The polymer and its degradation products should be nontoxic and nonabsorable from the GIT.

2. It should be nonirritant to the mucous 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 daily incorporation to the drug and offer no hindrance to its release.

6. The polymer must not decompose on storage or during the shelf life of the dosage form.

7. The cost of polymer should not be high so that the prepared dosage form remains competitive.

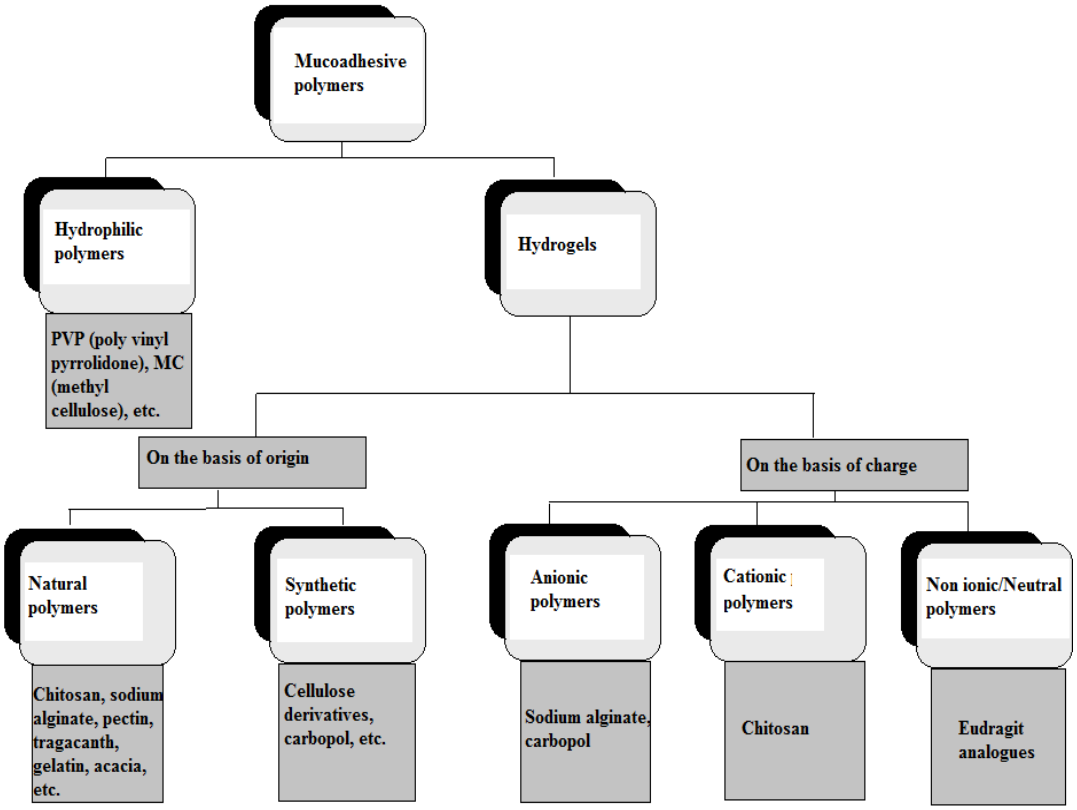

Fig. (5). Classification and examples of mucoadhesive polymers [42].

Microsphere Technology

Mucoadhesive microspheres:

Microspheres may bedefined as solid, spherical particles that range in size 1-1000µm and made of polymeric, waxy or other protectivematerials such as biodegradable synthetic polymers andmodified natural products such as polysaccharides, gums,proteins, fats and

waxes. For example, natural polymersinclude albumin and gelatin. Similarly the syntheticpolymers include polylactic acid and polyglycolic acid [43].

Microspheres are small in size and possess large surface tovolume ratio. The lower sized microspheres have colloidalproperties. The interfacial properties of microspheres areextremely important, often dictating their activity.

Microparticles are classified in two types [44, 45].

1. **Microcapsules:**The entrapped substance iscompletely surrounded by a distinct capsule wall.

2. Microspheres*:*The entrapped substance is dispersed throughout the microsphere matrix.

Mucoadhesive microspheres are the microparticles and/microcapsules ranging in size from 1-1000µm and consisteither entirely of a mucoadhesive polymer or having its outercoating, respectively and an inner core of drug.Mucoadhesive microspheres have the potential of being usedfor target specific and controlled drug delivery whichencouples the mucoadhesion properties of attachedpolymers. These mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of payer patches ingastrointestinal (GI) mucosa and this uptake mechanism hasbeen used for the delivery of high molecular weight drugs(proteins and peptides), antigens.

Methods of microsphere preparation:-

There areseveral techniques of microspheres preparation, but thechoice of these techniques mainly depends on several factorssuch as the nature of the polymer used, the drug, theintended use, and the duration of therapy as shown in Table.

These techniques of microsphere preparation and itschoice are determined by various formulation andtechnology related factors as mentioned below The physical, chemical and biological activity of theincorporated drugs should be maintained during themicroencapsulation method

The microspheres should have high encapsulationefficiency and yield enough for mass production.

The microspheres should possess the reasonable sizerange for the oral and parenteral administration. Itshould not be longer than 180 µm for parenteraladministration. Particles greater than 7 µmgetentrapped in the capillary. Particles coated with apolymer (poloxamer) and of size 60-150 nm are takerup to a considerable extent by the bone marrow.Similarly, particles of size larger than 250 nm can beused for spleen targeting.The release profile of the drug should be reproduciblewithout significant initial burst.

The technique employed for microspheres productionshould produce free-flowing microparticles effective for uniform suspension of microparticle.

The process should not adversely affect the stabilityof the drug.

No toxic reaction or product should be produced withthe final product.

Spray Drying (An anhydrous technique):-

Spray dryingis another method for preparation of microspheres. Itinvolves the use of volatile organic solvents such asdichloromethane, acetone, etc in which polymer is firstdissolved [46] as shown in Fig. (**5**). Then the solid drug isslowly dispersed in this polymer solution along withcontinuous stirring at high speed homogenization. After ahomogenous mixture is obtained, this dispersion is atomizedin a stream of hot air and the process is known asatomization [47]. Small droplets or the fine mist of drugpolymer solutions form after evaporation of volatile solventinstantaneously that leads to the formation of themicrospheres in a size range 1-1000 μm. These microparticles are then separated by means of the cycloneseparator from the hot air and the traces of the left oversolvent is removed by vacuum drying [48]. Spray dryingmethod has the major advantage of being rapid, feasibleunder aseptic conditions and leads to the formation of porousmicro particles. The spray drying obtained microspheres canbe improved in quality by the addition of plasticizers such ascitric acid, which promote polymer coalescence on the drugparticles and hence help in the formation of spherical andsmooth surfaced microspheres [49]. Further, the rate spraying, the feed rate of polymer drug solution, nozzle size,and the drying temperature affects the size of microspheres.This method of microencapsulation is however simple,reproducible, easy to scale up and independent of thesolubility characteristics of the drug and polymer [50].

Solvent Evaporation:-

Solvent evaporation method isagain similar to spray drying involving the use of volatileorganic solvent. This process is the most extensively used formicroencapsulation and carried out in a liquid manufacturingvehicle [51]. Here the process consists of two phases: first isthe buffered or plain aqueous solution phase of the drug withor without a viscosity building or stabilizing agent andsecond is the organic phase consisting of polymer solution involatile solvents like dichloromethane (or ethyl acetate orchloroform). This polymer solution dispersed in a volatilesolvent is immiscible with the liquid manufacturing vehiclephase. The core material that needs to be microencapsulatedis first dispersed in the liquid manufacturing vehicle phasewith vigorous stirring to form the primary water in oilemulsion. The emulsion mixture is then either added to alarge volume of water containing

an emulsifier like PVA(polyvinyl alcohol) or PVP (poly vinyl pyrrolidone) to formthe multiple emulsions (w/o/w) [52]

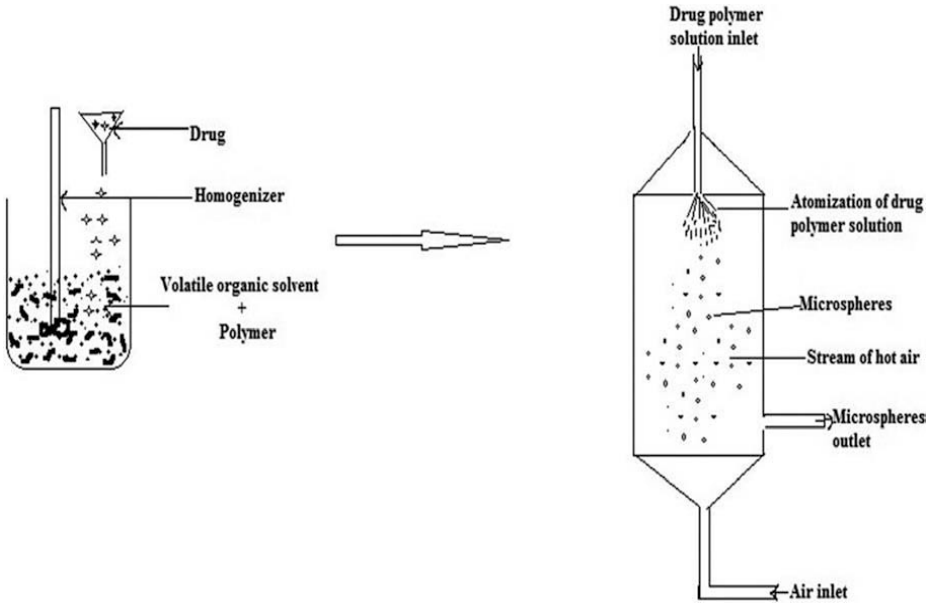

Fig. (6). Diagrammatic representation of spray drying method

.The double emulsionmixture is heated if necessary to evaporate the volatilesolvent under continuous stirring. The polymer shrinksaround the core material that may be either water soluble orwater insoluble materials [53].After a particular time whenwhole of the solvent evaporates the core materials getencapsulated by the polymer solution leaving solidmicrospheres. These microspheres can then be washed,centrifuged and lyophilized to obtain the free flowing anddried microspheres of appropriate size [54].

Hot Melt Microencapsulation:-

In this the polymer isfirst melted and then mixed with drug molecules that alreadyhave been sieed to a particular size. Then this mixture issuspended in a non-miscible solvent like silicone oil withcontinuous stirring, and heating at 5°C above the meltingpoint of the polymer [55]. After the emulsion gets stabilized,it is cooled to solidify polymer particles. The resultingmicrospheres obtained range in diameter from 1-1000 µm,are then washed by decantation with petroleum ether asrepresented in Fig. (**6**). This method is suitable formicroencapsulation of water labile polymers, *e.g*polyanhydrides. The only disadvantage of this method ismoderate temperature to which the drug is exposed [56].

Single emulsion technique:

In this method a dispersionor solution of natural polymers is prepared in aqueousmedium. This mixture is then dispersed in the non-aqueousmedium such as oil followed by cross linking of dispersedglobules either by means of heat or chemical cross linkingagents as shown in Fig. (7) [57].

Based on the type of crosslinking the method is classified as:

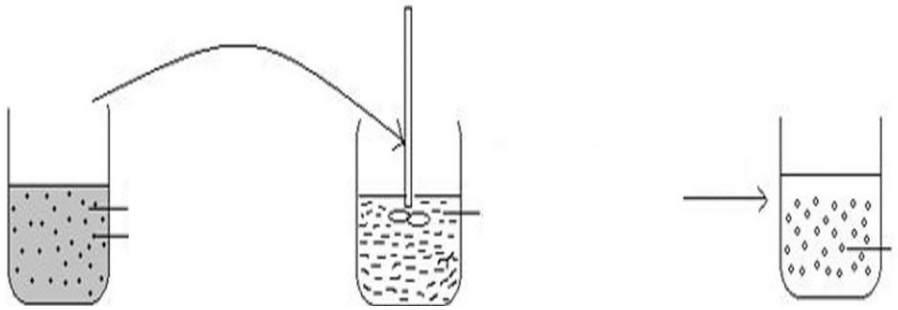

Fig. (7). Diagrammatic representation of hot melt microencapsulation method.

Thermal cross-linking method:

In this method crossmlinking is done by adding dispersion to previously heated oilunder continuous stirring to obtain microspheres of specificsize range. However this method is suitable for thermolabiledrugs as heat denaturation of drug occurs [58].

Cross linking agent method:

This method involves theuse of certain cross linkers such as glutaraldehyde,formaldehyde, di-acid chloride, etc. for microsphere preparation. This technique suffers from excessive exposureof active ingredients to chemicals if added at the preparationtime. First, a specific concentration solution of polymer inaqueous medium is prepared which is then added undercontinuous stirring to the continuous phase consisting of oiland surfactant to form water in oil (w/o) emulsion. Then adrop-by-drop solution of a measured quantity of aqueouscross linkers is added at specific time intervals to allowuniform mixing. Stirring was continued for a particular time until microspheres of specific size range are obtained which are then separated using a washing organic solvent [59].

Double emulsion method (A hydrous technique):-

Thismethod involves the formation of multiple emulsions ordouble emulsion of type water in oil in water (w/o/w) and isbest suited for water soluble drugs. It involves both naturalas well as synthetic polymers in formulation. First anaqueous drug polymer solution is dispersed in a lipophilicorganic continuous phase under vigorous stirring to form ahomogeneous mixture [60]. The continuous phase consists ofpolymer solution that eventually encapsulates the

drugpresent in dispersed aqueous phase as shown in Fig. (**8**). Thisprimary emulsion is then subjected to sonication beforeaddition to aqueous solution of polyvinyl alcohol (PVA) thatresults in the formation of double emulsion [61]. The laterdouble emulsion formed is subjected to solvent evaporationor solvent extraction process by maintaining emulsion atreduced pressure or stirring so that volatile organic phaseevaporates out. The emulsion is added to the large amount ofwater (with or without surfactant) into organic phase diffuseout and the solidmicrospheres are separated out by filtrationand washing [62]

Polymerization techniques:-This technique is furtherclassified into two types:

- Normal polymerization method
- Interfacial polymerization method

Normal polymerization method: -

This method usesdifferent techniques such as bulk, suspension precipitation,emulsion and miceller polymerization process.

Bulk polymerization:-

A monomer or a mixture ofmonomer containing an initiator is first heated toinitiate the polymerization reaction and carry out theprocess. The initiator or catalyst facilitates oraccelerates the rate of reaction. The polymer thusobtained is molded or fragmented as microspheres.Drug must be either adsorbed or added during theprocess of polymerization for better encapsulationefficiency. This method leads to the formation of purepolymer but suffers a difficulty in dissipating heat ofreaction which adversely affects the thermolabileactive ingredients [63].

Suspension polymerization:-

Also known as bead orpearl polymerization, this method involves theheating of monomer or mixture of monomers alongwith drugs as droplets dispersion in a continuousaqueous phase containing an initiator and otheradditives. This method can be carried out at lowtemperature since continuous external phase isnormally water through which heat can easilydissipate [64]. Suspension polymerization leads to theformation of high molecular weight polymer atrelatively faster rate. However it leads to theassociation of polymer with unreacted monomer andother additives thus creating a major disadvantage[65].

Emulsion polymerization:-

This method is similar tosuspension polymerization but differs in one step. Inthis method, the initiator present in the aqueous phaselater on diffuse to the surface of the micelles oremulsion globules [66].

Interfacial polymerization method:-

As the termdenotes, it involves reaction of monomers at the interface between the two immiscible liquid phases to form a polymerfilm enveloping the dispersed phase. Two reactionmonomers employed involve one which is soluble incontinuous phase and the other being dispersed in continuousphase. The continuous phase is generally aqueous in nature throughout which the second monomer is emulsified.Monomers in either phase diffuse and polymerize rapidly atthe interface [67].

Coacervation method:-

This technique is simple andbroadly applicable. The aqueous solution of drugs to beencapsulated is dispersed in a water immiscible solventcontaining the dissolved polymer. The polymer layer getsdeposited on the surface of the aqueous droplets onsubsequent evaporation of the volatile solvent [68].

Solvent Removal:-

Also known as non-aqueous methodof microencapsulation, it is particularly suitable for waterlabile polymers such as the polyanhydrides. In this method, adispersion or solution of drug is made in a selected polymerin a volatile organic solvent like methylene chloride. Thismixture is then suspended in oil phase containing surfactantand volatile organic solvent. Once the polymer solution ispoured into oil phase, petroleum ether is added and stirreduntil solvent is extracted into the oil solution. This results inthe formation of microparticles which are then dried invacuum [69].

Phase Inversion Microencapsulation:-

This is anothermethod of preparation of microspheres which involves theaddition of drug to a diluted polymeric solution (usually 1-5% w/v in methylene chloride) [69]. This mixture is then poured into an unstirred bath of strong non-solvent(petroleum ether) in a solvent to non-solvent ratio of 1:100,resulting in the spontaneous production of microspheres ofspecific size range which can then be filtered, washed withpetroleum ether and dried with air. It is the simplest and fastprocess of microencapsulation involving loss of drug, polymer [71].

Wet Inversion Technique:-

In this method a polymericsolution in acetic acid is added dropwise into an aqueoussolution of counter ion such as sodium tripolyphosphate through a small sized nozzle.Microspheres are formedwhich are allowed to stand undisturbed for some time andthen cross linked with cross linking agents such as 5%ethylene glycol diglysidyl ether. Microspheres obtained arethen washed and freeze dried [72].

Table 1.Various techniques for microspheres preparation [73-78].

Process	**Coating polymers/ Materials**	**Suspending Medium**	**Advantages**	**Disadvantages**
Spray-drying	Hydrophilic or hydrophobic polymers	Air, nitrogen	Rapid, reproducible and easy to scaleup, feasibility of process under asepticconditions, suitable for both batch andbulk manufacturing	Chances of crystallinity lose by polymers onfast drying, temperature-sensitive compoundsare degraded and control of the particle sizeis difficult
Solvent Evaporation	Hydrophilic or hydrophobic polymers	Organic/ aqueous	Suitable for thermolabile and hydrophobic compounds.	Not suitable for high hydrophilic drugs sincethe drug may not be dissolved in the organicsolvent and/or may diffuse into thecontinuous phase during emulsification,leading to a great loss of drug.
Hot melt microencapsulation	Hydrophilic or hydrophobic polymers	Aqueous/ organic	Reproducible with respect to yield and size distribution, also suitable for the water labile polymers, *e.g.* poly anhydrides.	Not suitable for thermolabile substances
Single emulsion Technique	Hydrophilic or hydrophobic polymers	Aqueous/ organic	Simple and easy	Excessive exposure of active ingredient tochemicals which degrades them
Double emulsion Technique	Hydrophilic or hydrophobic polymers	Aqueous/ organic	Suitable for aqueous soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural and synthetic polymers.	The particle formation process is quitecomplicated and influenced by a host ofprocess parameters.
Polymerization Technique	Hydrophilic or hydrophobic monomers	Aqueous/ organic	Highly spherical polymer microspheres can be obtained with	

			monodispersity in size and high drug encapsulation efficiency	
Coacervation Method	Hydrophobic polymers or hydrophilic polyelectrolytes	Organic/ aqueous	Simple and utilizes aqueous system for the preparation	
Solvent removal Method	Hydrophilic or hydrophobic polymers	Organic/ aqueous	Suitable for temperature sensitive compounds as there is no use ofelevated temperature	Organic
Phase inversion method/wet inversion method	Hydrophilic or hydrophobic polymers	Aqueous/ organic	Simple and fast process, involves relatively little loss of polymer and drug	

Table 2. List of some marketed microsphere formulations.

Commercial Name	**Drug**	**Company**	**Method of Preparation**	**Indications**	**Polymer**	**Delivery Route**
Vivitrol®	Naltrexone	Alkermes	Double emulsion (oil in water)	Alcohol dependence		i.m.
RISPERDAL® CONSTA®	Risperidone	Janssen®/ Alkermes, Inc	Double emulsion (oil in water	Schizophrenia; bipolar I disorder	PLGA	i.m.
Sandostatin® LAR	Octreotide	Novartis	Phase separation	Acromegaly	PLGA-glucose	i.v. or s.c.
Lupron Depot® Enantone Depot® Trenantone® EnantoneGyn	Leuprolide	TAP Takeda Takeda Takeda	Double emulsion (water in oil in water)	Prostate cancer/ endometrios	PLGA or PLA	i.m.
Nutropin®	Somatropin	Genentech/Alk	Cryogenic	Growth	PLGA	s.c.

Depot		ermes	spray-drying	deficiencies		
Somatuline® LA	Lanreotide	Ipsen-Beafour	Phase separation	Acromegaly	PLGA	i.m.
Suprecur® MP	Buserelin	Sanofi-Aventis		Endometriosis	PLGA	i.m.
Trelstar™ depot Decapeptyl® SR	Triptorelin	Pfizer Ferring	Phase separation	Prostate cancer	PLGA PLGA or PLA	i.m.
Parlodel LAR™	Bromocriptine	Novartis	Spray drying	Parkinsonism	PLGA-Glu	s.c. or i.m.
Arestin®	Minocycline	Orapharma		Periodontitis	PLGA	Subgingival
Zoladex®	Goserelin acetate	I.C.I.		Prostate cancer		s.c.
Posilac®	Recombinant bovine somatropin	Monsanto		To increase milk production in cattle		i.m. or s.c.

PLGA - Poly (lactide-co-glycolide), PLA - Polylactide, PLGA - Glu- Poly (D,L-lactide-co-glycolide-D-glucose), i.m. -intramuscular, s.c.- subcutaneous, i.v.- intravenous

Characterization/ evaluation of mucoadhesive microspheres:-[79]

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 tothe 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 acalibrated 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 (MeizerOPTIK) 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 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 theabove solution and swelling is allowed at 37 0C andchanges in weight variation between initial weight ofmicrospheres and weight due to swelling is measuredby taking weight periodically and soaking with filterpaper [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 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 tabletdisintegrating test apparatus. The disintegrating testapparatus is switched on and the tissue specimen isgiven up and down movements for 2 h in the beakerof the disintegration test apparatus, which containedthe stimulated gastric fluid (pH 1.2). Themicrospheres remaining at the surface of gastricmucosa are then collected, and the percentage of theremaining microspheres is calculated. The experiment is performed in triplicate. The percentagemucoadhesion 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 to detect six polymorphism, yittrium-90 microspheres is used to prevent tumourafter liver transplantation and it’s advanced way in delivery of vaccines and proteins.

CONCLUSION

Mucoadhesive microspheres serve as a unique carrier system for many pharmaceuticals that can be tailored to adhere to any mucosal tissue in the body. They can be used not only for sustained release but also for targeted delivery of the drugs to specific sites in the body. Though newadvancements in novel medicine have envisaged the development of mucoadhesive polymeric drug delivery system for various drugs, yet there are many challenges still to be explored ahead in this field. Apart from this, efforts have been initiated on the introduction of various new techniques for microsphere formulations that have also attracted researchers to develop microspheres of more efficiency, good mucoadhesive strength and required drug release. The time needs more research in polymeric science to find new mucoadhesive polymers with the added attributes of being biodegradable, biocompatible, mucoadhesive for specific cells or mucosa and which could be modified in a release pattern successful enough for the required delivery of various drugs. Hence, a multidisciplinary approach shall therefore be adopted to overcome the various challenges and to employ mucoadhesive microspheres as a cutting edge technology for targeted sustained release drug delivery of newer as well as existing.

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