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Review Article

# RECENT TRENDS IN MICROSPHERE DRUG DELIVERY SYSTEM AND ITS THERAPEUTIC APPLICATIONS – A REVIEW

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## ABSTRACT

Microspheres constitute an important part of novel drug delivery system by virtue of their small size and efficient carrier capacity. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 µm. The range of Techniques for the preparation of microspheres offers a Variety of opportunities to control aspects of drug administration and enhance the therapeutic efficacy of a given drug. 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 also known as micro particles. It is the 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. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs. The purpose of the review is to compile various types of microspheres used in the formulation of microsphere drug delivery system and its applications

**Keywords:** Microspheres, controlled release, target site, novel drug delivery.

## INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres (Fig No: 1) vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have

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numerous applications depending on what material they are constructed of and what size they are.

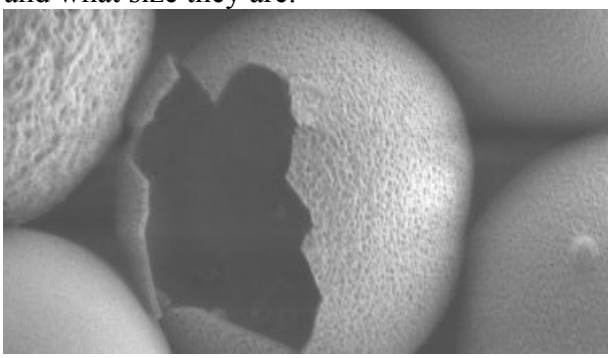

Fig No: 4 Hollow biodegradable capsules after core-liquid removal

With advances in biotechnology, genomics, and combinatorial chemistry, a wide variety of new, more potent and specific therapeutics are being created. Because of common problems such as low solubility, high potency, and/or poor stability of many new drugs, the means of drug delivery can impact efficacy and potential for commercialization as much as the nature of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery. Indeed, drug delivery systems—designed to provide a therapeutic agent in the needed amount, at the right time, to the proper location in the body, in a manner that optimizes efficacy, increases compliance and minimizes side effects.

Polyethylene and polystyrene microspheres are two most common types of polymer microspheres. Polystyrene microspheres are typically used in biomedical applications due to their ability to facilitate procedures such as cell sorting and immune precipitation. Proteins and ligands adsorb onto polystyrene readily and permanently, which makes polystyrene microspheres suitable for medical research and biological laboratory experiments. Polyethylene microspheres are commonly used as permanent or temporary filler. Lower melting temperature enables polyethylene microspheres to create porous structures in ceramics and other materials. High sphericity of polyethylene microspheres, as well as availability of colored and fluorescent microspheres, makes them highly desirable for flow visualization and fluid flow analysis, microscopy techniques, health sciences, process troubleshooting and numerous research applications.

Glass microspheres are primarily used as filler for weight reduction, retro-reflector for highway safety, additive for cosmetics and adhesives, with limited applications in medical technology. Microspheres vary widely in quality, sphericity, uniformity of particle and particle size distribution. The appropriate microsphere needs to be chosen for each unique application.

**a. Microsphere production for Drug Delivery**

Biodegradable polymer microspheres can work as miniature time release capsules for parenteral drugs, within pharmaceutical and biotechnology industries. Produced sometimes for injectable drugs, they require an aseptic manufacturing process and an accurate selection of the microsphere sizes, typically 5μm up to 250μm. Formulation of polymer microspheres is a complex process where classification of the correct size microspheres remains the most difficult task. Typically with microsphere processes, large liquid volumes with a small ratio of suspended solids have to be handled. Thus some processes require a scalping pre-filtration step in order to reduce the liquid volume and eliminate the over-sized microspheres. Suspended

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microspheres obtained from various micro encapsulation processes then require unique handling that differ from a typical filtration and drying operation. Following the synthesis stage, microspheres require being washed, classified by size, filtered then dried under appropriate conditions to gain the final free flowing injectable or inhalation microsphere product. Microspheres are random in size and need to be filtered and classified into the micron size range desired before drying. Microsphere Refiners are process equipment designed to meet these criteria from washing to classifying and drying [1].

**b. Pharmaceutical application**

Biologically active peptides and proteins have been delivered with the help of biodegradable microspheres. Sustained-release characteristics of microspheres reduce the need for frequent administrations and enhance patient compliance by maintaining in vivo drug levels in the therapeutic range. Poly (D, L lactide) (PLA) and poly (D, L-lactide-co-glycolide) (PLGA) are the most widely used and well characterized polymers for biodegradable micro-spheres [2-4].

Microspheres have also applications in injectable and inhalation products[5-7, 8]. Microcapsules are also used as diagnostics, e.g. temperature sensitive microcapsules for thermo graphic detection of tumours[9]. Scratch-n- sniff (microencapsulated aromas) has been used in children's books and food and cosmetic aroma advertising[10]. Microspheres are used for isolating materials until their activity is needed. The bio technology industry employs microspheres to contain organisms and their recombinant products to aid in the isolation of these products[11].

A number of pharmaceutical microencapsulated products are available in the market, such as aspirin, theophylline and its derivatives, vitamins, pancrelipase, antihypertensive, KCL progesterone and contraceptive hormone combinations[12]. Medically microsphere has also been used for the encapsulation of live cells and vaccines. The biocompatibility of artificial cells and biomolecules such as peptides, proteins, and hormones can be improved by encapsulation which can prevent unwanted immunological reactions that would lead to inactivation or rejection[13,14].

**APPLICATIONS OF MICROSPHERES:** Applications are explained as follows –

(A) **Microsphere-Based Vaccine Prevents and Reverses New-Onset Autoimmune Diabetes**

Type 1 diabetes is a disorder of glucose homeostasis caused by a chronic autoimmune inflammation of the pancreatic islets of Langerhans [15]. The ultimate outcome is the loss of insulin-producing cells to numbers below a threshold that is critically required to maintain physiological glucoregulation. Before this threshold, however, escalating inflammation around (peri-insulitis) and in the islets of Langerhans (insulitis) first renders the insulin-producing β-cells insensitive to glucose and incapable of appropriate insulin production mainly due to the actions of cytokines like interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β [16,17].

On clinical confirmation, a large number of type 1 diabetic patients still exhibit evidence of residual β-cell mass that, for a limited time, is functionally responsive to glucose and produces insulin (the so-

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called "honeymoon period")[18]. In fact, patients with a residual β-cell mass manifest better glycemic control and improved prognosis for diabetic complications including retinopathy and nephropathy. These observations have compelled investigation into agents that can be used at the time of clinical diagnosis to preserve residual β-cell mass primarily by intervening with the ongoing autoimmunity. The use of pharmacological systemic immunosuppressive drugs met with initial success in controlling autoimmunity, however, on withdrawal, the autoimmunity recurred, indicating that systemic agents would need to be administered long-term with their associated adverse effects [19,20].

More recently, clinical reversal of hyperglycemia has been achieved by anti-CD3 antibody administration, although some questions linger regarding mechanism of action in the transient immunodepletion and associated cytokine-related side effects[21,22].

Finally, despite the initial observations in adults, administration of a peptide derived from HSP60 into new-onset diabetic children failed to exhibit any benefit compared with control subjects [23,24].

A need therefore remains for a diabetes-suppressive immunotherapeutic agent that does not engender nonspecific systemic immunosuppression.

It is generally accepted that the initial wave of infiltrating immune cells in type 1 diabetes immunopathogenesis consists mainly of antigen-presenting cells homing into the islets in response to an as-yet-unidentified microenvironmental anomaly [25]. Although not completely resolved mechanistically and temporally, this anomaly, in a chronic process, compels migratory antigen-presenting cells, and most prominently dendritic cells, to acquire β-cell–resident antigens derived from apoptotic and/or necrotic β-cells. The migratory dendritic cells then undergo an intrinsic "maturation" program that renders them capable of activating T-cells (including autoreactive, β-cell–specific T-cells) as they accumulate inside the draining pancreatic lymph nodes [26-28].

Dendritic cells, however, also have the capacity to activate and maintain immunoregulatory, "suppressive" cell networks. Apparently, they are regulatory when in a state of functional "immaturity" [29-31].

Functional immaturity can be conferred to dendritic cells partly by downregulating costimulatory pathways using systemic and molecule-specific approaches [32]. Numerous studies have confirmed that exogenous administration of functionally immature dendritic cells can facilitate allograft survival and can also prevent autoimmune disease and its recurrence [32]. We have shown that administration of dendritic cells from NOD mice with low-level expression of CD40, CD80, and CD86 (induced by ex vivo treatment with antisense oligonucleotides targeting the 5′ ends of the respective primary transcripts) into syngeneic recipients can considerably delay and prevent the onset of disease [33,34].

This approach is now in a clinical trial in which autologous dendritic cells generated in vitro from leukapheresis products are being administered to established type 1 diabetic adult patients to determine safety (M.T. and N.G., personal communication; FDA IND BB-12858). Despite the promise of this study, it has encountered cumbersome logistical requirements to generate these

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dendritic cell embodiments. Concurrently pursuing an alternative method to stabilize dendritic cell immaturity directly in vivo.

Many studies confirm that microparticle carriers can direct dendritic cells to the administration site, and once phagocytosed, the contents can shape the dendritic cell functional phenotype [35,36].

Yoshida and Babensee [36] showed that biodegradable poly-(lactic-co-glycolic acid) (PLGA) microspheres actually induce dendritic cell maturation by upregulating the CD40, CD80, and CD86 costimulatory molecules. The studies required a nucleic acid delivery system that would be phagocytosed by dendritic cells without upregulating these costimulatory molecules. Therefore chose to incorporate antisense oligonucleotides directed against CD40, CD80, and CD86 into PROMAXX microsphere delivery system (Baxter Healthcare). The inert PROMAXX microsphere technology has been shown to be safe and effective in human trials [37]. More importantly, when administered in vivo, this technology is neutral with respect to dendritic cell maturation state compared with the known immunostimulatory properties of PLGA-based formulations [36]. This neutrality on dendritic cell maturation is a critical criterion in adapting microsphere chemistry for immunosuppressive objectives in which dendritic cells are involved as mediators. Herein, the report showsa PROMAXX-microsphere–based vaccine in which the antisense oligonucleotides were shown to render dendritic cells diabetes suppressive[33,34] and to prevent and even reverse new-onset autoimmune diabetes.

**(B) Microspheres in ocular delivery systems**

One of the main problems in ophthalmic drug delivery, the rapid elimination of conventional liquid eye drops from the eye, still remains unsolved. A number of factors, namely rapid tear turnover and the resulting precorneal loss, induction of tear flow due to irritation caused by the drug preparation, as well as the relatively large volume of the administered eye drop (-50 ~1 versus 7 ~1 of cornea1 tear film), lead to a high rate of lacrimal drainage. Due to the resulting elimination rate. the precorneal half life of drugs applied by these pharmaceutical formulations is considered to be between about 1-3 min. As a consequence, only the very small amount of about l-3% of the dosage actually penetrates through the cornea and is able to reach intraocular tissues [38,39,40]. The poor productive absorption, on the other hand, results in a high amount of drug that is drained into the nose or into the gut. Especially the nose but also the gut are very efficient absorption organs of the body. This in turn leads to an extensive systemic absorption and may result in unwanted side effects and toxicity of the drug [41]. Although these problems have been recognised for a long time, surprisingly little effort has been made by drug companies to improve the situation, and only very few alternative ocular drug delivery systems are on the market.

One possibility for such systems is the employment of small particles. These colloidal particles have the advantage that they may be applied in liquid form just like eye drop solutions. Thus they avoid the discomfort that is combined with the application of viscous or sticky

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preparations such as ointments. The latter preparations lead to a total blurring of vision if they are properly utilised. Large inserts, on the other hand. Are difficult to administer or if they are designed as non-dissolving inserts they are even more difficult to remove, especially by elderly patients. Microparticles and microcapsules have a particle size above 1um. Microspheres are monolithic particles possessing a porous or solid polymer matrix, whereas microcapsules consist of a polymeric membrane surrounding a solid or liquid drug reservoir.

**(c) Microspheres as a nasal drug delivery system.**

All types of microspheres that have been used as nasal drug delivery systems are water-insoluble but absorb water into the sphere's matrix, resulting in swelling of the spheres and the formation of a gel. The building materials in the microspheres have been starch, dextran, albumin and hyaluronic acid, and the bioavailability of several peptides and proteins has been improved in different animal models. Also, some low-molecular weight drugs have been successfully delivered in microsphere preparations. The residence time in the cavity is considerably increased for microspheres compared to solutions. However, this is not the only factor to increase the absorption of large hydrophilic drugs. The dextran microsphere system was as effective as an absorption enhancer for insulin as degradable starch microspheres (DSM). The mode of action for improved absorption found for starch microspheres is also applicable to dextran micro spheres. Microspheres also exert a direct effect on the mucosa, resulting in the opening of tight junctions between the epithelial cells.[42]

**(D) Magnetic microspheres**

These microspheres are used for delivering the drug at localised disease site. Magnetic drug delivery by particulate carriers is used for this very purpose. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then stopped in the target area with a powerful magnetic field[43]. Drug targeting is the delivery of drugs to receptors or organs or any other specific part of the body to which one wishes to deliver the drug exclusively. Magnetic microspheres are successfully utilized for drug targeting but they show poor site specificity and are rapidly cleared off by RES (reticuloendothelial system) under normal circumstances. Magnetic microspheres were developed to minimize reticulo-endothelial (RES) clearance and to increase target site specificity. They can be used to entrap a wide variety of drugs.

**Benefits of magnetic microspheres:**

1. Magnetic microspheres are site specific and by localization of these microspheres in the target area, the problem of their rapid clearance by RES is also surmounted.
2. Linear blood velocity in capillaries is 300 times less as compared to arteries, so much smaller magnetic field is sufficient to retain them in the capillary network of the target area.
3. Avoidance of acute toxicity directed against endothelium and normal parenchyma cell, controlled release within target tissue for intervals of 30 minutes to 30 hrs. As desired, adaptable to any part of body.
4. In case of tumour targeting, microsphere can internalize by tumour cells due to its much increased

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phagocytic activity as compared to normal cells.

5. Problem of drug resistance due to inability of drugs to be transported across the cell membrane can be surmounted.

1. **(E) Cancer Microsphere Technology**

One useful discovery made from the research of microspheres is a way to fight cancer on a molecular level. According to Wake Oncologists, SIR-Spheres microspheres are radioactive polymer spheres that emit beta radiation. Physicians insert a catheter through the groin into the hepatic artery and deliver millions of microspheres directly to the tumor site. The SIR-Spheres microspheres target the liver tumors and spare healthy liver tissue. Cancer microsphere technology is the latest trend in cancer therapy. It helps the pharmacist to formulate the product with maximum therapeutic value and minimum or negligible range side effects. A major disadvantage of anticancer drugs is their lack of selectivity for tumor tissue alone, which causes severe side effects and results in low cure rates. Thus, it is very difficult to target abnormal cells by the conventional method of the drug delivery system. Microsphere technology is probably the only method that can be used for site-specific action, without causing significant side effects on normal cells.[44]

**(F) Radioactive microspheres**

Therapeutic radioactive microspheres (radio labelled microspheres) are appropriate for therapy when the encapsulated diagnostic radioisotope has been exchanged for a therapeutic one from the α- or β-emitter group. Typical uses include local application for the treatment of rheumatoid arthritis, liver tumours and cystic brain tumour. However, their use remains experimental because of unwanted toxicity, smaller than expected target uptake and insufficient treatment effects that have resulted from radio chemical instability and suboptimal bio-distribution of the radiopharmaceutical moiety. In spite of proven superior results of many radiation therapies there exists a general negative attitude towards the use of radioactive substances[45-47].

**(G)Perfect count microspheres**

These microspheres are meant for invitro diagnostic use. These microspheres are meant for

determination of absolute counts of cells in peripheral blood, bone marrow, leukophoresis and culture medium samples using flow cytometry. These are micro-bead-based single platform system, which can be used in combination with monoclonal antibodies conjugated with different flurochromes for absolute counts, which helps to identify the cell subpopulations for which the absolute count is intended[49,50].

**(H) Fluorescent microspheres**

These are made of polystyrene or poly vinyl toluene, mono disperse system. There size ranges from 20nm to 4μm. Preparation of fluorescent microspheres involves swelling the polymeric microsphere followed by incorporation of fluorescent dyes in the microspheres pores. The main applications for Estapor® Fluorescent Microspheres (commercial fluorescent microspheres) are the following: Membrane-based technologies Flow Cytometry, Embolization, Confocal Microscopy FLISA (Fluorescent Linked Immunosorbent Assay), and Toxicology, Cell Biology, Microbiology, Biosensors, Biochips and Micro fluidics[51].

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**(I)Microspheres and Genomes**

Enhanced chemiluminescence (ECL), robotics, and magnetic microspheres recently have been applied successfully to the human genome project. The microspheres are used in the first step of rapid DNA purification.[52] Novagen's Straight A's™ mRNA Isolation System uses their Magnetight™ Oligo (dT) Particles, which are superparamagnetic microspheres covalently coated with oligo $(dT)_{25}$. The protocol is designed to selectively extract and purify mRNA from a variety of sources. After magnetic separation, the purified mRNA is eluted off the magnetic beads for recovery or for a second round of purification. Promega has a similar isolation procedure.[53] . Other techniques using magnetic microspheres include oligonucleotide[54]

A new approach to template purification for sequencing applications using paramagnetic particles. and DNA template purification,[55] "rapid genomic walking,"[56] and sequencing.[57] Wilson used uncoated magnetic particles *twice* to purify ss-DNA - first to collect aggregated $M_{13}$ phage and later to collect its DNA from ethanol. Magnetic particles are cited as being relatively inexpensive raw materials in a method which reduces labor cost by half. Streptavidin-coated magnetic particles are also used as a solid support in IGEN's human papilloma virus assay. This is yet another example of a DNA hybrid assay; it is based on polymerase chain reaction (PCR) and read by electrochemiluminescence.[58]

During the study of the underlying genetic causes of disease a need for multiplexed analytical genotyping methods have been increased. Although microarray platforms have attempted to fulfil this need, their acceptance in the clinical diagnostic setting has been limited. Microspheres have been used for the detection of six single nucleotide polymorphisms (SNPs) believed to be associated with venous thromboembolism, a classic example of a complex, multifactorial disorder involving multiple genetic abnormalities[59-62].

Pyrosequencing is real-time DNA sequencing by synthesis[63,64] . Pyrosequencing currently has many applications, including determination of single nucleotide polymorphisms, resequencing of PCR products, microbial typing, and analysis of secondary DNA structures such as hairpins. The pyrosequencing technique utilizes DNA templates which are attached to magnetic microspheres, which can easily be put on an electrowetting chip in solution. On a digital micro fluidic platform, pyrosequencing could be accomplished by merging droplet containing the magnetic microspheres with the wash droplets and then resolving the doublevolume droplet through droplet splitting.

**(J) Controlled-Release Microsphere Vaccines**

Vaccination has been highly successful for controlling or even eradicating many important types of infectious diseases, and new or improved vaccines are being heavily investigated for AIDS [65], hepatitis B [66] anthrax and SARS [67]. A frequent problem is the need for repeated administrations—usually injections—to ensure long-lasting immunity. For example, the current anthrax vaccine requires a series of boosters at 2 and 4 weeks, and at 6, 12, and 18 months following the first inoculation; and the Recombivax HBr_ vaccine for hepatitis

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B—required for most healthcare workers in the U.S.—is administered in three injections at 0, 1, and 6 months. The need for multiple injections poses a serious problem for patients in developing countries with limited access to medical care, where awareness is lacking, or for transient populations.

One promising alternative is a single-shot vaccine in which a drug delivery device provides the necessary boosters at specified times after administration [68] Further, the ability to more precisely control the time course of vaccine delivery may lead to more effective vaccination with current antigens and may allow utilization of antigens that were previously ineffective [69]. Single-shot Vaccine delivery systems should provide the antigen(s) and adjuvant on a prescribed schedule and maintain the bioactivity of the antigen, both during fabrication of the delivery device and during the often prolonged residence time of the device in the body. In recent years, much work has focused on developing microsphere-based, single-administration, vaccine delivery vehicles [70,71,72,73] using a variety of materials including hydroxypropyl cellulose/PLG [74] , poly(ε-caprolactone) [75], . PLA [76], chitosan [77], and collagen [78], though the majority have been fabricated with PLG [79-83]. Maintenance of antigen bioactivity has been problematic due to contact of the proteins with organic solvents and the hydrophobic polymer, and the use of strong physical forces to produce the microspheres [84-86]. To enhance vaccine stability, researchers have been focusing on several approaches, including the use of adjuvants to protect the protein antigens or by choosing different microsphere materials [87-89]. A major advantage of microspheres for vaccination is that they can be passively targeted to antigen-presenting cells (APCs) such as macrophages (M_) and dendritic cells. The ability of APCs to phagocytose particulates is dependent on the particle size. In particular, 1- to 10-μm diameter microspheres are optimally taken up by APCs in a number of tissues [90] and have been shown to enhance antigen-specific T-helper lymphocyte (Th) responses [91] (thus leading to an enhancement in antigen-specific antibody responses) and elicit a cytotoxic T lymphocyte (CTL) response (Nixon et al. 1996). T-cell activation in response to antigen-encapsulating microspheres has been shown to be 100-1000 fold better than antigen alone [92].

**(K) Mapping Microclimate Ph In Biodegradable Polymeric Microspheres**

The microclimate inside microspheres prepared from biodegradable polymers (e.g., poly(lactic---*co*---glycolic acid) PLGA) often becomes acidic owing to the accumulation of water---soluble polymer degradation products, which can induce the destabilization of encapsulated therapeutic agents. The objective of this is to quantitatively evaluate the microclimate pH (μpH) inside biodegradable polymeric microspheres in order to facilitate the development of microsphere formulations that control μpH and stabilize acid-labile drugs.

When PLGA microspheres are injected into the body, water will penetrate into the polymer matrix and dissolve the encapsulated protein rapidly. Generally, it is well accepted that proteins are most stable in their solid state [93,94]. The rehydration of protein will mobilize the protein and enhance its reactivity significantly, resulting in destabilization. Another

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important detrimental factor for protein stability is the microclimate pH inside aqueous pores of the PLGA matrix. The presence of acid impurities (often monomers and Dimmers of glycolic acid and lactic acid) plus the degrading products of PLGA containing carboxylic acids create an acidic microenvironment, which could be deleterious to acid labile proteins. Acid-induced instability mechanisms for proteins include acid-catalyzed peptide bond hydrolysis, deamidation, aggregation, and denaturation[95,96]. For example, simulations of BSA in a very acidic microclimate pH (pH=2) showed denaturation, peptide bond hydrolysis, and noncovalent aggregation [97]. Evidence for acidification within degrading PLGA microparticles has become increasingly notable recently

## CONCLUSION

Microsphere offer vast advances in the pharmaceutical field. The recent use allows targeting the delivery of such drugs which offers difficulties in their normal delivery. Now higher dose can be administered as microspheres thus limiting gastrointestinal side-effects and allowing a full course of antibiotics to be given in a single dose. In recent years, studies of microspheres have been increased so that it may be used in more diverse applications and it is evident that the range of its applications is vast and enormous. For biologists, microspheres have emerged as an exciting new platform in the investigation of cellular processes and bimolecular interactions. The future certainly looks bright for microspheres, particularly in the areas of proteomics, genomics and drug discovery.

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