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MUCOADHESIVE MICROSPHERES- A VIRTOUS BIOAVAILABILITY EMBELISHING TOOL

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ABSTRACT

Mucoadhesive drug delivery has versatile potential for efficient drug release because of the properties provided by their small particle size for various diseases. Ex., *Helicobacter pylori* infection is currently the cause of 75% peptic ulcers. In an effort to augment the anti *Helicobacter pylori* effect, microspheres reside in the gastrointestinal tract with mucoadhesion mechanism and exhibits sustained release effect over a period of time. Microspheres are the carrier linked drug delivery system in which particle size ranges from 1-1000 µm in diameter having a core of drug and entirely outer layers of polymer as coating material. Due to their short residence time, bioadhesive characteristics can be coupled to microspheres to develop mucoadhesive microspheres. The polymers with excellent mucoadhesive properties and good entrapment efficiency. This review article focusses various aspects of mucoadhesion, theories of mucoadhesion preparation methods and applications along with its future trends.

Keywords: Mucoadhesion, Microspheres, Helicobacter *pylori.*

INTRODUCTION

Controlled release or targeted release is more reliable than traditional drug delivery systems. Delivery by using carrier technology is an effective way of achieving this approach in which the drug is coupled with carrier particles such as microsphere, nanoparticles and liposomes.[1] In the drug delivery system, Mucoadhesion is of current interest and challenging endeavour by the pharmaceutical scientists. The term mucoadhesion means polymer adhesion to the mucosal surface layer. The major advantage being that the drug absorbed through mucosal lining of tissues enter in to blood stream directly will not be degraded by enzymes in the gastrointestinal tract.[2] Microspheres are carrier linked drug delivery system which has particle size between 1-1000 µm with core of drug surrounded with polymer layers as coating material.[3] The process by which a natural or synthetic polymer adhere to biological susbstrate is called Bioadhesion. If mucosal layer acts as biological substrate, this phenomenon is known as mucoadhesion.

Advantages of Mucoadhesive drug delivery system

- Residence time of drug gets prolonged at drug absorption site
- Strong contact with mucous layer
- Localisation of drug at given target site
- Reduces drug frequency and dose
- Increased drug bioavailability
- Improved patient compliance.[4]

Microspheres are used for both oral and parenteral controlled release of drugs. In mucoadhesive microspheres, physical entrapment of drugs in microsphere pores occurs or it may by chemical conjugation to polymer matrix. Interfacial forces held drug together with mucosal layer for prolonged period of time. Due to polymer interaction with mucosa a prolonged contact time is achieved for sustained drug action.[5]

THEORIES OF MUCOADHESION

Wetting theory: This theory explains the importance of adhesion mechanism. The adhesive agents which

penetrate in to liquid surface substrate act as adhesive by lowering the contact angle of liquid on the surface of substrate. [6]

Electronic theory: This theory explains the phenomenon of electron transfer process occurs across the adhesive surface and adhering surface which result in electrical double layer formation. Attractive forces play a prominent role to establish contact between the two layers.[7] This theory explains that mucoadhesion occurs due to diffusion coefficient. An adhesive bond of semipermanent nature is formed due to the integrity mixing of polymer chains and mucosal layer. The penetration extent depends on two important factors like diffusion coefficient and contact time.[8]

Adsorption theory: This theory explains the phenomenon of surface force acting between the atoms in two surfaces. It also states that material adherence is due to net result of hydrogen and hydrophobic bonding nature.[9]

Fracture theory: This theory stress the impact of force on surface after adhesion. To separate the two forces a strong force is needed in which fracture strength is equivalent to adhesive strength.

DRUG RELEASE MECHANISM IN MUCODHESIVE MICROSPHERES

- Pore diffusion mechanism
- By osmotically driven burst mechanism
- Erosion or degradation of polymers[10]

Polymers in Mucoadhesive drug delivery: Mucoadhesive polymers are water soluble or water insoluble polymers with swellable network joined by crosslinking agents.

Ideal characteristics of Mucoadhesive polymer

- Non irritant to mucus membrane
- The polymer and its degradation products should be nontoxic and nonabsorbable to GIT
- Capability of adhering quickly to most tissues and has site specific action
- Should form a noncovalent bond with mucin – epithelial surface.
- Non-degradation of polymer during storage condition [11].

Mechanism of action of mucoadhesive microspheres: Mucus layer covering the mucosal epithelium surface and mucin molecules has great affinity and it interacts with mucoadhesive microspheres at the absorption site. It releases the the drug loaded in sustained action and hence mucoadhesive microspheres has lucrative attention by the pharmaceutical scientists[13-15]

The mechanism of Mucoadhesion exists in three different steps:

Step 1: Strong affinity and interaction between the polymer of mucoadhesive and the mucus membrane

Step 2 : Interpenetration of mucoadhesive linkage chain with that of mucus membrane.

Step 3 : Existence of strong chemical bond between polymer chain and molecules of mucin.[16]

PREPARATION OF MUCOADHESIVE MICROSPHERES

Incorporation of solid, liquid or gases in to 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 the above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co-precipitation etc.

- Preparation of mucoadhesive microspheres should satisfy the following criteria
- It should have potential ability to incorporate reasonably high concentration of drug
- Stability of the preparation after synthesis with a clinically acceptable shelf life
- Controlled particle size and dispersibility in aqueous vehicles for injection
- Drug release with good control over time scale
- Biocompatibility with polymers

Various methods employed for the preparation of Mucoadhesive microspheres are as follows:

1. Phase inversion method
2. Solvent removal technique
3. Spray drying
4. Complex coacervation
5. Hot melt microencapsulation
6. Hydrogel methods
7. Solvent evaporation

In the preparation of Microspheres following factors are to be considered such as drug particle size, release profile of the drug, stability condition and its relevant methods and final product should be toxic free[17-19]

Phase inversion method: Drug is added to polymer mixture slowly in dilute concentration. It is then stirred slowly and pured in to methylene chloride

solution.This mixture is added to solvent-nonsolvent ratio 1:100 slowly. The obtained microspherses washed with petroleum ether and air dried suitably.

Advantages

- Drug and polymer loss is minimum
- Microcarrier in size 0.5-5.0 µm can be obtained.[20]

Solvent removal technique: It is a versatile non-aqueous technique of preparation of microspheres. This technique comprises of drug is dissolved suitably in a polymeric solution and in volatile oraganic solvent. The resultant mixture is suspended in silicone oil containing span 85,Finally, petroleum ether was added till all the solvent was completely extracted in to the oil solution. The microspheres are dried by vacuum drying

Advantages

Since this is non-aqueous method it is suitable for water liable polymers[21]

Spray drying: In this method drug is dissolved or dispersed in polymer solution which was spray dried. A liitle quantity of plasticizer was added to enhance the coalescence of polymer. The major factors to be considered are spraying rate, feed rate of polymer drug solution, nozzle size and the drying temperature. Microcarrier size can be controlled.

Advantages

- Easily scale up technique
- This process is independent of solubility of drug and the polymer[22]

Complex coacervation: This method employs the phase separation of a liquid precipitate or phase when solutions of two hydrophilic colloids were mixed under appropriate conditions. This process takes place in two steps. In first step the core material is dispersed in the solution of coating polymer in the liquid manufacturing vehicle phase.

The second step the coating material phase is prepared by dissolving the immiscible polymer in a suitable vehicle under the influence of strong stirring, mixing of coating material phase and core material phase were done. The process of microencapsulation can be achieved either by changing the polymer solution temperature or by adding a salt or by intiating polymer-polymer interaction. The coating obtained finally was strengthened by thermal, cross linking or desolvation methods. This will leads to the formation of microcarrier with very good sustaining drug release capacity.

Advantages

Microspheres formed by this method have very good stability[23]

Hot melt microencapsulation: This process of mucoadhesive microspheres preparation comprises of good polymer selection compatible with drug. The polymer is first melted carefully and dispersion of solid particles of drug slowly in to it. The particle size of drug should be less than 50 µm. The resultant mixture was suspended in immiscible solvent with vigorous stirring. The solution was heated well to get a stable emulsion. It is then cooled to solidify polymer particles and decantation done. The resulting microspheres were washed with petroleum ether.

Advantages

Stable microspheres are prepared by this method[24]

Hydrogel method: This method ensures prominent suitability to encapsulate living cells by avoiding residual solvents in microcarriers. It involves by dissolving gel type polymers such as alginate in aqueous solution then to the solution active ingredient is added slowly. This is extruded through a precision device, results in microdroplet formation. The stirring should be done relatively slow speed.

Advantage

Avoidance of residual solvent [25]

Solvent evaporation method: This process involves the formation of an emulsion between polymer solution and an immiscible continuous phase either it may be aqueous or non-aqueous nature. The core material used may be water soluble or water insoluble in nature. In the first step, a core material to be microencapsulated is dissolved or dispersed in coating polymers solution.

The next step comprises of the coating of microcapsule is dispersed in a volatile solvent which is immiscible with liquid manufacturing vehicle phase. Due to the strong agitation, the core material mixture is dispersed in liquid manufacturing vehicle phase, thereby forming appropriate size microcapsule. In case of necessary the mixture may be heated for the evaporation of the solvent for the polymer of the core material is dispersed in the polymer solution results in the shrinkage of polymer around the core [26]

EVALUATION

Drug Polymer Interaction (FTIR Study): FTIR Study aims to find out the interaction of drug with polymers by carrying the study on physical mixture, pure drug and also the microsphere formulations by Fourier Transform Infrared Spectrophotometer. In KBR press the drug pellets and potassium bromide were compressed at 20 psi for 10 min. In the

wavelength range of 4000 – 600 cm^{-1}, the spectras were scanned [27]

Particle size, shape and surface morphology: Scanning Electron Microscopy as used to find out the size, shape and morphology of microspheres. A small amount of microspheres were spread on a double side adhesive tape stucked on aluminium stubs. It is placed on to fine coat ion sputter for gold coating. A scanning electron photomicrograph was taken at 20 KV acceleration potential with pressure of 0.6 mm Hg. For the determination of particle size optical microscope fitted to an ocular micrometer and a stage micrometer is used. For more than 100 microspheres the particle diameter can be calculated. Widely used technique is Confocal laser scanning microscopy used for characterising not only the surface but also inside particles[28]

Thin layer chromatographic study: It is an very efficient method of testing the drug stability. By this method the R_f value of microsphere is compared with Rf value of pure drug [29]

Density determination: A multivolume pycnometer is used to determine the density of microspheres. Accurately weighed sample is placed in a cup of multivolume pycnometer**.** By introducing Helium gas at constant pressure causes gas to expand and thus reduction in pressure is observed. Two consecutive readings of reduction in pressure at initial and final were noted. From this density and volume of microsphere were calculated[30]

Drug Capture efficiency: To determine the capture efficiency, the microspheres were subjected to repeated washing and then allowed to lyse. The lysate was observed for the active constituents as per the requirement of monograph. The supernatant liquid was assayed spectrophotometrically for drug contents. The Entrapment efficiency was calculated according to the following equation[31]
Entrapment Efficiency = Practical drug content / theoretical drug content x 100

Swelling Index: Swelling index study aims to find out the swelling extent rate of microspheres in given microspheres. Accurately weighed microspheres were allowed to swell in a buffer ensuring complete equilibrium is achieved. Blotting paper is used to wipe out the excess adhered liquid drops and a microbalance is used to weigh the swollen microspheres. Drying is done until there was no change in dried mass of the sample. Swelling index was calculated from the following formula[32]
Swelling Index = (mass of sweollen microspheres – mass of dry microspheres / mass of dried microspheres) x 100

Angle of contact: Angle of contact is an important parameter in the evaluation of microspheres as it is used to measure the wetting property of microspheres. By measuring the angle of contact at solid/ air/water interface the microspheres nature can be found out in terms of hydrophilicity or hydrophobhicity. In an inverted microscope, a droplet is placed in a circular cell mounted above the objective and the contact angle is measured at 20 within a minute of microspheres deposition.

Bulk density: Accurately weighed microspheres were transferred in to a 10 ml graduated cylinder. Autotrap is used to tap the microspheres to stabilize its bed volume. Bulk density can be found out measuring the ratio of intial weight of microspheres to final volume of tapped microspheres[33-34]

In vitro mucoadhesive test: *In vitro* wash off test was performed to find the mucoadhesiveproperty. Polymer adhesion with mucus membrane is due to hydration and promoting the sticky nature. To a glass slide 1cm x 1cm stomach mucosa of rat was tied using a thread to the glass slide. The washed microspheres were spreaded slowly and it was hung on to grooves of USP tablet disintegration test apparatus. The tissue specimen in disintegration test apparatus moves up and down at regular intervals of time in a beaker containing simulated gastric fluid. At the end , number of microspheres adhered to tissue specimen was counted and its adhesive strength was determined[35]

Isoelectric point: Microelectrophoresis apparatus is used for measuring the isoelectric point of microspheres. When the particles moves over a distance of 1mm the mean velocity was calculated at different pH values ranging from 3 – 10. The elecrophoretic mobility is attributed to surface ionisable charge or ion absorption character of the microspheres[36]

In vitro drug release: It is an important evaluation parameter for microspheres. Depending on the shape and application of dosage form, following methods were used.

Beaker method: The drug loaded microspheres were dispersed in a dissolution fluid containing beaker with the stirring speed rate ranges from 30 - 600 rpm. At specified time intervals samples were withdrawn using a hypodermic syringe fitted with 0.4 µm

milllipore filter. The samples were analysed by spectrophotometric method. A standard calibration curve was drawn to determine the drug release content[37]

Interface diffusion system: This method was designed by Deardon and Tomlinson representing oral cavity, buccal membrane with octanol, body fluids with 0.2 ml Hcl, protein binding with 1 – octanol as four compartments. Before using the aqueous phase and octanol were saturated with each other, samples were withdrawn slowly and again returned to first compartment containing oral cavity with syringes and samples were analysed [38.]

Stability studies: The microspheres were stored at different conditions of temperature like 4^{o}_{o} C (refrigerator), room temperature and 40^{o}C (thermostatic oven). At the end of 15, 30 and 60 days all the formulations were inspected and observed for the changes. The drug content was analysed by spectrophotometric method[39]

APPLICATIONS OF MICROSPHERES

Some of the applications of microspheres are as follows

- In developing controlled and sustained release dosage forms very effectively
- 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
- Very effective of drugs against environmental hazards such as humidity, light, oxygen or heat
- The separations of incompatible substances done by encapsulation method.
- Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation
- The hygroscopic properties of many core materials may be reduced by microsphere
- Many drugs have been microencapsulated to reduce gastric irritation
- 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 [40]
- Radio-embolization of liver and spleen tumours, local radiotherapy, local restenosis prevention in coronary arteries.
- Blood flow determination, tracing, *in vivo* imaging and calibration of imaging . Hollow microspheres used to decrease material density. Monodisperse microspheres used to calibrate particle sieves and determines in particle counting[41]

CONCLUSION

Mucoadhesive microspheres has witnessed spectacular developments in novel drug delivery systems Mucous membranes of human organism are relatively permeable and allow fast drug absorption , hence mucoadhesive microspheres creating a great impact providing endless opportunity in treatment of various diseases. It is an lucrative alternative for noninvasive drug delivery of protein peptide molecules. Mucoadhesive microspheres is a promising area for continued research with the aim of achieving controlled release with enhanced bioavailability over longer periods of time and drug targeting action. Advances in experimentation and computation methodologies will helpful in clinical areas of research will create a wider potential in drug delivery . Drug companies strives hard in formulating more smaller complex molecules, proteins and peptides, and DNA for future technological advancement in the ever-evolving drug delivery arena. Hence, drugs when formulated as mucoadhesive microspheres efficiently eradicates diseases and find widespread applications in biomedicine.

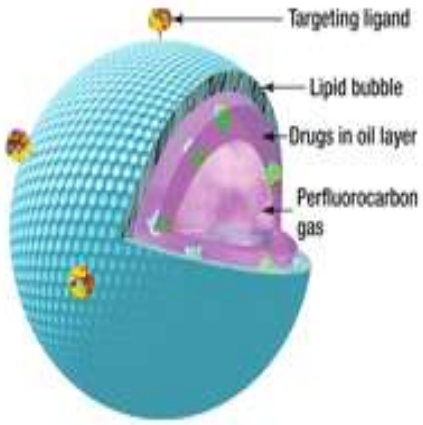

Fig 1 : **Section of Microsphere**

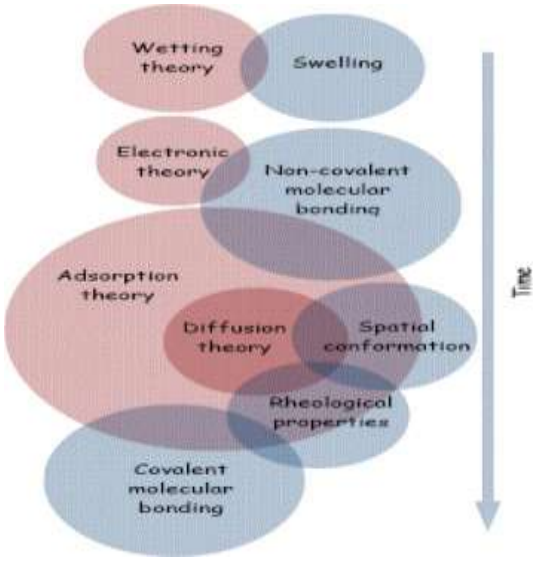

Fig 2 : **Theories of Mucoadhesion**

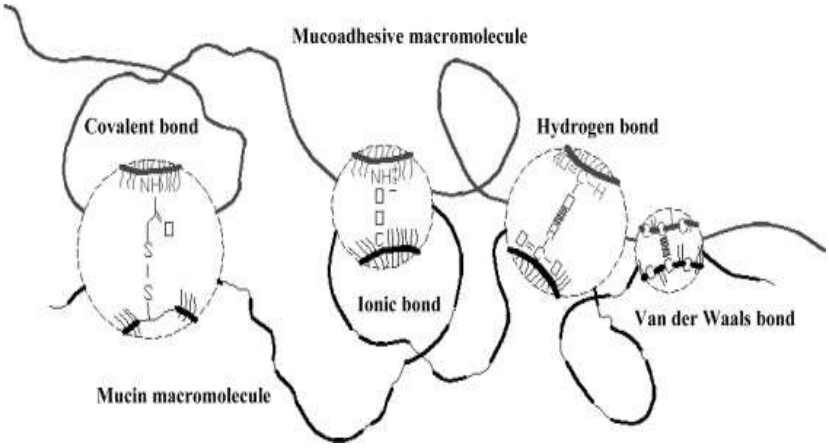

Fig 3: Electronic theory

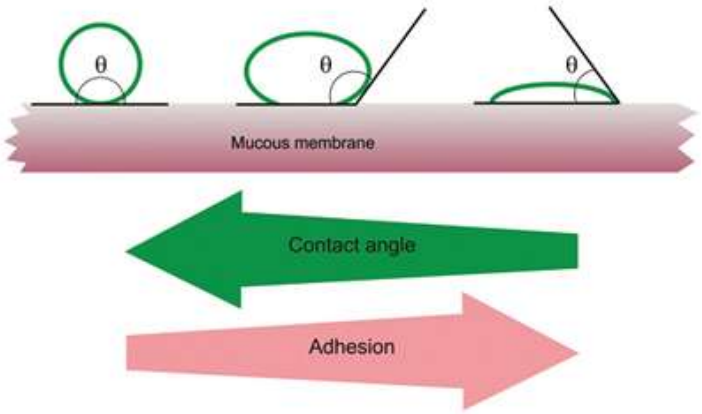

Fig 4 : Adsorption theory

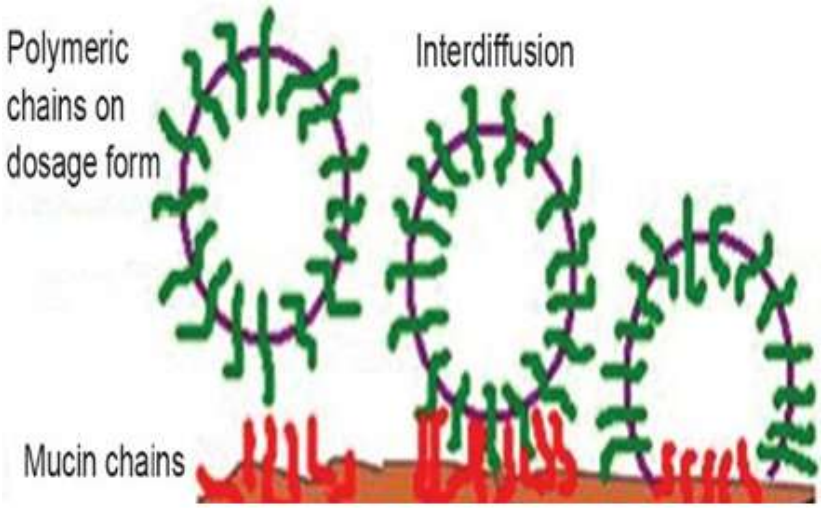

Fig 5 *:* Fracture theory

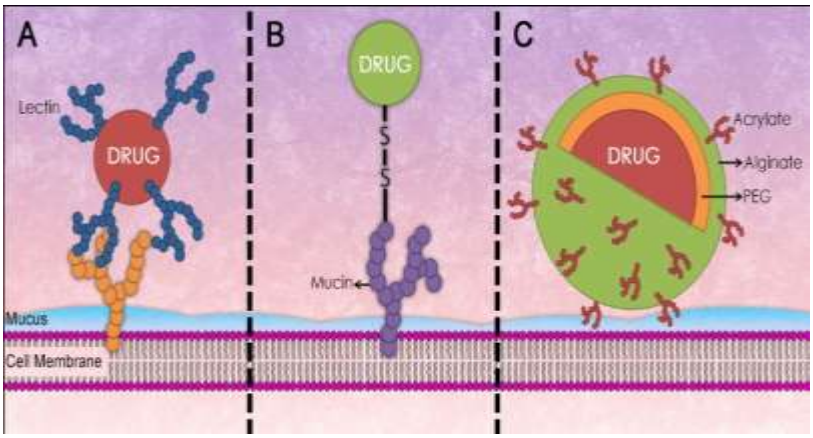

Fig 6 : Mechanism of mucoadhesion

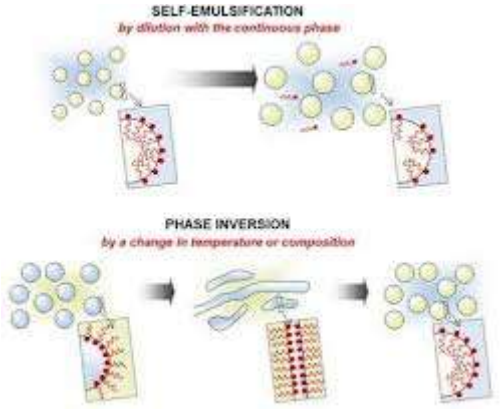

Fig 7 : Phase inversion method

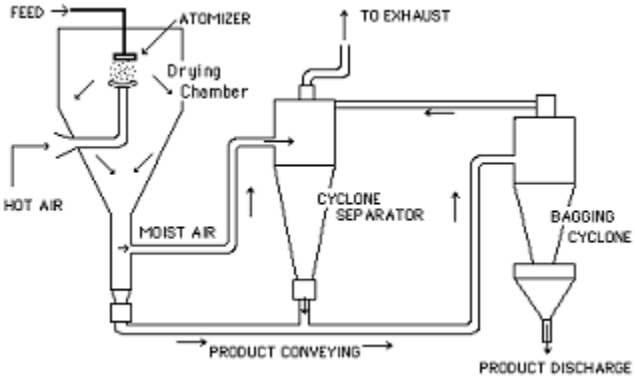

Fig 8 : Spray drying

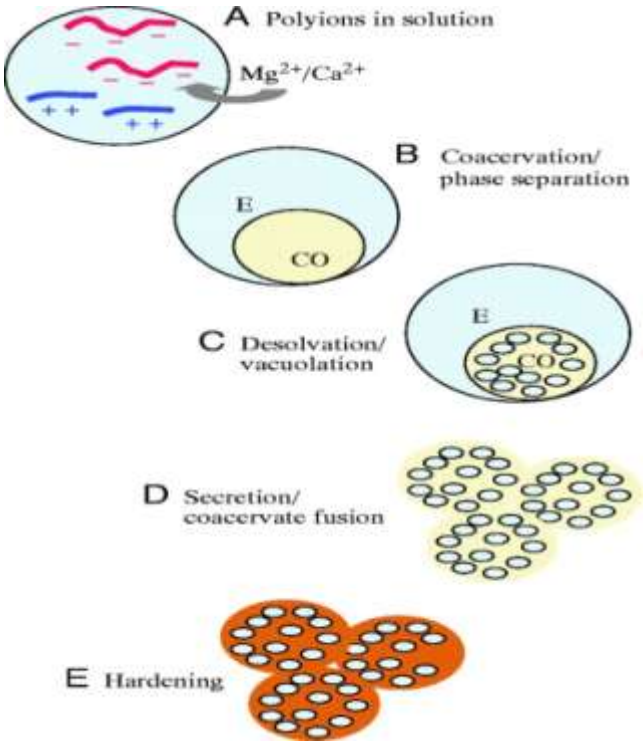

Fig 9: Complex coacervation

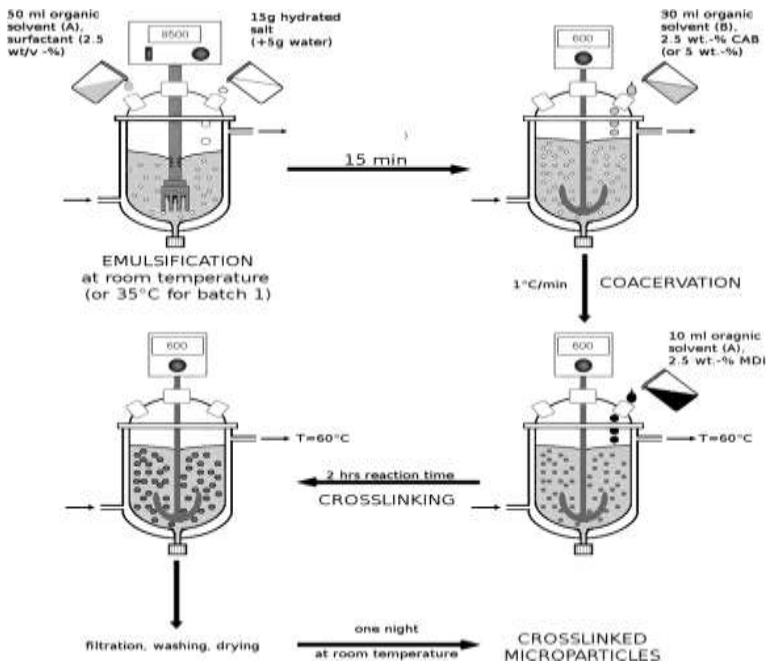

Fig 10: Hot melt microencapsulation

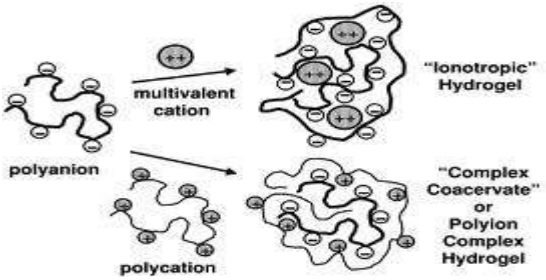

Fig 11. Hydrogel methods

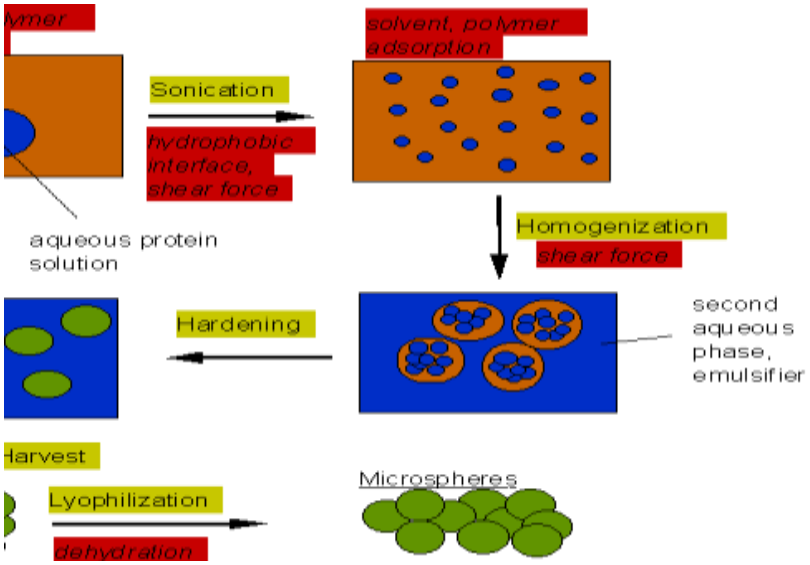

Fig 12 : Solvent evaporation method

Table 1 : List of Mucoadhesive polymers[12]

Synthetic polymer	Natural polymer	Biocaompatible polymer	Biodegradable
Polycarbophil	Tragacanth	Cellulose based polymer	Chitosan
Polyethylene oxide	Sodium alginate	Ethylene glycol copolymer	Polyorthoesters
Polyvinyl pyrollidone	Karaya gum	Esters of Hyaluronic acid	Polyalkylcyanocrylate
Polyacrylic acid	Guargum	-	Polyphosphazene
Polyhydroxy ethyl methacrylate	Gelatin	-	Polycarbolactone
Hydroxy propyl cellulose	Soluble starch	-	-
Ambrellite resin	-	-	-
Sodium Carboxy methyl cellulose	-	-	-
Polyamide	--	-	-

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