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NANOPARTICLES IN MICROPARTICLES SYSTEM: AN OVERVIEW

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ABSTRACT

Nanoparticles-in-Microparticles System (NiMS) is the delivery system in which the particles of nano- and micro- ranges are ensembled for drug and gene delivery in specific parts of the body. Nanoparticle-in-microparticle (NIM) systems, offers the possibility of dual or multiple functionalities within a formulation. For example, multiple release profiles (burst release from outer particles and sustained release from internal components) and/or combinations of features allowing site specificity. When the microencapsulated drug is consumed, it does not interact with taste receptors as it is insoluble in mouth. The moment the microencapsulated drug reaches the stomach the acidic pH conditions favour dissolution and thus the drug is released. Thus microencapsulation is a useful technique for masking the unpleasant taste.

INTRODUCTION

Nanoparticles-in-Microparticles System (NiMS) is the delivery system in which the particles of nano- and micro- ranges are ensembled for drug and gene delivery in specific parts of the body. The drug can be dissolved, entrapped, encapsulated or attached to a nanoparticles matrix [1].

The major goals in designing NiMS as delivery system are to control particles size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen [2]. Despite the more complex and onerous production of the multiple-unit drug delivery systems they have several advantages over the single-unit systems[3]. NiMS, offers the possibility of dual or multiple functionalities within a formulation. NiMS can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. The synthetic and natural polymers can be used to formulate NiMS. The NiMS can be developed into ODTs. NiMS have been successfully formulated for the development of orally disintegrating tablets of Scopolamine and box behnken designed nanoparticles-in-microparticles system (NiMS) for formulating mouth dissolving tablets of acetazolamide [1,4].

Significance

Advantages of using nanoparticles in microparticles as a drug delivery system

- protect drug payloads and prevent physical and chemical instability phenomena in the biological environment,
- improve the release profile of the encapsulated agent,
- reduce or eliminate the burst effect
- target specific cells, tissues and organs[3].

PREPARATION OF NANOPARTICLES IN MICROPARTICLES

The extent of nanoparticle entrapment within the NIMs was found to be dependent on the state (wet vs dry) in which the nanoparticles were introduced to the formulation. The technique was readily adaptable to produce NIMs of different morphologies. It is proposed that NIMs and this method to produce them have broad application in drug delivery research[6].

The methods used for the preparation of NiMS are as:

- Ionic gelation
- Emulsion techniques

i. Emulsion cross-linked method
ii. emulsification diffusion method
iii. Emulsion droplet coalescence

- Coacervation/ precipitation
- Nanoprecipitation
- Spray-drying

Spray drying techniques have been used to produce NIMs for aerosols [7,8,9,10,] and oral [11,12,] and and intravitreal formulations[13].

- Salting-out method
- Supercritical fluid techniques[14,15,16,].

The method being utilized for the preparation of nanoparticles can also be used for the development of NiMS.

1. **Spray drying techniques:** In this method, polymer is first dissolved in a solvent, drug is dissolved or dispersed in solution and then, suitable cross- linking agent is added, this solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates leading to the formation of free flowing powders. The particle size depends upon size of the nozzle, spray flow rate, atomization pressure, and inlet air temperature and extent of cross-linking [17].

   The advantage of this method is that the both hydrophilic and hydrophobic polymer can be used with proper selection of the solvent [18]. Spray drying is useful for encapsulating even heat-sensitive drugs, such as proteins or peptides, because it involves mild temperature [19].

   The limitation of this method is that the considerable amounts of the material can be lost during the process due to sticking of the nanoparticles/ microparticles to the wall of the drying chamber [20].

2. **Emulsion techniques**

a. **Emulsion cross-linked method** In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the polymer solution in oil phase. Aqueous droplets are stabilized using a suitable surfactant, the stable emulsion is cross-linked by appropriate cross-linking agent such as glutaraldehyde to harden the droplets, and the particles are filtered and washed repeatedly. In this method, the particle size can be controlled by controlling the size of aqueous droplets. However, the particle size of the final product depends upon the extent

of the cross-linking agent used while hardening in addition to the speed of stirring during the formation of emulsion.

The drawback of this method involves tedious procedure as well as use of harsh cross-linking agents, which might possibly induce chemical reaction with agents, however complete removal of the un-reacted cross-linking agent may be difficult in this process [21,22].

b. **Emulsion-droplet coalescence** In this method, instead of cross-linking the stable droplets, precipitation is induced by allowing the coalescence of polymer droplets with NaOH droplets. Firstly, a stable emulsion containing aqueous solution of chitosan along with drug is produced using paraffin oil and then, another stable emulsion containing aqueous solution of NaOH is produced in same manner. When both the emulsion are mixed under high speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating chitosan droplets to small size particles. The advantages of this technique include high yield and encapsulation efficiency. The limitations of this method are poor aqueous solubility, lack of site specific targeting [17].

c. **Emulsification diffusion method** In this method, polymer is dissolved in measured amount of solvent. This organic phase is added into required amount of aqueous phase containing the stabilizer. After mutual saturation of organic and continuous phase, the mixture is emulsified with a high speed homogenizer. For full diffusion into water phase, excess amount of water is added to the oil in water emulsion under magnetic stirring, leading to the nanoprecipitation of the polymer [17]. This technique presents several advantages, such as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution[23]. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency [24].

3. **Ionic gelation**: This method involves preparation of two aqueous phases. One phase contains polysaccharides dissolved in water or in weak acidic medium and other phase contains a polyanion dissolved in water. These solutions are then added dropwise under constant stirring. When electrostatic interaction take place between two aqueous phases coacervates are formed, and when two molecules interact due to ionic force, it results in transition from liquid phase to gel phase at room temperature. The beads particles of nano

range/ micro range are removed by filteration or centrifugation washed with distilled water and dried using lyophilizer [25].

The advantages of this method includes:

- reversible physical crosslinking by electrostatic interaction occur instead of chemical crosslinking;
- absence of organic solvents avoids the possible toxicity of reagents and other undesirable effects and
- distribution can be easily monitored by changing the amount of counter ions [21].

The limitation of this method is that:

- it can only be used for ionic species and not for neutral particles;
- the size of particles will only be depends on physical interaction and not any involvement of chemical reaction and
- only water soluble substances are used for this method [26].

4. **Coaceravation/ precipitation** This method utilizes the physiochemical property of polymer. Since polymer is insoluble in alkaline pH and as it comes in contact with alkaline solution, it precipitates or coaceravates. Particles are produced by blowing the chitosan solution into the alkaline solution using a compressed air nozzle to form coacervates droplets. Separation and purification can be done by filteration/ centrifugation followed by successive washing with hot and cold water [27]. The advantages of this technique include high yield and encapsulation efficiency; however, it involves complex and multi-step manufacturing procedures[28]. The major disadvantages of coacervation method include the difficulties in scaling-up and the use of large amount of organic solvent [29].
5. **Nanoprecipitation** The nanoparticles formation by nanoprecipitation is instantaneous and the entire procedure is carried out in only one step. Ideally, both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent [24]. The advantage of this method is that it is a single step not requiring extended shearing/ stirring rates, sonification, or high temperatures [30,31]. This method is mostly suitable for hydrophobic compounds that are soluble in ethanol or acetone [32]. The limitations of this method are poor aqueous solubility, lack of site specific targeting, rapid systemic clearance, intestinal metabolism and systemic toxicities [33,34].

6. **Salting-out method** In this method, acetone is used as the water-miscible organic solvent. This method consists of the addition of water soluble polymer in a highly concentrated salt solution in water (aqueous phase) to a polymer solution in acetone (organic phase). Although acetone is miscible with pure water in all ratios, the high salt concentration of the aqueous phase prevents mixing of the phase. After emulsification, the addition of pure water in a sufficient quantity causes acetone to diffuse into the aqueous phase, resulting in the formation of nanoparticles [35]. The major advantages of this technique are the possible incorporation of high amounts of polymer and drug, excellent yields and the easy scale up in an industrial setup [35]. This technique is limited to lipophilic drugs, salting out agents that enable phase separation without precipitation and soluble stabilizers that are compatible with saturated aqueous solutions and do not coacervates in the presence of the solvent. Also, the process requires intense purification to ensure complete removal of the electrolytes [35].

Characterization and evaluation of Nanoparticles-in-microparticles system(NiMS)

The NiMS are generally characterized for ;size, morp- hology, electrophoretic mobility, angle of contact and specific surface area, in vitro release, encapsulation efficiency, drug loading etc [37,38].

Size and morphology The particle size and morphology is one of the most important parameters of NiMS. Two main techniques are being used to determine the particle size distribution which includes photon correlation spectroscopy (PCS) and electron microscopy (EM) [24]. This latter includes scanning electron microscopy (SEM), transmission electron microscopy (TEM). The size evaluation of NiMS dispersion demonstrates better results with freeze- fracturing microscopy and photon correlation spectroscopy as quantitative methods [38]. The electron microscopy however, could be adopted as an alternative option, which measures individual particles for size and its distribution, it is relatively less time consuming. In combination with freeze-fracture procedures, TEM permits differentiation among nano-capsules, nanoparticles and emulsion droplets. On the other way SEM is much less time consuming. However, since particles are based on organic and non-conductive material, they require gold coating [39].

Specific surface area: The specific surface area of freeze-dried NiMS is generally determined with the help of Sorptometer [40]. The equation (1) can be used in the calculation of specific surface area.

$$A = 6/Ə.d \quad \text{eq (1)}$$

Where A is the specific surface area. Ə is density and d is the diameter of the particles.

Surface charge and electrophoretic mobility: The nature and intensity of the surface charge of NiMS is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The surface charge of NiMS can be determines by measuring the particle velocity in an electric field. Laser light scattering technique has become available as fast and high resolution technique for the determination of NiMS velocities [41].

Surface hydrophobicity: The surface hydrophobicity of NiMS has an important influence on the interaction of colloidal particles with the biological environment. The hydrophobicity and hydrophilicity collectively determine the bio-fate of NiMS and their contents. Several methods, including hydrophobic interaction chromatography, two-phase partition, adsorption of hydrophobic fluorescent of radio- labeled probes, and contact angle measurements have been adopted to evaluate surface hydrophobicity [42].

NiMS recovery and drug incorporation efficiency: The encapsulation efficiency, drug loading and % yield were calculated according to the following equations [43];

Loading Capacity (%) = (Mass of drug in NiMS)/ (Mass of NiMS recovered)×100 eq (2)

Incorporation Efficiency (%) = (Mass of drug in NiMS)/ (Mass of drug used in formulation) ×100 eq (3)

Percentage yield (%) = (Total NiMS weight)/ (Total solid weight) ×100 eq (4)

In vitro release: In vitro release profile can be determined using standard dialysis, diffusion cell of recently introduced modified ultra filtration technique. In vitro release from the NiMS can be evaluated in acidic medium as well as in neutral medium utilizing double chamber diffusion cells on a shaker stand. A Millipore hydrophilic low protein binding membrane is placed between two chambers. The donor chamber is filled with nanoparticulate suspension and the receptor chamber with plain buffer. The receptor chamber is assayed at different intervals for the released drug sing standard procedures. Modified ultra filtration technique can also be used to determine in vitro release behaviour of the NiMS. The NiMS suspension is added directly into a stirred ultra filteration cell containing buffer. At different time intervals aliquots of the dissolution medium are filtered through the ultra filteration membrane using less than and assayed for the released drug using standard procedures [44].

VARIOUS DRUG DELIVERY SYSTEMS WHICH UTILIZED NiMs

Nanoparticle-in-microparticle system is administered by different routes such as oral, pulmonary or even parenteral.

- **ORAL:**a novel nanoparticles-in-microsphere oral system (NiMOS) has been developed using a "double emulsion-like" technique and evaluated as potential drug and gene delivery in specific regions of the GI tract for therapeutic and vaccina- tion purposes. The advantages of NiMOS might involve in the achieved high drug entrapment efficiency, controlled drug release and potential enhanced absorption.
- **GIT:**Ulex europaeus agglutinin (UEA) anchored CS-NiMPs might be used as a potential drug delivery system targeted to the specific regions of gastrointestinal tract.
- **PULMONARY:** Disease-responsive microgels with encapsulated nanoparticles for intracellular drug delivery to the deep lung. It is well appreciated that delivery of therapeutic agents through the pulmonary route could provide significant improvement in patient compliance and reduce systemic toxicity for a variety of diseases. Many inhalable drug formulations suffer from low respirable fractions, rapid clearance by alveolar macrophages, target non-specificity, and difficulty in combining aerodynamic properties with efficient cellular uptake. To overcome these challenges, an enzyme-responsive, nanoparticle-in-microgel delivery system is used. This system is designed to provide optimal aerodynamic carrier size for deep lung delivery, improved residence time of carriers in the lungs by avoiding rapid clearance by macrophages, and reduction of side effects and toxicity by releasing encapsulated therapeutics in response to disease-specific stimuli. The resulting microgels have a highly porous internal structure and an optimal aerodynamic diameter for effective deep lung delivery. They also exhibit triggered release of various nanoparticles and biologics in the presence of physiological levels of enzyme. In addition, the nanoparticle-carrying microgels showed little uptake by macrophages, indicating potential for increased lung residence time and minimal clearance by alveolar macrophages. Collectively, this system introduces a rationally designed, disease-specific, multi-tiered delivery system for use as an improved pulmonary carrier for biologic drugs

Applications of nanoparticles in microparticles system

- **Reduction of burst release:** lower diffusion of the drug from double polymeric wall formed by the nanoparticles matrix followed by another diffusion step through the microparticle polymeric wall.

  eg: microencapsulation of theophylline loaded nanoparticles on the reduction of burst release[46,47].
- **Epidermal powder immunisation (EPI):**integrating the advantages of nanoparticles and microparticles into one vaccine delivery system for epidermal powder immunization[48].

The present nano-in-micro system allows

(1) nanoparticles to be immobilized and finely distributed in microparticles,

(2) microparticle formation and

(3) re-dispersion of nanoparticles without subsequent aggregation.

The nanoparticles inside microparticles can

(1) adsorb proteins to cationic shell/surface voids in spray-dried products without detriment to ovalbumin stability,

(2) deliver antigens in nano-sized modes to allow recognition by the immune system.

- protect drug payloads and prevent physical and chemical instability phenomena in the biological environment,
- improve the release profile of the encapsulated agent,
- reduce or eliminate the burst effect and
- target specific cells, tissues and organs
- **for improved local retention after intra-articular injection:**

  a) this system(NiM) increase the intra-articular (IA) retention time of osteoarthritis drugs in the synovial cavity and

  b) slow down the burst release of microspheres (MPs)[49].

- **Novel protease inhibitor-loaded Nanoparticle-in-Microparticle Delivery System leads to a dramatic improvement of the oral pharmacokinetics in dogs:** With the advent of the Highly Active Antiretroviral Therapy, the morbidity and the mortality associated to HIV have been considerably reduced. However, 35-40 million people bear the infection worldwide. One of the main causes of therapeutic failure is the frequent administration of several antiretrovirals that results in low patient compliance and treatment cessation. An innovative Nanoparticle-in-Microparticle Delivery System (NiMDS) comprised of pure drug nanocrystals of the potent protease inhibitor indinavir free base produced by nanoprecipitation that were encapsulated within mucoadhesive polymeric microparticles. NiMDSs displayed an encapsulation efficiency of approximately 100% and a drug loading capacity of up to 43% w/w. In addition, mucoadhesiveness assays ex vivo conducted with bovine gut showed that film-coated microparticles were retained for more than 6 h. Finally, pharmacokinetics studies in mongrel dogs showed a dramatic 47- and 95-fold increase of the drug oral bioavailability and half-life, respectively, with respect to the free unprocessed drug. These results support the outstanding performance of this platform to reduce the dose and the frequency

of administration of protease inhibitors, a crucial step to overcome the current patient-incompliant therapy.

- This NiMs protects molecules of interest from degradation in the digestive tract

CONCLUSION

Microparticles have exhibited a better stability in the biological environment, and their highly reproducible formulation methods provide support to encapsulate hydrophilic and hydrophobic drugs, which gives them a wide range of therapeutic applications. The release of drugs from microparticles shows several benefits , which include their ability to modulate the rate of drugs release for a long time periods and their capacity to reduce the drug toxicity. Their combination, as nanoparticle-in-microparticle (NIM) systems, offers the possibility of dual or multiple functionalities within a formulation. For example, multiple release profiles (burst release from outer particles and sustained release from internal components) and/or combinations of features allowing site specificity. When the microencapsulated drug is consumed, it does not interact with taste receptors as it is insoluble in mouth. The moment the microencapsulated drug reaches the stomach the acidic pH conditions favour dissolution and thus the drug is released. Thus microencapsulation is a useful technique for masking the unpleasant taste. NiMS have caused a fundamental change in manufacturing and have an enormous impact on drug delivery, diagnostics, nutraceuticals and production of biomaterials. They have advantages over conventional drug delivery systems and can increase the solubility, bioavailability, and permeability of many potent drugs. NiMS based drug delivery systems will also reduce the drug dosage frequency and will increase the patient compliance. Moreover, NiMS can be used to alter the kinetic profiles of drug release leading to more sustained release of drugs. There are now numerous simple, safe and reproducible preparation methods available for producing NiMS, and important technological advances have been achieved. In future, NiMS based drug delivery systems can be used for exploiting many therapeutically active agents which have poor aqueous solubility, permeability and less bioavailability. Overcoming the obstacles in conventional drug delivery systems, NiMS will have better application and effective drug delivery and would ultimately enhance treatment and patient compliance.

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