Drug Delivery System: A Review on Microspheres

P Lakshmikanth Reddy, S Sangeetha
1 Research Scholar, Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur, 603203
2 Professor, Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur, 603203

Abstract
Oral modified-release multiple-unit dosage forms have always been more effective therapeutic alternative to conventional or immediate release single-unit dosage forms. With regards to the final dosage form, the multiparticulates are usually formulated into microspheres and filling them into hard gelatin capsules. Microspheres received much attention not only for prolonged release, but also for targeting of drugs. In future microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, genetic materials, targeted and effective drug delivery. The current aim of this review is to study various aspects of the microparticulates drug delivery system including method of formulation, evaluation & characterization.

Key-Words: Microspheres, Controlled release, Novel Drug Delivery, Therapeutic Efficacy.

INTRODUCTION
To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bioerodible polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones. For example, by taking advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behaviour. Microspheres are defined as “Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles” (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticulates. Biodegradable polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin, the synthetic polymer include poly lactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials chosen according to the polymer and drug solubility and stabilities, process safety and economic considerations. Microspheres for oral use have been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided. Microencapsulation is used to modify and retard drug release. Due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa.

Materials Used
Microspheres used usually are polymers. They are classified into two types.
1. Synthetic Polymers
2. Natural polymers

Fig - 1: Polymers used in Microspheres
Development Synthetic polymers are divided into two types.

i. Non-biodegradable polymers
   • Poly methyl methacrylate (PMMA)
   • Acrolein
   • Glycidyl methacrylate
   • Epoxy polymers

ii. Biodegradable polymers
   • Lactides, Glycolides & their co polymers
   • Poly alkyl cyano Acrylates
   • Poly anhydrides

Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.7,8

A] Proteins:
   • Albumin
   • Gelatin
   • Collagen

B] Carbohydrates:
   • Agarose
   • Carrageenan
   • Chitosan
   • Starch

C] Chemically modified carbohydrates:
   • Poly dextran
   • Poly starch.

TYPES OF MICROSPHERE12, 13, 14
1. Bioadhesive Microspheres15, 16, 17
   Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bioadhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action.

2. Magnetic Microspheres18
   This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different types are therapeutic magnetic microspheres and diagnostic microspheres.

i. Therapeutic Magnetic Microspheres: It is used to deliver chemotherapeutic agent to liver tumor. Drugs like proteins and peptides can also be targeted through this system.

ii. Diagnostic Microspheres: It can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.

3. Floating microspheres19, 20
   In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of striking and dose dumping. One another way it produces prolonged therapeutic effect and therefore reduces dosing frequencies.

4. Polymeric Microspheres21
   The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and synthetic polymeric microspheres.

i. Biodegradable Polymeric Microspheres22:
   Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release.

ii. Synthetic Polymeric Microspheres:
   The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantage of these kinds of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

ADVANTAGES23
1. Microspheres provide constant and prolonged therapeutic effect.
2. Reduces the dosing frequency and thereby improve the patient compliance.
3. They could be injected into the body due to the spherical shape and smaller size.
4. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
5. Microsphere morphology all owes a controllable variability in degradation and drug release.

LIMITATION23
Some of the disadvantages were found to be as follows:
1. The modified release from the formulations.
2. The release rate of the controlled release dosageform may vary from a variety of factors like food and the rate of transit though gut.
3. Differences in the release rate from one dose to another.
4. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
5. Dosage forms of this kind should not be crushed or chewed.
CHARACTERISTICS OF MICROSPHERES:

Table 1: Microsphere property

<table>
<thead>
<tr>
<th>S. No</th>
<th>Property</th>
<th>Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Size Diameter</td>
<td>Uniformity/distribution</td>
</tr>
<tr>
<td>2</td>
<td>Composition</td>
<td>Density, Refractive Index,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrophobicity/hydrophilicity</td>
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<tr>
<td></td>
<td></td>
<td>Nonspecific binding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autofluorescence</td>
</tr>
<tr>
<td>3</td>
<td>Surface Chemistry</td>
<td>Reactive groups Level of functionalization Charge</td>
</tr>
<tr>
<td>4</td>
<td>Special Properties</td>
<td>Visible dye/fluorophore Super-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>paramagnetic</td>
</tr>
</tbody>
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1. Microsphere size may be critical to the proper function of an assay, or it may be secondary to other characteristics. Considering traditional diagnostic methods, the test or assay format commonly dictates particle size, such as the use of very small spheres (~0.1-0.4μm) to ensure satisfactory wicking in lateral flow tests, or the use of larger, cell-sized spheres (~4-10μm) for bead based flow cytometric assays.

2. Common microsphere compositions include polystyrene (PS), poly(methyl methacrylate) (PMMA), and silica. These materials possess different physical and optical properties, which may present advantages or limitations for different applications. Polymer beads are generally hydrophobic, and as such, have high protein bindingabilities. However, they often require the use of some surfactant (e.g. 0.01-0.1% Tween® 20 or SDS) in the storage buffer to ensure ease of handling. During synthesis, functional monomers may be co-polymerized with styrene or methyl methacrylate to develop beads with surface reactive groups. Functional groups may be used in covalent binding reactions, and also aid in stabilizing the suspension. Silica microspheres are inherently hydrophilic and negatively charged. Consequently, aqueous silica suspensions rarely require use of surfactants or other stabilizers. Carboxyl- and aminefunctionalized silica spheres are available for use in common covalent coating protocols, and plain silica microspheres may be modified using a variety of silanes to generate functional groups or alter surface properties.

3. Microspheres may be coated with capture molecules, such as antibodies, oligonucleotides, peptides, etc. for use in diagnostic or separation applications. Microsphere coatings are typically optimized to achieve desired specific activity, while minimizing nonspecific interactions. Consideration should also be given to the required stability, development time frame and budget, and the specific biomolecule to be coated. These factors will aid in determining the most fitting coating strategy for both short- and long-term objectives. Standard microsphere products support three basic coating strategies: adsorption, covalent coupling, and affinity binding.

4. Many applications in the life sciences demand added properties, such as fluorescence or a visible color, or iron oxide inclusions for magnetic separations. Polymer spheres (and polymer based magnetic spheres) are often internally dyed via organic solvent swelling, and many standard products are available. Dye concentrations can be adjusted to produce beads with different intensities to meet special needs, such as QuantumPlex™ for multiplexed flow cytometric assays, or our Dragon Green or Flash Red Intensity Standards, which support imaging applications and associated instrument QC. Many surface- or internally labelled fluorescent beads are also available as specialized flow cytometry standards.

CRITERIA FOR MICROSPHERE PREPARATION:

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. Preparation of microspheres should satisfy certain criteria:

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersability in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability and
6. Susceptibility to chemical modification.

METHOD OF PREPARATION:

The various methods of preparations are:

**Emulsion Solvent Evaporation Technique:**

In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0.2 % sodium of PVP as emulsifying agent. The above mixture was agitated at 500 rpm then the drug and polymer (eudragit) was transformed into fine droplet which solidified into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralized water and desiccated at room temperature for 24 hrs.

![Image](image.png)

Fig - 2: Microspheres by Double Emulsion Technique
Emulsion Cross Linking Method:
In this method drug was dissolved in aqueous gelatine solution which was previously heated for 1 hr at 40°C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35°C, results in w/o emulsion then further stirring is done for 10 min at 15°C. Thus the produced microspheres were 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 was treated with 100mL of 10mm glyciene solution containing 0.1%w/v of tween 80 at 37°C for 10 min to block unreacted glutaraldehyde. Examples for this technique is Gelatin A microspheres.

Coacervation Method:
Co-acervation thermal change: Performed by weighed amount of ethyl cellulose was dissolved in cyclohexane with vigorous stirring at 80°C by heating. Then the drug was finely pulverised and added with vigorous stirring on the above solution and phase separation was done by reducing temperature and using ice bath. Then above product was washed twice with cyclohexane and air dried then passed through sieve (sieve no. 40) to obtain individual microcapsule. Co-acervation non solvent addition: Developed by weighed amount of ethyl cellulose was dissolved in toluene containing propylisobutylene in closed beaker with magnetic stirring for 6 hr at 500 rpm and the drug is dispersed in it and stirring is continued for 15 mins. Then phase separation is done by petroleum benzoin 5 times with continuous stirring. After that the microcapsules were washed with n-hexane and air dried for 2 hr and then in oven at 50°C for 4 hr.

Spray Drying Technique:
This was used to prepare polymeric blended microsphere loaded with ketoprofen drug. It involves dispersing the core material into liquefied coating material and then spraying the mixture in the environment for solidification of coating followed by rapid evaporation of solvent. Organic solution of poly (epsilon-caprolactone) (PCL) and cellulose acetate butyrate (CAB), in different weight ratios and ketoprofen were prepared and sprayed in different experimental condition achieving drug loaded microspheres. This is a rapid but may lose crystalinity due to fast drying process.

Emulsion-Solvent Diffusion Technique:
In order to improve the residence time in colon floating microperticles of ketoprofen were prepared using emulsion solvent diffusion technique. The drug polymer mixture was dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture was added dropwise to sodium lauryl sulphate (SLS) solution. The solution was stirred with propeller type agitator at room temperature at 150 rpm for 1 hr. Thus the formed floating microspheres were washed and dried in a dessicator at room temperature. The following microparticles were sieved and collected.

Multiple Emulsion Method:
Oral controlled release drug delivery of various drugs was prepared by this technique. In the beginning powder drug was dispersed in solution (methyl cellulose) followed by emulsification in ethyl cellulose solution in ethyl acetate. The the primary emulsion was then re-emulsified in aqueous medium. Under optimised condition discrete microspheres were formed during this phase.

Ionic Gelation Method:
Alginate/chitosan particulate system for Nateglinide release was prepared using this technique. Different % (w/v) of Nateglinide was added to 2 % (w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it was added dropwise to a solution containing Ca²⁺ and chitosan solution in acetic acid. Microspheres which were formed were kept in original solution for 6 hrs & 24 hr for internal gellification followed by filteration for separation. The complete release was obtained at pH 7.4 but the drug did not release in acidic pH.

Hydroxyl appetite (HAP) Microspheres in Sphere Morphology:
This was used to prepare microspheres with peculiar spheres in sphere morphology microspheres were prepared by o/w emulsion followed by solvent evaporation. At first o/w emulsion was prepared by dispersing the organic phase (Diclofenac sodium containing 5% w/w of EVA and appropriate amount of HAP) in aqueous phase of surfactant. The organic phase was dispersed in the form of tiny droplets which were surrounded by surfactant molecules this prevented the
droplets from co-solvening and helped them to stay individual droplets. While stirring the DCM was slowly evaporated and the droplets solidify individual to become microspheres. A novel quasi-emulsion solvent diffusion method to manufacture the controlled releasemicrospheres of drugs with acrylic polymers has been reported in the literature. Microsponges can be manufactured by a quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol. The internal phase is consisting of drug, ethanol and polymer is added at an amount of 20% of the polymer in order to enhance plasticity. At first, the internal phase is manufactured at 60°C and then added to the external phase at room temperature. After emulsification process, the mixture is continuously stirred for 2 hours. Then the mixture can be filtered to separate the microsphers. The product is then washed and dried by vacuum oven at 40°C for a day.

**Physicochemical Evaluation:**

**i. Characterization**

The characterization of the microparticulate carrier is an important phenomenon, which helps to design a suitable carrier for the proteins, drug or antigen delivery. These microspheres have different microstructures. These microstructures determine the release and the stability of the carrier. The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM). Both can be used to determine the shape and outer structure of microparticles. LM provides a control over coating parameters in case of double walled microspheres. The microspheres structures can be visualized before and after coating and the change can be measured microscopically. SEM provides higher resolution in contrast to the LM. SEM allows investigations of the microspheres surfaces and after particles are cross-sectioned, it can also be used for the investigation of double walled systems. Confocal fluorescence microscopy is used for the structure characterization of multiple walled microspheres. Laser light scattering and multi size coulter counter other than instrumental methods, which can be used for the characterization of size, shape and morphology of the microspheres.

**Electron Spectroscopy for Chemical Analysis:**

The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA). ESCA provides a means for the determination of the atomic composition of the surface. The spectra obtained using ESCA can be used to determine the surface degradation of the biodegradable microspheres.

**Attenuated total reflectance Fourier Transform-Infrared Spectroscopy:**

FT-IR is used to determine the degradation of the polymeric matrix of the carrier system. The surface of the microspheres is investigated measuring alternated total reflectance (ATR). The IR beam passing through the ATR cell reflects many times through the sample to provide IR spectra mainly of surface material. The ATR-FTIR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions.

**Density Determination:**

The density of the microspheres can be measured by using a multi volume pycnometer. Accurately weighed sample in a cup is placed into the multi volume pycnometer. 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 hence the density of the microsphere carrier is determined.

**Isoelectric Point:**

The microelectrophoresis is an apparatus used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined. The mean velocity at different pH values ranging from 3-10 is calculated by measuring the time of particle movement over a distance of 1 mm. By using this data the electrical mobility of the particle can be determined. The electrophoretic mobility can be related to surface contained charge, ionisable behaviour or ion absorption nature of the microspheres.

**Surface Carboxylic Acid Residue:**

The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugates is prepared by the reaction of c14-glycine ethyl ester hydro chloride with the microspheres. The glycine residue is linked using the water soluble condensing 1-ethyl-3 (3-dimethyl amino propyl) carbodiimide (EDAC). The radioactivity of the conjugate is then measured using liquid scintillation counter. Thus the carboxylic acid residue can be compared and correlated. The free carboxylic acid residue can be measured for hydrophobic or hydrophilic or any other derivatized type of the microspheres.

**Surface Amino Acid Residue:**

Surface associated amino acid residue is determined by the radioactive c14-acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly. EDAC is used to condense the amino group and the c14-acetic acid carboxylic acid residue. The method used for determining the free amino or the free carboxylic acid residues are based on indirect estimation, by measuring the radioactivity of the c14 having acetic acid or the glycine conjugate. The accuracy of the method however, dependson the time allowed for conjugation of the radioactive moiety and the reactivity of free functional group.

**Capture 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:

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

**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 200°C within a minute of deposition of microspheres.

**In - Vitro methods**

There is a need for experimental methods which allow the release characteristics and permeability of adrug through membrane to be determined. For this purpose, a number of in vitro and in vivo techniques have been reported. In vitro drug release studies have been employed as a quality control procedure in pharmaceutical production, in product development etc. Sensitive and reproducible release data derived from physico chemically and hydro dynamically defined conditions are necessary. The influence of technologically defined conditions and difficulty in simulating in vivo conditions has led to development of a number of in vitro release methods for buccal formulations; however no standard in vitro method has yet been developed. Different workers have used apparatus of varying designs and under varying conditions, depending on the shape and application of the dosage form developed.

**Beaker Method**

The dosage form in this method is made to adhere at the bottom of the beaker containing the medium and stirred uniformly using overhead stirrer. Volume of the medium used in the literature for the studies varies from 50-500 ml and the stirrer speed form 60-300 rpm.

**Interface Diffusion System**

This method is developed by Dearden & Tomlinson. It consists of four compartments. The compartment A represents the oral cavity, and initially contained an appropriate concentration of drug in a buffer. The compartment B representing the buccal membrane, contained 1-octanol, and compartment C representing body fluids, contained 0.2 M HCl. The compartment D representing protein binding also contained 1-octanol. Before use, the aqueous phase and 1-octanol were saturated with each other. Samples were withdrawn and returned to compartment A with a syringe. Modified Keshary Chien Cell

A specialized apparatus was designed in the laboratory. It comprised of a Keshary Chien cell containing distilled water (50ml) at 37°C as dissolution medium. TMDDS (Trans Membrane Drug Delivery System) was placed in a glass tube fitted with a 10# sieve at the bottom which reciprocated in the medium at 30 strokes per min.

**Dissolution Apparatus:**

Standard USP or BP dissolution apparatus have been used to study in vitro release profiles using both rotating elements, paddle, and basket etc. Dissolution medium used for the study varied from 100- 500 ml and speed of rotation from 50-100 rpm.

**Other Methods:**

Few other methods involving plexi glass sample blocks placed in flasks, agar gel method, Valia-Chein cell USP n2 III dissolution apparatus etc have also been reported. Although a number of methods have been reported, the ideal method would be one where sink condition is maintained and dissolution time in vitro simulates dissolution time in vivo.

**In -vivo methods**

Methods for studying the permeability of intact mucosa comprise of techniques that exploit the biological response of the organism locally or systemically and those that involve direct local measurement of uptake or accumulation of penetrants at the surface. Some of the earliest and simple studies of mucosal permeability utilized the systemic pharmacological effects produced by drugs after application to the oral mucosa. However the most widely used methods include in vivo studies using animal models, buccal absorption tests, and perfusion chambers for studying drug permeability.

**Animal Models**

Animal models are used mainly for the screening of the series of compounds, investigating the mechanisms and usefulness of permeation enhancers or evaluating a set of formulations. A number of animal models have been reported in the literature, however, very few in vivo (animal). Animal models such as the dog, rats, rabbits, hamster, cat, pigs, and sheep have been reported. In general, the procedure involves anesthetizing the animal followed by administration of the dosage form. In case of rats, the oesophagus is ligated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed.

**In vitro-In vivo correlations**

Correlations between in vitro dissolution rates and the rate and extent of availability as determined by blood concentration and or urinary excretion of drug or metabolites are referred to as “in vitro-in vivo correlations”. Such correlations allow one to develop product specifications with bioavailability.

**Percent of Drug Dissolved In Vitro Vs Peak Plasma Concentration:**

One of the ways of checking the in vitro and in vivo correlation is to measure the percent of the drug released from different dosage forms and also to estimate the peak plasma concentrations achieved by them and then to check the correlation between them. It is expected that a poorly formulated dosage form releases amount of drug than a well formulated dosage form, and, hence the amount of drug available for absorption is less for poorly formulated dosage form than from a well formulated dosage form.
Percent of Drug Dissolved Vs Percent of Drug Absorbed:
If the dissolution rate is the limiting step in the absorption of the drug, and is absorbed completely after dissolution, a linear correlation may be obtained by comparing the percent of the drug absorbed to the percent of the drug dissolved. If the rate limiting step in the bioavailability of the drug is the rate of absorption of the drug, a change in the dissolution rate may not be reflected in a change in the rate and the extent of drug absorption from the dosage form.

Dissolution Rate Vs Absorption Rate:
The absorption rate is usually more difficult to determine than the absorption time. Since the absorption rate and absorption time of a drug are inversely correlated, the absorption time may be used in correlating the dissolution data to the absorption data. In the analysis of in vitro and in vivo drug correlation, rapid drug absorption may be distinguished from the slower drug absorption by observation of the absorption time for the dosage form. The quicker the absorption of the drug the less is the absorption time required for the absorption of the certain amount of the drug. The time required for the absorption of the same amount of drug from the dosage form is correlated.

Percent of Drug Dissolved Vs Serum Drug Concentration:
For drugs whose absorption from GIT is dissolution rate limited, a linear correlation may be established between the percent of drug dissolved at specified times and the serum drug concentrations at corresponding times.

Percent of Drug Dissolved Vs Percent of the Dose Excreted in Urine:
The percent of a drug dissolved and the percent of drug absorbed are linearly correlated. There exists a correlation between the amount of drug in body and the amount of drug excreted in the urine. Therefore, a linear relation may be established between the percent of the drug dissolved and the percent of the dose excreted in the urine.

Advantages
1. Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.
2. Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.
3. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor.
4. The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles in vivo.
5. Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intracellularly.
6. Blood flow determination: Relatively large microspheres (10-15 μm in diameter) are useful for regional blood flow studies in tissues and organs. In most cases the microspheres are injected at desired locations in the circulatory system and eventually lodge in the capillaries. The microspheres and fluorescent dyes they contain are first extracted from the tissue sample, and then fluorescence is quantitated on a spectrofluorometer or fluorescence microplate reader. Traditionally, this type of study has been carried out using radiolabelled microspheres; however fluorescent microspheres have been shown to be superior in chronic blood flow measurements.

APPLICATIONS
1. Microspheres in Vaccine Delivery:
The prerequisite of a vaccine is protection against the micro organism or its toxic product. An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. The aspect of safety and minimization of adverse reaction is a complex issue. The aspect of safety and the degree of the production of antibody responses are closely related to mode of application. Biodegradable delivery systems for vaccines that are given by parenteral route may overcome the shortcoming of the conventional vaccines. The interest in parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages including:
   1. Improved antigenicity by adjuvant action
   2. Modulation of antigen release

2. Targeting using Microparticulate Carriers:
The concept of targeting, i.e. site specific drug delivery is a well established dogma, which is gaining full attention. The therapeutic efficacy of the drug relies on its access and specific interaction with its candidate receptors. The ability to leave the pool in reproducible, efficient and specific manner is center to drug action mediated by use of a carrier system. Placement of the particles indiscrete anatomical compartment leads to their retention either because of the physical properties of the environment or biophysical interaction of the particles with the cellular content of the target tissue.

Monoclonal Antibodies Mediated Microspheres Targeting:
Monoclonal antibodies targeting microspheres are immune microspheres. This targeting is a method used to achieve selective targeting to the specific sites. Monoclonal antibodies are extremely specific molecules. This extreme specificity of monoclonal antibodies (Mabs) can be utilized to target microspheres loaded bioactive molecules to selected sites. Mabs can be directly attached to the microspheres by means of covalent coupling. The free aldehyde groups, amino groups or hydroxyl groups on the surface of the microspheres can be linked to the antibodies. The Mabs can be attached to microspheres by any of the following methods
   1. Non specific adsorption
   2. Specific adsorption
   3. Direct coupling
4. Coupling via reagents

3. Chemoembolisation:
Chemoembolisation is an endovascular therapy, which involves the selective arterial embolisation of a tumour together with simultaneous or subsequent local delivery the chemotherapeutic agent. The theoretical advantage is that such embolisations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of the tumour. Chemoembolisation is an extension of traditional percutaneous embolisation techniques.

4. Imaging:
The microspheres have been extensively studied and used for the targeting purposes. Various cells, cell lines, tissues and organs can be imaged using radio labelled microspheres. The particle size range of microspheres is an important factor in determining the imaging of particular sites. The particles injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is exploited for the scintiographic imaging of the tumour masses in lungs using labelled human serum albumin microspheres.

5. Topical Porous Microspheres:
Microsponges are porous microspheres having myriad of interconnected voids of particle size range 5- 300 μm. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils etc., are used as the topical carries system further, these porous microspheres with active ingredients can be incorporated into formulations such as creams, lotions and powders. Microsponges consist of non collapsible structures with porous surface through which active ingredients are released in a controlled manner.

6. Surface Modified Microspheres:
Different approaches have been utilized to change the surface properties of carriers to protect them against phagocytic clearance and to alter their body distribution patterns. The adsorption of the poloxamer on the surface of the polystyrene, polyester or poly methyl methacrylate microspheres renders them more hydrophilic and hence decrease their MPS uptake. Protein microspheres covalently modified by PEG derivatives show decreased immunogenicity and clearance. The moststudied surface modifiers are:
1. Antibodies and their fragments
2. Proteins
3. Mono-, oligo- and polysaccharides
4. Chelating compounds (EDTA, DTPA or Desferroxamine)
5. Synthetic soluble polymers Such modifications are provided surface of microspheres inorder to achieve the targeting to the discrete organs and to avoid rapid clearance from the body.

RECENT ADVANCEMENT IN MICROSPHERE

1. Important utilizations of chitosan polymer

Cholesterol-lowering effects
Chitosan and cellulose were used as examples of fibers with high, intermediate and low bile acid-binding capacities, respectively. The serum cholesterol levels in a control group of mice fed a high fat/high cholesterol diet for 3 weeks increased about 2-fold to 4-3mM and inclusion of any of these fibers at 7-5% of the diet prevented this increase from occurring. In addition, the amount of cholesterol accumulated in hepatic stores due to the HHFC diet was reduced by treatment with these fibers. The three kinds of fibers showed similar hypocholesterolaemic activity; however, cholesterol depletion of liver tissue was greatest with cholestyramine. The mechanisms underlying the cholesterol lowering effect of cholestyramine were, Decreased cholesterol (food) intake, Decreased cholesterol absorption efficiency, and Increased faecal bile acid and cholesterol excretion. The latter effects can be attributed to the high bile acid- binding capacity of cholestyramine.

In contrast, incorporation of chitosan or cellulose in the diet reduced cholesterol (food) intake, but did not affect either intestinal cholesterol absorption or faecal sterol output. The present study provides strong evidence that above all satiation and satiety effects underlie the cholesterol lowering.

2. Increase Stability of Drug
Chitosan polymer is used to increase the stability of the drug in which the drug is complexed with chitosan and make slurry and kneading for 45 minutes until dough mass. This dough mass is pass through sieve no.16 and make a granules is completely stable at different condition.

3. Orthopaedic Patients
Chitosan is a biopolymer that exhibits osteo conductive, enhanced wound healing and antimicrobial properties which make it attractive for use as a bioactive coating to improve Osseo integration of orthopedic and craniofacial implant devices. It has been proven to be useful in promoting tissue growth in tissue repair and accelerating wound-healing and bone regeneration.

4. Cosmetics industry
Cosmetic compositions are disclosed for the treatment of hair or skin, characterized by a content of new quaternary chitosan derivatives of the formula. The chitosan derivatives have a good substantial, particularly to hair keratin, and prove to have hair strengthening and hair conditioning characteristics. e.g.: Hair setting lotion, Oxidation Hair-coloring Composition, Hair toning Composition, Skin Cream, Hair treatment Composition, Gel-form.

5. Dental Medicine
Chitosan have been recognized to accelerate wound healing to attain an aesthetically valid skin surface, and to prevent excess scar formation. In dental medicine, chitosan is also applied as a dressing for oral mucous wound and a tampon following radical treatment of maxillary sinusitis. Furthermore, it is being investigated as an absorbing membrane for periodontal surgery. Chitosan has a variety of biological activities and advertised as a healthy food that is effective for improvement and/or care of various disorders, arthritis,
cancer, diabetes, hepatitis, etc.

6. Chitosan as Permeation Enhancer
It has been reported that chitosan, due to its cationic nature is capable of opening tight junctions in a cell membrane. This property has led to a number of studies to investigate the use of chitosan as a permeation enhancer for hydrophilic drugs that may otherwise have poor oral bioavailability, such as peptides. Because the absorption enhancement is caused by interactions between the cell membrane and positive charges on the polymer, the phenomenon is pH and concentration dependant. Furthermore increasing the charge density on the polymer would lead to higher permeability.

7. Chitosan as Mucoadhesive Excipient
Bioadhesivity is often used as an approach to enhance the residence time of a drug in the GI tract, thereby increasing the oral bioavailability. A comparison between chitosan and other commonly used polymeric excipients indicates that the cationic polymer has higher bioadhesivity compared to other natural polymers, such as cellulose, Xantham gum, and starch.

8. Effect of chitosan: citric acid ratio on drug Release
It has been demonstrated that polymer with appropriate viscosity and expanding property can be used as osmotic agents for the release of water-insoluble drug. Due to its high molecular weight and a linear unbranchedstructure, chitosan is completely biodegradable, toxicologically harmless and low cost, and exhibits an excellent gelation characteristic. Hence the potential for chitosan to be used as a polymeric osmotic agent in osmotic pump is obvious. The hydration and gel formation of chitosan are very much dependent on the pH of surroundings. It is insoluble at an alkaline and neutral pH but soluble at acid condition. Upon dissolution, amine groups of the polymer become protonated, forming a resultant viscous and soluble polysaccharide. Inclusion of citric acid as pH-regulating excipient in the developed formulations was expected to decrease the microenvironmental pH of the core to a suitable level at which chitosan could form appropriate viscous gelling solution and hence, to enhance the osmotic pressure of core tablets.

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10. Enhanced Bone Formation by transforming growth factor (TGF-pl)
Chitosan composite microgranules were fabricated as bone substitutes for the purpose of obtaining high bone-forming efficacy. The chitosan microgranules were fabricated by dropping a mixed solution into a NaOH/ethanol solution. TGF-pl was loaded into the chitosan microgranules by soaking the microgranules in aTGF-pl solution.

11. Direct Compressible Excipients and as Binder:
Chitosan has an excellent property as excipients for direct compression of tablets where the additions of 50% chitosan result in rapid disintigration. The degree of deacetylation determine the extent of moisture absorption Chitosan higher then 5%, was superior to corn starch and microcrystalline cellulose as a disintigrant .The efficiency was dependent on chitosan crystalinity, degree of deacetylation, molecular weight and particle size Chitosan is found to be excellent tablet binder as compared to other excipients with the rank order co-relation for binder efficiency. Hydroxy propyl methyl cellulose >chitosan> Methyl cellulose>Sodium carboxy methyl cellulose.

12. Wound Healing Properties
Efficacy of chitosan in the promotion of wound healing was first reported in 1978. Chitosan acetate films, which were tough and protective, had the advantage of good oxygen permeability, high water absorptivity.

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, eg: microsphere based genotyping platform is used to detect six single nucleotide polymorphism, yttrium-90 microspheres is used to prevent tumour after liver transplantation and it’s advanced way in delivery of vaccines and proteins.

CONCLUSION
Drug absorption in the gastrointestinal tract is a highly variable procedure and prolonging gastric retention of the dosage form extends the time for drug absorption. Microspheres by ionotropic gelation technique promises to be potential approach for gastric retention. Although there are number of difficulties to be worked out to achieve prolonged gastric retention, a large number of companies are focusing toward commercializing this technique. In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective in vivo delivery and supplements as miniature versions of diseased organ and tissues in the body. The Chitosan Coated Alginate microspheres of Nateglinide were successfully prepared by Ionotropic Gelation Method confirmed that it is a best method for preparing Nateglinide loaded microspheres from its higher percentage yield. The formulation E₁ has highest milligram of drug content followed by other formulations. The percentage of encapsulation of all formulations was found to be in the range of 85 to 97%. Higher percentage of loading was obtained by increasing the amount of Nateglinide with respect to polymer and Co-polymer concentration. The particle size of a microsphere was determined by optical microscopy and
all the batches of microspheres show uniform size distribution. The average particle size was found to be within the Microparticle range. The prepared microspheres had good spherical geometry with smooth as evidenced by the scanning electron microscopy. The \textit{in vitro} dissolution studies showed that Chitosan Coated Nateglinide loaded Alginate microsphere formulation showed better Controlled Release Effect (>96%) over a period of 6.5 hours than other formulations.

\textbf{REFERENCES}
