Methods and Evaluation Parameter of Sustained Release Muco-Adhesive Microsphere

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Abstract: Microsphere are free flowing powder, consist of spherical particle of size ideally less than 1 to 1000 µm. Each particle of microspheres is basically a matrix of drug dispersed in a polymer from which release obtained by a first order kinetic process. In the microsphere, the polymer used are biodegradable and biocompatible. Internal structure of microspheres is a matrix of drug and polymeric excipient. The microspheres are meant to reduce the dosing frequency and improve patient compliance by designing and evaluating sustained release mucoadhesive microspheres for effective control of disease. Mucoadhesive drug delivery system are delivery system which utilizes the property of bio-adhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time.

Key words: Mucoadhesive Microsphere • Mucoadhesion • Biodegradable • Mucoadhesive polymer • Bio-Adhesive Polymer

INTRODUCTION

Mucoadhesive microsphere is a targeting drug delivery system in a particular surface of the body for extended period of time. A good Sustained Release Mucoadhesive (SRM) drug dosage form has three properties, from a technological point of view. The delivery system must maintain drug position in the mouth for a few hours, release the drug in a controlled manner and provide the drug release in a unidirectional way towards the mucosa. Microspheres play an important role in the novel drug delivery system. Success of these microspheres is limited owing to their short residence time at the site of absorption [1]. Mucoadhesive drug delivery system are delivery system which used the property of bio adhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body. The term “mucoadhesion” was coined for the adhesion of the polymers with the surface of the mucosal layer. Bio adhesion is a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate such as adhesion between polymer and a biological membrane in case of polymer attached to the mucin layer of mucosal tissue. The term mucoadhesion is used when the mucosal layer lines a number of regions of body including a gastrointestinal tract, urogenital tract, the airways, the ears, nose and eye [2].

Advantages [3]:

- Increase bioavailability
- Efficient absorption
- Prolonged gastrointestinal time
- High surface to volume ratio which ensure a much more intimate contact with mucosal layer
- Specific targeting of drug to absorption site
- Better control of systemic drug delivery

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Polymer Used for Mucoadhesive Microsphere System:
There are different types of polymer used in mucoadhesive microsphere [4].

**Synthetic Polymers Natural Polymers:**
- Cellulose derivative
- Polycarbophil
- Sodium alginate
- Poly (Ethylene oxide)
- Poly (Vinyl pyrrolidone)
- Poly (Vinyl alcohol)
- Poly (Hydroxyethylmethylacrylate)
- Hydroxyl propyl cellulose

**Natural Polymers:**
- Tragacanthe
- Sodium alginate
- Karaya gum
- Guar gum
- Gelatin
- Chitosan
- Soluble starch

**MATERIALS AND METHODS**

**Coacervation:** This method is performed in mainly three steps carried out under continuous agitation which are formulation of three immiscible chemical phases, deposition of coating and rigidization of the coating. Three immiscible phases include a liquid manufacturing vehicle, a core material phase and a coating material phase. The core material is dissolved in a solution of the polymer, the solvent for the polymer being the liquid manufacturing vehicle phase. By changing the temperature of the polymer solution, microsphere can be prepared, by adding salt, using a non solvent and also by the addition of an incompatible polymer to the polymer solution and polymer-polymer interaction [15].

**Ionic Gelation:** In this ionic gelation method drug is added to aqueous solution of sodium alginate. Alginate/chitosan particulate systems for diclofenac sodium release have been prepared using this ionic gelation technique. In this order to get the complete solution stirring is continued and after that it is added drop wise to a solution containing Ca$^{2+}$/Al$^3$. Microspheres which are formed, kept in original solution

Table 1:

<table>
<thead>
<tr>
<th>Patent No.</th>
<th>Title</th>
<th>Result</th>
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<tbody>
<tr>
<td>8197435</td>
<td>Methods and devices for drug delivery to ocular tissue using microneedle.</td>
<td>Methods and devices are provided for targeted administration of a drug to a patient's eye [5].</td>
</tr>
<tr>
<td>7691811</td>
<td>Transporter-enhanced corticosteroid activity and methods and compositions for treating dry eye.</td>
<td>Methods and compositions for enhancing the activity and/or duration of action of loteprednol etabonate and other soft anti-inflammatory steroids of the haloalkyl 17α-alkoxy carbonyloxyl-11β-hydroxy androst-4-en-3-one 17β-carboxylate type and the corresponding Δ1, 4-compounds are described [6].</td>
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<tr>
<td>8603778</td>
<td>Anti-TNF antibodies, compositions, methods and uses.</td>
<td>The present invention relates to anti-TNF antibodies comprising all of the heavy chain variable CDR regions of SEQ ID NOS:1, 2 and 3 and/or all of the light chain variable CDR regions of SEQ ID NOS:[4, 5, 6] specific for at least one human tumor necrosis factor alpha (TNF) protein or fragment thereof, as well as nucleic acids encoding such anti-TNF antibodies, complementary nucleic acids, vectors, host cells, production methods and therapeutic methods [7].</td>
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<tr>
<td>4743545</td>
<td>Hollow porous microspheres containing biocatalyst</td>
<td>Biocatalyst such as enzymes or cells are immobilized in hollow porous microspheres for use as bioreactors in biochemical processes [8].</td>
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<tr>
<td>6235313</td>
<td>Bioadhesive microspheres and their use as drug delivery and imaging systems.</td>
<td>Bioadhesive polymers in the form of, or as a coating on, microcapsules containing drugs or bioactive substances which may serve for therapeutic, or diagnostic purposes in diseases of the gastrointestinal tract, are described [9].</td>
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<tr>
<td>6315981</td>
<td>Gas filled microspheres as magnetic resonance imaging contrast agents.</td>
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<td>5922304</td>
<td>Gaseous precursor filled microspheres as magnetic resonance imaging contrast agents.</td>
<td>Novel gas filled microspheres useful as magnetic resonance imaging (MRI) contrast agents are provided [11].</td>
</tr>
<tr>
<td>6365187</td>
<td>Bioadhesive microspheres and their use as drug delivery and imaging systems.</td>
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<tr>
<td>5972387</td>
<td>Modified hydrolyzed vegetable protein microspheres and methods for preparation and use thereof.</td>
<td>Modified hydrolyzed vegetable protein microspheres and methods for their preparation and use as oral delivery systems for pharmaceutical agents are described [13].</td>
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<tr>
<td>5186922</td>
<td>Use of biodegradable microspheres labeled with imaging energy contrast materials.</td>
<td>The invention relates to an inexpensive and easy to use method of visualizing an arterial circulation, using biodegradable microspheres which are permeated with an imaging energy absorbent contrast material, such as an X-ray absorbent material, which enables the diagnosis of pulmonary embolism [14].</td>
</tr>
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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 [16].

**Wet Inversion Technique:** In this method, a polymeric solution in acetic acid is added drop wise into an aqueous solution of counter ion such as sodium tripolyphosphate through a small sized nozzle. Microspheres are formed which are allowed to stand undisturbed for some time and then cross linked with cross linking agents such as 5% ethylene glycol diglycidyl ether. Microspheres obtained are then washed and freeze dried [17].

**Pan Coating:** In this method, the coating material is applied as solution in the coating pan. Warm air is passed over the coated materials to remove the coating solvent [18].

**Complex Coacervation:** CS (Core cell) micro particles can also be prepared by complex coacervations. Sodium alginate, sodium CMC and sodium polyacrylic acid can be used for complex coacervation with CS (Core cell) to form microspheres. By interionic interaction between oppositely charged polymers solutions and KCl & CaCl solutions, these micro particles are formed. The obtained capsules are hardened in the counter ion solution before washing [19].

**Hot Melt Microencapsulation:** Here the melted polymer is mixed with solid particles of the drug that have been sieved to less than 50 μm. The mixture is suspended in a non-miscible solvent (like silicone oil) continuously stirred and then heated to 5°C above the melting point of the polymer. When the emulsion is stabilized, it is cooled until the polymer particles solidify. The obtained microspheres are washed by decantation with petroleum ether. The necessary objective for developing this method is to develop a microencapsulation method suitable for the water labile polymers [20].

**Air Suspension:** In this process the dispersion of solid particles of core materials in a supporting air stream is done followed by the spray coating of the air suspended particles [21].

**Solvent Removal:** Solvent removal is a non-aqueous method of microencapsulation, mainly preferred for water labile polymers. Solvent removal, drug is dissolved in a solution of the selected polymer in a volatile organic solvent. This mixture is then suspended in silicone oil containing Span 85 and volatile organic solvent. After added the polymer solution into silicone oil, petroleum ether is mixed and stirred until solvent is obtained into the oil solution. The resulting microspheres can then be dried in vacuum [22].

**Preparation of Microspheres by Glutaraldehyde Cross Linking:** By aqueous acetic acid, a 2.5 % (w/w) chitosan solution are prepared. This dispersed phase is mixed to continuous phase (125 mL) making of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (w/v) Span 85 to develop a water in oil (w/o) emulsion. Stirring is stirred at 2000 rpm using a 3-blade propeller stirrer. A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% v/v) is mixed at 15, 30, 45 and 60 minutes. Stirring is stirred for 2.5 hours and separated by filtration under vacuum and washed with petroleum ether (60 °C- 80 °C) and then with distilled water to remove the liquid paraffin and glutaraldehyde. Then the microspheres are dried in vacuum desiccators [23].

**Hydrogel Microspheres:** Microspheres consist of gel-type polymers, such as alginate are developed in an aqueous solution by dissolving the polymer, suspending the active material in the solution and extruding by a precision technique, developing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually takes calcium chloride solution, whereby the divalent calcium ions crosslink the polymer developing gelled microspheres. The hydrogel microspheres method involves an all-aqueous system and avoids residual solvents in microspheres [24].

**Emulsion Cross Linking Method:** Emulsion cross linking method, drug is dispersed in aqueous gelatin solution which is previously heated for 1 hr. at 40°C. The solution is mixed drop wise to liquid paraffin while stirring the solution at 1500 rpm for 10 min at 35°C, results in w/o emulsion stirring is done for 10 min at 15°C. Thus the develop microspheres are washed three times with acetone and isopropyl alcohol, then air dried and dissolved in 5mL of aqueous glutaraldehyde at room temperature for 3 hr., for cross linking and then treated with 100mL of 10mm glycine solution containing 0.1%w/v of tween 80 at 37°C for 10 min to block unreacted glutaraldehyde. An example for this emulsion cross linking technique is Gelatin microspheres [25].
Preparation of Microspheres by Tripolyphosphate: By 2.5% w/v concentration, chitosan solution is prepared. Microspheres are developed by mixing the bubble-free dispersion of chitosan by a disposable syringe (10 mL) onto a gently agitated (magnetic stirrer) 5% or 10% w/v TPP solution. After 2 hrs, Chitosan microspheres separated, by filtration and washed with distilled water, after that they were air dried [26].

Preparation of Ethyl Cellulose Microspheres: In acetone, a solution of Ethyl cellulose is mixed to liquid paraffin containing emulgent (Span 85) while stirring at a speed of 1500 rpm. The emulsion has been stirred for 5 to 6 hours at 25°C to 30°C. Subsequently, a proper amount of petroleum ether has been mixed to the dispersion, filtered and dried at ambient temperature. The obtained microspheres washed with petroleum ether to remove traces of liquid paraffin [27].

Evaluation
Yield of Microspheres: The developed microspheres has been collected and weighed. By the total amount of all non-volatile components, the measured weight are divided, which were used for the development of the microspheres [28].

\[
\text{% Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of microsphere method}} \times 100
\]

Particle Size, Shape and Morphology: All the develop microspheres has been considered with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The diameter of particle more than 100, optical microscopy is being used for measured microspheres. Scanning Electron photomicrographs of drug-loaded microspheres taken. A little amount of microspheres has been spread on gold stub. The, the stub obtained the sample is placed in the Scanning electron microscopy (SEM) [29].

Entrapment Efficiency: The capture efficiency of the microspheres can be measured by allowing washed microspheres to lyse. The lysate is then subjected to measure the active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation [30].

\[
\text{% Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100
\]

Bulk Density: By three tap method, bulk density can be measured. After filling the weighed amount of microspheres in a graduated cylinder, the volume occupied by microspheres should be measured [31].

Surface Topography by Scanning Electron: Scanning electron microscope of the microspheres determines the surface morphology of the microspheres like their shape and size. The surface morphology and structure are shown by scanning electron microscopy (SEM). The samples are consisting of double side adhesive type by lightly sprinkling the microspheres powder which already shucked to on aluminum stubs. Then, the stubs are kept into fine coat ion sputter for gold coating. After gold coating samples are randomly scanned for determination of particle size and surface morphology [32].

Stability Studies of Microsphere: The prepared microspheres divided into 3 sets and stored at 4°C (Refrigerator), room temperature and 40°C (thermostatic oven). After 15, 30 and 60 days drug content of all the preparation measured spectrophotometrically [33].

Scanning Electron Microscopy (SEM): Surface morphology is measured by the scanning Selections microsphere method. In this microcapsule has been placed directly on the scanning electron microsphere slab with the help of double sided sticking tape and coated with gold film under reduced pressure [34].

Swelling Index: Swelling index technique is being used for determination and characterization of sodium alginate microspheres. Various types of solution like (Distilled water, buffer solutions of pH (1.2, 4.5 and 7.4) take and alginate microspheres (100 mg) are kept in a wire basket and placed on the above solution. After that swelling is allow at 37°C and changes in weight variation between initial weight of microspheres and weight due to swelling has been determined by taking weight randomly and soaking with filter paper [35].

In vitro Wash-Off Test: A piece of rat stomach (1 cm x 1 cm) is tied onto a glass slide (3 inch x 1 inch) using a thread. Prepared slide of microsphere is hung onto one of the groves of the USP tablet disintegrating test apparatus. The tissue specimen regular up and down movements in a beaker obtaining the simulated gastric fluid by operated
the disintegrating test apparatus. After the end of every time period, the number of microsphere still covering on to the tissue is calculate and there covering strength measured [36].

**In vitro Diffusion Studies:** By using in vitro nasal diffusion cell, in-vitro diffusion studies are performed. The receptor chamber is filled with buffer maintained at 37±2°C. 10 mg microspheres are spread on sheep nasal mucosa. Accurately weighed 0.5 ml of diffusion samples are withdrawn by a hypodermic syringe and replaced with the same quantity of prepared fresh buffer solution to fix a constant volume of the receptor compartment. The samples are determined spectrophotometrically [37].

**Drug Polymer Interaction (FTIR) Study:** By Fourier transformed Infrared spectrophotometer, IR spectroscopy can be shown. The drug and potassium bromide pellets 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 [38].

**CONCLUSION**

Mucoadhesive microspheres show as a selected carrier system for many pharmaceuticals. They can be targeted to adhere to any mucosal tissue in the body surface. The control of drug releasing properties has been the main aim of pharmaceutical research and development in the past two decades. Mucoadhesive microspheres have been showed as promising candidate in delivery of drugs to a particular surface in the body in sustained release manner, as they reached the drug to a particular surface for longer period, the absorption of drug increased and hence, the bioavailability of the drug get influenced. So we can say that in future also mucoadhesive microspheres will play an important role in the development of new pharmaceuticals techniques and material. Microsphere is the promising candidate for sustained and as a targeted drug delivery in colon, GIT, liver, nasal and ocular drug delivery etc. These are also performed as diagnostic agent and for therapy of cancer too. Mucoadhesive drug delivery systems have obtained popularity day by day in the pharma field and a current area of further research and development.

**REFERENCES**


