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Preparation and Characterization of Microspheres for the Anticancer Activity

Garima Soni[1], Pooja Yadav[2], Manisha Dagar[3]
[1, 2, 3]*School of Medical and Allied Sciences, K. R. Mangalam University, Sohna Road, Gurugram.122103, Haryana, India*

Abstract: ***Microspheres are spherical in shape and having free flowing powder characterstics. Their particle size ranging from 1-1000µm. Microspheres are made up of two polymers natural and synthetic polymer. It contains carbohydrates, proteins, as well as biodegradable polymer. It consist of micrometric matrix system and it provides the protection for unstable drug, before and after the administration of the drug .Various methods are involved in the microspheres are single emulsion, double emulsion, spray drying solvent evaporation. Different types of microspheres are Mucoadhesive, Floating, Radioactive, Magnetic, Polymeric etc . Microspheres which have different microstructures determine the stability of carriers and it play a major role in enhancing the bioavailability of the drug. This article outlines the evaluation of microspheres, highlights method of preparation of microspheres, advantages, disadvantages, types, and characterization method of microspheres.***
Keywords: Microsphere, cancer, anticancer, polymer.

I. INTRODUCTION

Cancer is a disease that possesses the potential to enter or spread to other parts of the body which leads to abnormal cell growth. Cancer is considered as second leading cause of mortality and it is accountable for over 9.6 million deaths in 2018 globally, and about 1 out of 6 deaths is caused due to cancer. (1). the most prevalent types of cancer found among men are Lung, prostate, colorectal, stomach and liver cancer whereas breast, colorectal, lung, cervical and thyroid cancer are commonly found among women. Anticancer drugs are the weapon to fight against cancer. Currently, about 130–150 anticancer drugs have been approved for sale in various countries (2, 3). In the process of drug research and development, the anticancer drug fails due to unsuitable drug targets, unsuitable molecules, misleading conclusions, and unmatched patients. In anticancer chemotherapy, most important goal is to provide medication to a specific area. One of the major drawbacks of anticancer drugs is the lack of selectivity for a tumour tissues alone due to which severe side effects and low cure rate can be experienced. To achieve particular drug delivery profiles, examples of few approved approaches with cytotoxic drugs is mentioned (mainly doxorubicin, mitomycin C, cisplatin, methotrexate and 5-fluorouracil). However, it is understandable that certain cytotoxic drugs are encapsulated in systems with unsuitable pharmaceutical properties for the particular mechanistic class. Because of smoking and air pollution, the leading cause of cancer death is lung cancer. Therefore, for this purpose we use stereo complexed spherical microsphere with sizes between 0.5 and 10 µm loaded with doxorubicin (DOX) to be administered through the nasal route. Microspheres are envisioned to provide remarkable contribution in the future to the systemic, oral and loco-regional treatment of cancer with cytotoxic drugs and biological response modifiers. Colorectal cancer, the third most common malignancy and the fourth most recurrent cause of cancer deaths worldwide, accounts for about 945,000 new cases and 492,000 deaths per year in industrialized countries, where the lifetime incidence of the disease is 5%. (4). Microspheres are free flowing particles mainly consisting of proteins or synthetic polymers, which are spherical in shape and perishable in nature. They are multi particulate drug delivery systems, developed to attain prolonged or controlled drug delivery to improve bioavailability, stability and to target the drug at a fixed rate. Microparticles are commonly administered either intraperitoneally, intramuscularly, subcutaneously (or) on the targeted organ. They are fundamentally free flowing powders ranging in size from 1- 1000µm. As an approach, microspheres can be used as drug carriers for a sustained controlled release. Biodegradable microspheres will be then used to bound organs through capillary blockade using direct medication. They can be injects by needle no 18 or 20. Every particle could be a drug mixture that is largely spread in polymer form with dispense it that occurs by first order process. Microspheres obtains abundant heed not only for extended release, however conjointly that seek for metastatic tumour medication. Microparticles will be additional divided into two varieties specifically, microcapsules and microspheres. Microcapsules are heterogeneous particles wherever a membrane shell is contiguous the core forming a reservoir. Microspheres are solid sphere having micrometric matrix systems within which the drug is homogeneously distributed, either dissolved or homogenously suspended. For imaging the drug delivery and biomedical contrast, it seems for extreme interest in bio-stable vesicles. (5-7). Microsphere that most biocompatible structures are protein core-shell in which outer shell is made from disulphide ,crosslinked with the thickness of 50 nm of protein , which contain air or non-aqueous liquid core . (8,9)

In the area of Nano medicine, Macromolecular therapeutics are rapidly gaining importance for their assisting abilities as an alternatives to traditional drug regimens. For targeting large molecular nexuses to a specific cell populations in organ systems, it can assist with the attachment of macromolecular carriers to drug moieties. (10)

A. Advantages

1) The dose is dispersed in many small separate particles, which carry and release a part of the dose, hence the malfunction of an individual subunit does not cause the failure of the whole dosage**.**
2) Solubility of the poorly soluble drugs can enhance by reduction in size which leads to increased surface area.
3) Increase patient compliance by providing constant drug concentration in blood.
4) Due to less dosing frequency it improve patient compliance.
5) Securing the GIT from aggravating effects of the drug.
6) Decrease dose and toxicity
7) Bioavailability will be improved by better drug utilization and helps to reduce the intensity of adverse effects.
8) They could be easily administered to the body due to smaller size and spherical shape.
9) Odour and taste of unpleasant drugs can be effectively concealed.

B. Disadvantages

1) The firmness of core particles can be encapsulated by some states like pH, temperature modification, solvent addition and evaporation/ turbulence.
2) Variations within the unleash rate from one dose to a different.
3) IV of microspheres may interrelate or form complexation with blood components.
4) Some factors like intrinsic or extrinsic factors of food, rate of transit through gut, mucin turnover rate etc. can occur controlled release rate of microsphere.
5) Material used in Preparation of controlled release and processing are substantially costly than those of standard formulations.

II. CLASSIFICATION OF POLYMER

Polymers are classified into two types:

A. Synthetic Polymers

These type of polymers are widely used in clinical applications, furthermore they can also use as bulking agent, fillers, embolic particles, drug delivery vehicles etc. and proved to be biocompatible and safe. But the main detriment of such kinds of microspheres is they tend to deviate from injection site and cause potential risk of further organ damage. (11)

1) *Non-Biodegradable:* Acrolein, epoxy polymers, glycidyl methacrylate, poly methyl meth acrylate (PMMA), polyimminocarbonates.
2) *Biodegradable:* Glycolides and their copolymers, polyanhydrides, polyalkyl cyano acrylates, lactides, poly- ε-caprolactone, PEG, Polyurea, polyurethane, Carbopol 940

a) One of the potential drug carrier for ophthalmic, oral and parenteral preparations is poly alkyl cyano acrylates. Poly lactic acid is a proper carrier for sustained release of anti-neoplastic agents such as cisplatin, cyclo phosphamide, doxorubicin and narcotic antagonist. (12,13)
b) In late 70s, Injectable microsphere systems were developed. Poly lactide and/or poly (lactide-co-glycolide) was used to encapsulate an ester of norethisterone called NET via oil-in water emulsion/solvent evaporation process. These microcrystals of NET were encapsulated into microspheres of PLA and PLGA. In microspheres the biodegradation rate is directly linked to its composition which in turn disturbs the release profiles of API. The range for effectiveness of injection is between 20 and 90 μm. (14)

B. Semi Synthetic Polymers

They are biocompatible and non-biodegradable polymers and broadly used as encapsulating material for the controlled release of pharmaceuticals.
Ex- ethyl cellulose, Na carboxy methyl cellulose

C. Natural Polymers

They are biocompatible, biodegradable and also bio adhesive in nature. Biodegradable polymers increase the residence time when come in contact with mucous membrane due to its high degree of swelling property with aqueous medium, resulting in formation of gel. (15)

Natural polymers acquired from different sources like carbohydrates, proteins and chemically modified carbohydrates.

1) *Carbohydrates:* Starch, carrageenan, chitosan, agarose, alginate
2) *Proteins:* Gelatin, albumin and collagen
3) *Chemically Modified Carbohydrates:* Poly dextran, poly acryl starch

One of the most widely distributed natural protein is albumin. It is considered as the potential carrier for drugs or proteins and widely used in the targeted drug delivery to the tumour cells in cancer. Chitosan, a deacylated product of chitin. The chitosan impact has been thought-about owing to its charge. Gelatine microspheres may be used as carrier system capable of delivering the biological response modifiers or drugs as antiviral agents to phagocytes. (16)

NATURAL POLYMERS		Reference
Chitosan	Chitosan microspheres for the delivery of chemotherapeutic agents: Paclitaxel as a model	17
Alginate	Alginate microspheres loaded with diloxanide furoate for colon-specific drug delivery	18
Agarose	Preparation of berbamine loaded chitosan-agarose microspheres and in vitro release study	19
Carrageenan	Loading and evaluation of meloxicam and atorvastatin in carrageenan microspherical aerogels particles	20
Starch	Formulation of epichlorohydrin cross-linked starch microspheres	21
Gelatin	Formulation and evaluation of gelatin microspheres loaded with fenofibrate	22
Albumin	Preparation and Characterization of Albumin Microspheres Encapsulated with Propranolol HCl	23
Collagen	Preparation and Characterization of Collagen Microspheres for Sustained Release of Steroidal Saponins	24

III. CROSS LINKING AGENT

The crosslinking reaction is predominantly altered by the type and size of cross linker agent and the functional groups of chitosan. The smaller the molecular size of crosslinker, speedy the crosslinking reaction, since its diffusion is much easier. Depending on the nature of the crosslinker, the main interactions forming the network are ionic or covalent bonds (25). Increase in particle size results in increasing the cross-linking time and eventually increasing the drug loading. Cross-linking reactions are usually promoted through heating or photo irradiation, though the inclusion of catalysts and the irradiation of radioactive rays are also used for raise of cross-linking.

Classification- Cross linking agents are mainly classified into two categories-

1) *Physical Crosslinking:* It is achieved using interactions other than the covalent bond, such as ionic interaction or hydrogen bonding. Physical cross-links can be reversibly dissociated and recombined on heating/cooling. Physical crosslinking is preferred because it shuns the use of crosslinking agents, which are often poisonous compounds and have to be extracted or removed from gels before use.
2) *Chemical Crosslinking:* In between polymer and cross linker or polymer chains, cross linking covalent bonds are present. During the cross linking counter ions diffused into the cross linking and polymeric agents, react with polysaccharides forming either intramolecular or intermolecular linkages.

Example- glutaraldehyde, calcium chloride, barium chloride, strontium chloride, aluminum chloride, formaldehyde, vanillin, genipin, 1, 6 hexamethylenediisocynate, sulphuric acid,etc.

Because of nontoxic behaviour of calcium chloride, it is the most widely used crosslinking agent, and beads or films or thus obtained are more condense in nature

EX

- Chitosan microspheres are crosslinked with the three crosslinking agents viz, glutaraldehyde, sulphuric acid. (26)
- Tripolyphosphate (TPP), formaldehyde (FA) and glutaraldehyde (GA) preparing alginate chitosan microspheres. (27)
- Preparation of alginate chitosan microsphere through Comparison between two cross linkers, i.e. calcium ions and genipin. (27)
- Different cross-linking agents that used for the chitosan micro particulates preparation are Glutaraldehyde (28-30), 2,3-dihydroxy propanal (31), calcium chloride (32), citric acid (33), vanillin (34), sodium sulphate, sulphuric acid, genipin, tripolyphosphate and epichlorohydrin.

IV. TYPES OF MICROSPHERES

A. Bio Adhesive Microspheres

It displays an extended residence time at application site and causes familiar contact with the absorption site and improves cure. It can be used to attach the water-soluble polymers, in which the drug clings to the membrane. Bio-adhesion may be defined as the process by which a natural/ synthetic polymer can cling to a biological membrane and the mucosal layer in biological layer is called muco adhesion. Bio-adhesion may be named as drug delivery adhesion device to the membrane of mucosal such as buccal, ocular, rectal, nasal *etc.* (35-37)

1) Applications

- Design and assessment of repaglinide mucoadhesive microspheres for oral controlled release. (38)
- Nasal insulin administration as a delivery system using bio adhesive microsphere. (39)

B. Magnetic Microspheres

The delivery system that locates the drug in the disease site is critical. In this a smaller quantity of a magnetically targeted medicine, conveying a magnetic response to a magnetic field of integrated materials used for magnetic microspheres, is chitosan, dextran etc. replaced larger amount of freely circulate drug. This technology is focused on binding the establishment of a Ferro fluid anti-cancer compound, which uses magnetic fields to focus the drugs in the tumour site. In these more freely circulating drug can be substituted by smaller amount of drug targeted magnetically. A newer approach, the microsphere magnetically controlled releasable supramolecular particles with a particle size between1-1000 μm is a newer approach in pharmaceutical field. Magnetic microsphere drug delivery system is captured in micro vessels and magnetic fields below 0.5-0.8 tesla drawn into adjoining tissues. (40)

1) Magnetic therapeutic microspheres can be used to target drugs such as protein and peptides, and also for chemotherapeutic products in the liver tumour supply.

2) Diagnostic microspheres that have been used to imagine liver metastases, as well as to distinguish bowel loops from other abdominal structures by forming supra magnetic iron oxide particles of Nano size. (41,42)

C. Radioactive Microspheres

This type of microspheres gives the target areas high radiation doses without affecting the surrounding tissues. For the therapeutic purposes, radioactive particles (10–30 μm) are used in the veins connected to the target organ or tissue directly through injection. Three types of waves emit from these radioactive particulates: α emitters, β emitters and μ. The microsphere 10-30 nm are larger than the capillary radio emobilisation treatment and is tapped when they are met in the first capillary bed. (43)

1) Application: Hepatocellular carcinoma loco regional therapy: radio embolization with yttrium-90 microspheres. (44)

D. Floating Microspheres

This type of microphone is advantageous in Gastro retention due to the fact that the bulk density is less than the gastric fluid and therefore keeps the stomach buoyant without affecting the gastric vacuum rate. The medication is released at the optimal rate slowly and the device is observed to float at gastric content and to increase gastric residence and fluctuation in plasma concentration. The risks of hitting and dose dumping are also decreased. (45,46)

1) Application: Formulation and assessment as Gastro retentive Dosage of floating cephalexin microspheres. (47)

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E. Polymeric Microspheres

The polymer microspheres are graded as biodegradable and synthetic polymer microspheres.

1) *Biodegradable Polymeric Microspheres:* It consists of biodegradable, biocompatible and bio adhesive natural polymerics such as starch. Due to its high degree of swelling property and its aqueous medium, biodegradable polymers extend the duration when interaction with the mucous membrane results in gel formation. The rate and extent of the release of drugs are regulated on a sustained basis via polymer concentration and release pattern. (48). Application- An infectious disease application of Biodegradable polymer microsphere-based vaccines. (49)
2) *Synthetic Polymeric Microspheres:* They consist of synthetic polymers used as bulking agents, fillers, embolic particles, medicinal products, etc. It is disadvantageous that they tend to migrate from the injection site and cause risk, embolism and additional organ damage.

V. TECHNIQUES USED FOR THE PREPARATION OF MICROSPHERE

The method selection for the preparation of microsphere depends on particle size, polymer nature as well as drug nature, interlinking stage, route of administration, drug release duration, evaporation time etc.

Various methods of preparation are-

A. Solvent Evaporation Method

This is the best-known method of preparing drug-loaded microparticles from water insoluble polymers such as PLA, PLGA and polycaprolactone. It is commonly used for the encapsulation of insoluble or poorly water-soluble active ingredients.

1) *Single Emulsion Technique:* Through this technology, natural polymers are produced by this technique, the micro particulate carriers of the proteins and the carbohydrates.

It involves three steps-

a) Firstly, select any aqueous polymer solution that is followed by non-aqueous oil scattering.
b) Then heat it. After heating, cross linking step is followed by two methods- either by heat (it is inappropriate for the thermo labile drugs) or by chemical cross linkers (agents are glutaraldehyde, formaldehyde, di acid chloride etc.). Chemical cross-related disadvantages of the excessive exposure to chemicals of the active ingredient if added during preparation.
c) Microsphere is then produced in an organic phase and then centrifuged, washed and separated. (50)

2) *Double Emulsion Technique:* In this multiple emulsion or the double emulsion type w/o/w are produced. It is produced by dispersing in an organic stage any aqueous polymer or drug solution. Then there is primary emulsion. Then the primary emulsion is homogenised or sonicated before being added to the polyvinyl alcohol (PVA) aqueous solution. The first emulsion then becomes a multiple emulsion. Either solvent evaporation or solvent extraction evaporates the solvent. Then Addition of a large aqueous phase there is a microsphere in a solution and subsequently removed, washed and dried. (51)

a) Colon-targeted anti-cancer microsphere Capecitabine has been developed successfully by using HPMC, EC separately and in combination for colonic drug delivery. Emulsion solvent evaporation approach was used to prepare spherical and free-flowing microspheres. (52)
b) Preparation and microsphere characterisation of anti-inflammatory aceclofenac drug. The ultimate purpose of this formula was to limit the release of drugs in the upper GI area and thereby prevent their local side effect. The drug used for the formulation of the microsphere was O / W emulsion solvent evaporation, and targeted colon encapsulation within Eudragit S100, RS 100 and RL100. (53)

B. Spray Drying and Spray Congealing

The technique of sprays drying relies on the solvent removal or cooling of solution, the methods are spray drying & spray congealing. In spray drying, evaporation is the fundamental process, whereas a reversal phase from a liquid to a solid is a spray congealing. Firstly, the polymer is dissolved into an appropriate organic volatile solvent, like dichloromethane, acetone, etc. The presence of drug is mainly in solid form. Then the solution is scattered under homogenization at high speed and then atomised in a hot air stream. At that time, atomization results in the formation of the small droplets or the fine mist, from which the solvent instantly evaporates, resulting in microsphere formation. The cyclone separator is used to extract a residue of the solvent by drying the vacuum from all microparticles. (54).

He et al. has prepared the spray drying method for the delivery of cimetidine, Famotidine and Nizatidine for both uncross linked and linked chitosan microparticles. (55)

Conti et al. has developed Cetylpyridinium Chloride, a spray-drying technology to expose spray dried particulate matter to vapours with cross linking agents, as an anti-infective agent chitosan microspheres. During the time of exposure to cross-linkers, the extent of cross linking was controlled. (56)

C. Polymerization Techniques

The polymerization techniques are primarily categorised as for microsphere preparation:

1) Normal polymerization

2) Interfacial polymerization. Both are carried out in liquid phase

a) *Normal Polymerization:* The normal polymerisation process is carried out using a variety of technologies such as bulk, suspension, precipitation, emulsion and micellar polymerisation. In bulk, in order to initiate polymerisation, a monomer or mixture of monomers together along with the initiator or catalyst is normally heated. The polymer can be moulded as microsphere thus obtained. During the polymerization process, drug loading can be carried out. In Suspension polymerization, it is also referred as bead or pearl polymerization. It is achieved in a continuous aqueous process by heating the monomer or mixture sof monomers as droplets dispersion. The suspension techniques are less than 100 μm of the microsphere size. In Emulsion polymerization, it differs from suspension polymerization because of the aqueous stage presence of the initiator which later diffuses to the micelles surface.

b) *Interfacial Polymerization:* The interface between the two immiscible liquid phases requires the reaction of different monomers to form a polymer film, which basically envelops the dispersed phase.

D. Solvent Extraction Method

It is most widely used in a preparation of microspheres. It involves removal of solvent which is to be an evaporated. First polymer dissolve in a volatile organic solvent with addition of drug particles. Then add the solution which contain surfactant and continuous stirring on it then the solvent particles are subjected to an agitating method, to the aqueous phase to be extracted during the solvent evaporation phase. Then the microspheres are removed and dried. (57)

Preparation and assessment of metformin microspheres to achieve their long formulation of release. Microspheres for prolonged releases have been prepared with polymers such as ethyl cellulose, HPMC, carbopol934 and chitosan and method used was non-aqueous solvent evaporation. (58)

E. Coacervation phase Separation Method

The coacervation theory decreases polymer solubility in the organic phase to impact polymer-rich phase formation known as coacervates. The first is to disperse drug particles in a solution of polymer and an incompatible polymer is added to the system that allows the first polymer to phase out and absorbs the drug particles. This process was used for preparing poly-lactic acid microspheres (PLA), which was incompatible polymer such as butadiene. The agglomeration must be avoided by stirring the suspension with an adequate speed stirrer, as the forming of the microsphere starts to begin and formed polymerize globules start to stick and form the agglomerates. Thus, the process variables are very critical as the rate at which coacervates are achieved determines the distribution of the polymer film, its particle dimensions and its agglomeration.

F. Ionic Gelation Method

It is most important method that is to be used in a preparation of microspheres. Firstly, in distilled water, sodium alginate or any other polymer can be dissolved. On continuously stirring viscous dispersion is formed. Then add any active pharmaceutical ingredient. After that add any active pharmaceutical ingredient. At that moment take drop wise calcium chloride through syringe. Then added droplets are retained and curing reaction get take place. Therefore microspheres are formed and collected by decantation. Product separated, washed & dried for 12 hours at 45˚c. (59)

Formulation and assessment of microspheres Nateglinide. The ultimate goal was to formulate sustained release microspheres which help to enhance patient compliance. Microspheres have been formulated by the use of different proportions of biodegradable polymers, such as Olibanum gum and Guar gum. The method used was the ionic gelation method for calcium chloride / sodium alginate. (60)

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VI. CHARACTERIZATION

A. UV Spectra

A UV – visible spectrophotometer was used to determine drug loading in composite microspheres and encapsulation efficiency. Briefly, whether 20 or 50mg of the microsphere were accurately weighed in solvent mixture in a conical flask. They were put in an orbital shaking incubator and shaken for 24 hours at 100 rpm.

The resulting solution was filtered by using a Whatman filter paper, diluted with respective solvent system and by using UV–visible spectrophotometer, concentration was determined at 214nm. By comparing the quantity of drugs actually incorporated to that of the initial products, the efficiency of drug encapsulation was calculated.

1) Drug loading (%) = (Weight of drug loaded (g)) / (weight of microspheres(g))
2) Encapsulation efficiency (%) = (weight of drug loaded (g)) / (weight of initial drug(g))

B. Scanning Electron Microscopy (SEM)

A scanning microscope was used for visual analysis of surface morphology and cross-sectional structures of the monolithic system with and though not the drug for micrographs in microspheres. Samples were mounted on metal stubs through double-sided adhesive tape and vacuum-coated with gold film under reduced pressure and analysed (61). Samples were then cross-sectioned using a blade to examine the internal structure. The size was then determined by the microspheres average size within the micrographs (three micrographs each containing approximately 20 to 25 micrographs).

C. Raman Spectroscopy

Raman spectroscopy is used in recent years for the screening of anti-cancer products. It describes the interaction between light and molecular or atomic vibrations in a solid. Aromatic or conjugated domains with high Raman dispersion activity are found in active pharmaceutical ingredients. Therefore, Raman spectroscopy is an enticing alternative conventional pharmaceutical analysis approach (62) that contains information regarding molecular vibration and offers a very precise fingerprint of the target molecule (63). Because of the advantages of a narrow spectra and a near-infrared excitation method, Raman spectroscopy is commonly used in cell and in vivo imaging. However, its low signal intensities which can be overcome by increasing the intensity of Raman scattering, whether by resonant excitation or surface enhancement, severely limit direct applications of Raman spectroscopy. (64)

D. FTIR

Samples were dispersed by potassium bromide (KBr) powder in the Fourier transform infrared spectroscopy and 5-ton pressure were applied to form pellets. Powder diffuse reflection of the FTIR spectrophotometer has been obtained from the FTIR spectra. The drug polymer interaction and while processing for microencapsulation the degradation of drug can be determined by FTIR.

E. Optical Microscopy

The OM method for particle size and counting includes:

1) The homogenisation in a non-dissolving liquid of test particles;
2) Identification and image capture, using a combination of brightfield microscopy and traceable counting chambers of a representative test ensemble followed by
3) Conversion of the captured images into a particle size distribution using image processing.

Briefly, as little as 10 µL of the homogenous dispersed particles, by capillary action, into a counting chamber, following a haemocytometer. The range of observable dimensions is defined only by a microscope, as all microscopic techniques can in principle be used. (65)

F. Particle size (Master Sizer)

For particle size analysis, Mastersizer 2000 uses the Mie theory which assumes spherical particles. For the determination of particle dimensions, a homogenous aqueous dispersion of microspheres was used. For non-spherical particles, Mastersizer uses an equivalent spherical method for estimating the diameter of the particles based on the volume of an equivalent sphere. A laser scattering by a Mastersizer 3000 measured the mean volume diameter and particle size distribution of the microsphere.

G. In Vitro Release Studies

To measure the amount of drug released through the microspheres, well-known samples have been placed into a cell of 50mL of buffer solution (pH 1.2, pH 6.8 and pH 9.0).

The samples (1mL) were removed at set times and thus fresh release media replaced the solutions. At 37ºC, all release studies have been done in a shaker at 100rpm.

The release experiments have been performed for 2 hours with simulated gastrical fluid (pH 1,2), followed by 4 hr and 6 hr with simulated intestinal fluids (pH 6,8 and pH 9.0) respectively. The total residence time in buffer solution was 12 h.

In each sample the drug concentration was then determined by spectrophotometrically at 280 nm. The cumulative drug release percentage was determined and the mean of three determinations were used for data analysis.

The amount of drug discharged from the calibration curve in combination with the absorbance has been measured at any selected time (Mt). The maximum weight available for release (M∞) was determined as Mt, the fractional release (Ft) was then calculated as Ft = Mt / M∞. (66)

VII. EVALUATION

A. Particle Size And Shape

1) Mainly two techniques, i.e. conventional light microscopy (LM) and electron microscopy scanning (SEM) are used to visualise microparticles. Using both technique, shape and outer structure of microparticles can be determined.

2) LM allows in the case of double walled microspheres to resolve the coating parameters. Structures can be seen before and after the coating in this microsphere and the change can be microscopically measured.

3) In comparison to LM, SEM gives higher resolution. Dry microspheres were mounted in a black adhesive tape covered electron microscope stub and observed under a microscope after the application of vacuum. SEM facilitates investigation of the surfaces of the microsphere and cross-sectioned particles. (67)

B. Electron Spectroscopy for Chemical Analysis

It is also called an X-ray photoelectron Spectroscopy (XPS) that can be used for the determination of surface chemistry, atomic surface composition, and the spectra provided by ESCA used for the determination of biodegradable microsphere surface degradation.

C. Micrometric Method

1) *Bulk Density:* It's a known mass of microspheres in graduated measuring cylinder. A ratio from microsphere weight in gram to bulk volume of microspheres in cm3 is determined to determine the bulk density.

2) *Tapped Density:* Tapped density is the powder quantity calculated by tapping with a cylinder with pre-weighted sample quantity. A ratio of the weight of microsphere in gram to the volume of microsphere after tapping in cm3 was calculated for tapped microsphere density.

3) *Carr's Index:* At the initial stage & after 1250 tapping to constant volume, the Carr's index was calculated by powder volumes. Carr's index was calculated by following equations:

Carr's index= (Tapped density - Bulk density) × 100/Tapped density

The density of the particles of the apparent microspheres has been measured with Pycnometer.

4) Hausner's ratio - Hausner's ratio was determined by following equations: Hausner's ratio= Tapped density/Bulk density

D. Isoelectric Point

Micro electrophoresis is an apparatus for determining the electrophoretic mobility of microsphere which can be used for the determination of the isoelectric point. The electrophoretic mobility can be correlated with the surface charge, an ionising action or the nature of the microspheres' ion absorption.

E. Angle of Contact

In evaluating the wetting property of a micro-particle carrier, the angle of contact is measured. In terms of hydrophilicity or hydrophobicity, it determines the nature of microsphere. At the solid air / water/ interface, the contact angle is measured.

F. In vitro

As a quality control technique in pharmaceutical manufacturing, growth etc., in vitro drug releases studies were used.

1) *Beaker Technique:* This technique is used to adhere to the beaker 's bottom containing the medium and to stir the overhead stirrer evenly. The literature used for the analysis ranges in volume from 50 to 500 ml and the speed of the stirrer ranges from 60 to 300 rpm.
2) *Interface Diffusion System:* Dearden & Tomlinson have developed this system. There are four compartments. A is the oral cavity which held a appropriate drug concentration initially in a buffer. Compartment B of the buccal membrane containing 1-octanol and compartment C representing body fluids of 0.2 M HCL. The compartment D representing protein binding also included 1-octanol. Prior to application, both the aqueous phase and 1-octanol were saturated. Samples were removed and returned with a syringe to compartment A. (68)
3) *Modified Keshary Chien Cell:* In the laboratory a specialised apparatus was created. It consisted of a Keshary Chien cell containing distilled water (50ml) at 370 C as dissolution medium. G lass tube fitted with a 10 # sieve in the bottom, TMDDS (Trans Membrane Drugs Delivery System) was reciprocated at 30 strokes per min in the medium. (69)
4) *Dissolution Apparatus:* For in-vitro release profiles with rotating elements, paddle and basket, standard USP or BP dissolution apparatus were used. Dissolution medium used in the study ranged between 100 and 500 ml and a rotational speed between 50 and 100 rpm. (70)

G. Entrapment Efficiency

For each batch, drug entrapment efficiency in microspheres was determined by the percentage of drug entrapment in accordance with the following formula, % entrapment efficiency= (practical drug content/ theoretical drug content) ×100.

The microsphere-containing drug had been powdered and suspended in 100 ml of methanol water solvent. The dispersion of Whatman filter was retentioned for 20 minutes for continuous mixing with magnetic stirrer, while the UV spectroscopy against blank methanol containing 10 mg of blank microsphere was analysed. (71)

H. Swelling Index

Firstly, dried microsphere has been carefully weighed in the given buffer and performed. It determines the drug mucilage content. The exact weighted quantity of microspheres in a given buffer was allowed to swell to confirm the complete equilibrium. Excess liquid surface drops were eliminated by blotting & the swollen microspheres weighed by using microbalance. Its diameter is routinely determined by means of a laser particle sizing distribution analyser until erosion and dissolution have decreased it. The swelling index (S %) can be calculated using formula- Swelling index (S %) = (mass of swollen microspheres – mass of dry microspheres/mass of dried microspheres) 100. (72,73)

VIII. DRUGS AND THEIR ACTIVITY

DRUG	EXCIPIENTS	METHOD OF PREPARATION	CHARACTERIZATION	IN VIVO/IN VITRO ACTIVITY	REFERENCE
Paclitaxel	PLGA, polycaprolactone, Dichloromethane	emulsion solvent evaporation method	FTIR spectrophotometer, DSC, SEM	In vivo drug release study was conducted in rats.	74
Methotrexate	Bovine serum albumin, Span80, and glutaraldehyde	emulsion cross-linking method	SEM, IR spectrophotometer, compound microscope	*In vivo* drug release study was conducted in Bagg Albino mice.	75
Docetaxel	Chitosan, liquid paraffin, glutaraldehyde , mannitol	emulsion crosslinking method	TEM, dynamic light scattering, UV spectrophotometer	i.v. administration of the DTX-loaded microspheres to mice was investigated with DTX injection. In vivo experiments have shown that microspheres have been found to release the drug to a maximum extent into the target tissue (lung)	76
5-fluorouracil	PEG , formaldehyde,chitosan, Tween-80, liquid paraffin oil, formic acid	emulsion-cross-linking followed by the solvent evaporation technique	SEM, UV spectrophotometer, DSC	Dimethyl-hydrazine-induced colorectal cancer in albino male Wistar rats was evaluated for in vivo activity	77

6-thioguanine	PLA-co-PEG	continuous solvent evaporation technique	SEM, UV-Vis spectrophotometer	Study of in-vitro release of drug using the phosphate buffer pH (7.4) as dissolution media was conducted using dialysis bag diffusion method.	78
Cisplatin	Poly(benzyl l-glutamate),PVA, tween 80	Solvent evaporation	SEM, UV spectrophotometer, DSC	*In vivo* drug release study was conducted in mice.	79
Anastrozole	Polyvinyl alcohol, iron(II/III)oxide, Poly ε-caprolactone, dichloromethane	O/W emulsion- solvent evaporation technique	SEM, optical microscopy, UV/ Vis spectrophotometer, FTIR, DSC	The phosphate buffer (pH 7.4) was used in in vitro release experiments for formulate magnetic microspheres at 37.5 ± 0.5 ° C.	80
Sulforaphane	Bovine serum albumin, glutaraldehyde, Ammonium hydroxide, Iron oxide	spray-drying	SEM, optical microscopy, UV/ Vis spectrophotometer, DSC	To induce tumors in C57BL/6 mice, 2 × 106 B16 melanoma cells was suspended in RPMI-1640 medium and implanted subcutaneously between the ears of the mice.	81
Dexamethasone	PLGA, PEG, PVA, Dichloromethane	oil in water (o/w) emulsion solvent evaporation technique	XRD, FTIR, RP-HPLC, TEM, UV/Vis spectrophotometer	In 6 mL of PBS in screw-capped test tubes, 10 mg of DEX-PLGA MS is suspended. In a shaking water bath at 37 ° C, the tubes were held under constant shaking (60 rpm)	82
Vancomycin	Bovine serum albumin, glutaraldehyde, albumin	spray-drying method	SEM, UV spectrophotometer, DSC, optical microscopy	Tablet dissolution tester fitted with eight baskets were used for dissolution. At a constant 100 rpm speed, dissolution rates were calculated at 37±0.5 ° C. Microsphere releases of drug at 1.2 pH as well as a phosphate buffer solution at 7.4 pH have been investigated.	83
Tamoxifen	PCL/PHB, Gelatin, Dichloromethane	w/o/w modified double emulsion solvent evaporation method.	FT-IR, DSC, SEM, X-RD, UV spectrophotometer, optical microscopy	Tablet dissolution tester fitted with eight baskets were used for dissolution. At a constant 100 rpm speed, dissolution rates were calculated at 37±0.5 ° C. Microspheric releases of drug at 1.2 pH as well as a phosphate buffer at 7.4 pH have been investigated.	84
Capecitabine	Ethyl cellulose, PVA,PEG, hydroxyl ethyl cellulose, Dichloromethane	emulsion solvent evaporation method	SEM, FTIR, DSC, optical microscopy, UV spectrophotometer	In SGF (0.1N HCl, pH 1.2) for the first 2 hours, followed by SIF (0.05 M potassium dihydrogen phosphate, pH 7.4 phosphate buffer), up to maximum dissolution was investigated for in-vitro drug release	85
Idarubicin	ethyl cellulose, dichloro methane, ethanol, HPMC	solvent evaporation meth od	optical microscopy, UV spectrophotometer, SEM	A USP XXVI rotating basket method at 50 rpm at 37 ± 0.05o C was used for the in-vitro release study of microsphere.	86
Imatinib mesylate	Poly (Lactic Acid), Gelatin and Collagen	W/O/W double emulsion solvent evaporation method	FTIR, DSC, SEM, XRD, UV spectrophotometer	The Tablet dissolution tester fitted with six paddles with a paddle speed of 100 rpm was investigated for in vitro release studies at 370 C.	87
Gemcitabine	Chitosan, acetic acid, dimethylsulfoxide	Spray drying method	SEM, UV-vis spectroscopy,	The phosphate buffer solution (PBS, pH 7.4) at 37±0.5 ° C was investigated for in vitro release tests.	88

TABLE 2 Works on natural polymers based microspheres as shown in the Table 2

POLYMER	CROSS LINKING AGENT	CHARACTERIZATION	METHOD OF PREPARATION	REFERENCE
Sodium alginate	Calcium chloride	UV spectrophotometer, SEM, Malvern Instrument	Modified emulsification method	89
Guargum	Glutaraldehyde	optical microscopy, SEM, UV spectrophotometer	Emulsification method	90
Chitosan	Glutaraldehyde	DSC-60, SEM, UV spectrophotometer, optical microscopy	emulsion crosslinking method	91
Drumstick gum	calcium chloride	UV-VIS spectrophotometer, Mechanical sieve , SEM	orifice ionic gelation method	92
Gum Acacia	Glutaraldehyde	SEM, FTIR and DSC	single step emulsion in-situ crosslinking technique	93
Albumin	Glutaraldehyde	optical microscope, Metler DSC-7, SEM	heat denaturation method	94
Chitosan (CS) and Carrageenan (CR)	Glutaraldehyde	FTIR, DSC, X-RD, SEM, UV spectrophotometer	water-in-oil emulsion technique	95
Bael fruit gum	sodium trimeta phosphate	FTIR, DSC, SEM, UV spectrophotometer	emulsification method	96
Gellan	Calcium chloride	UV spectrophotometer, SEM, Malvern Instrument, optical microscopy	water-in-oil emulsification	97
Tragacanth	Barium chloride	UV spectrophotometer, SEM, optical microscopy	Orifice ionic gelation method	98

IX. CONCLUSION

The current review article demonstrates that microspheres are a superior drug delivery system. Microspheres have received a lot of attention, not only for their long-term release, but also for targeting anticancer drugs to the tumor. In the gastrointestinal tract drug absorption is a highly variable procedure, and prolonging gastric retention of the dosage form prolongs drug absorption time. These natural polymers have proven to be effective in biomedical applications as well as as a carrier for pharmaceutical preparations.

A. Abbreviation

1) FT-IR = Fourier transform infrared spectroscopy
2) DSC = Differential scanning calorimetry
3) SEM = Scanning electron microscopy
4) TEM= Transmission electron microscope
5) X-RD= X-ray diffraction studies
6) PCL= Poly(ε-caprolactone)
7) PHB= Poly ((R)-3-Hydroxybutyric acid)

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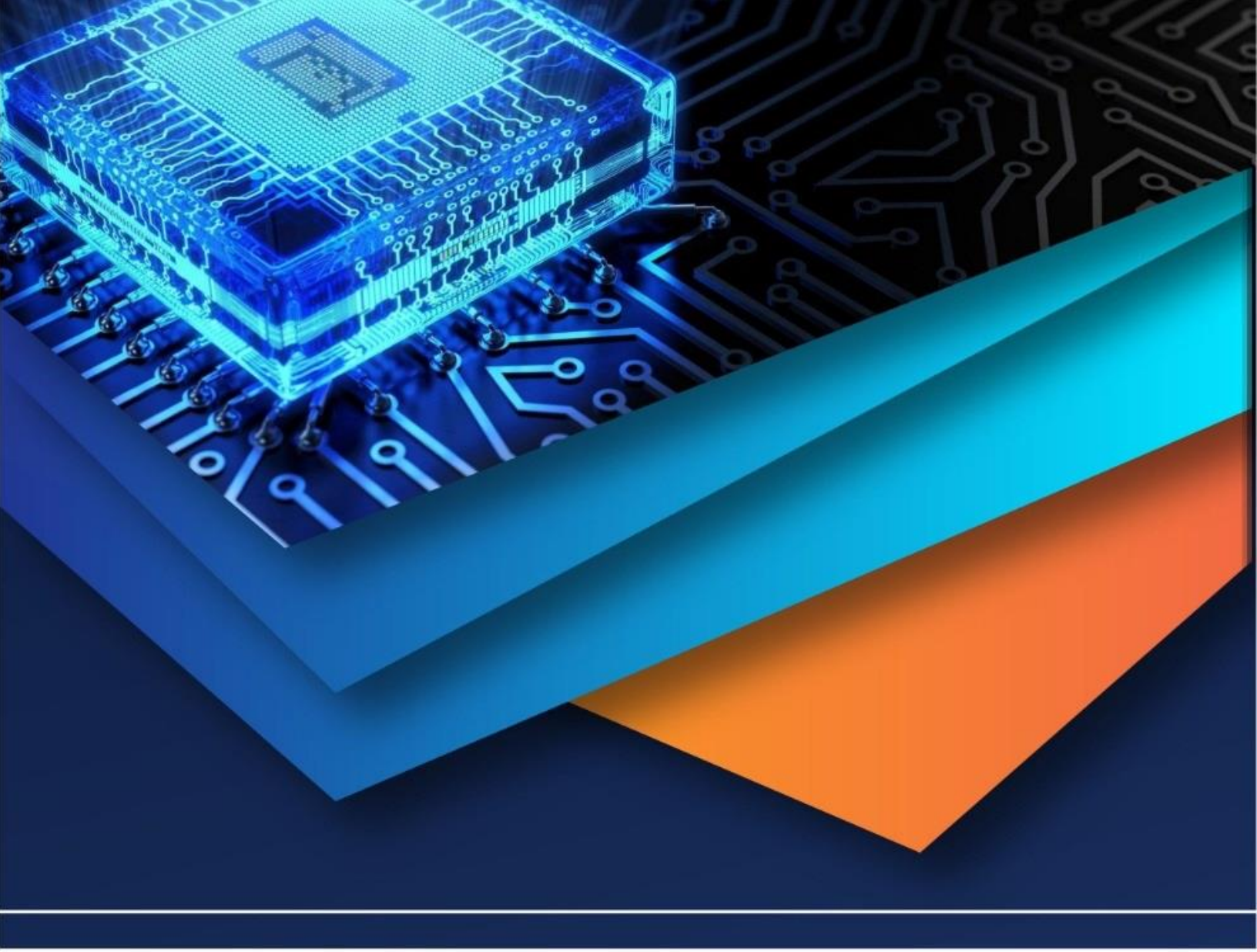

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