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Evaluation Parameters of Nanoparticles

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ABSTRACT

Nanotechnology as defined by the National Nanotechnology Initiative (NNI) is the study and use of structures roughly in the size range of 1 to 100 nm. Nanoparticles render a promising drug delivery system of controlled and targeted drug release. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanoparticles specially designed to release the drug in the vicinity of target sites. In this article we discuss about nanoparticles and its evaluation parameters.

Keywords: Nanoparticles, Nanotechnology, Polymers, Nanocapsules.

INTRODUCTION

The field of Novel Drug Delivery System is emerging at an exponential rate with the deep understanding gained in diversified fields of biotechnology, biomedical engineering and nanotechnology [1]. Many of the recent formulation approaches utilize nanotechnology that is the preparation of nano sized structures containing the API [2]. Nanotechnology as defined by the National Nanotechnology Initiative (NNI) is the study and use of structures roughly in the size range of 1 to 100 nm. The overall goal of nanotechnology is the same as that of medicine: to diagnose as accurately and early as possible and to treat as effectively as possible without any side effects using controlled and targeted drug delivery approach [3].

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved,

entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes [4-7]. Nanoparticles are not simple molecules itself and therefore composed of three layers i.e. (a) The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers, (b) The shell layer, which is chemically different material from the core in all aspects and (c) The core, which is essentially the central portion of the NP and usually refers the NP itself.

LITERATURE REVIEW

Method of preparation

There are several techniques for the preparation of nanoparticles which can be broadly classified into two main categories

- Polymerization of monomer
- Dispersion of preformed polymer.
- Polymerization method.
- Dispersion of preformed polymer.
- Solvent evaporation method.
- Spontaneous emulsification or solvent diffusion method.
- Coacervation or ionic gelation method.
- Supercritical fluid technology.
- Emulsion droplet coalescence method.
- Ionic cross linking method.
- Salting out method.
- Nano precipitation.
- Controlled jellification method.
- Modified high shear homogenization and ultra-sonication method.

Evaluations

Particle size analysis: The particle sizes of the nanoparticles were evaluated by scanning electron microscope were ranging from 350 nm to 600 nm, particle size varies depending on the polymer load [8, 9].

Scanning Electron Microscopy (SEM) studies: The particle shape and surface morphology of nanoparticles were examined by scanning electron microscopy. Lyophilised and completely moisture free samples were consigned on aluminium stubs using adhesive tapes and coated with gold using sputter coater and observed for morphology at an acceleration voltage of 20 kV.

Differential scanning Calorimetry (DSC) studies: The physical status of the native drug inside the nanoparticles was ascertained by the DSC analysis (DSC-60, Shimadzu, Japan). Approximately, weighed 2 mg of native drug, polymer and nanoparticles were placed separately into the different sealed standard aluminium pan and were scanned between 25°C and 300°C with heating rate of 10°C/min under nitrogen atmosphere. An empty aluminium pan served as reference.

Atomic Force Microscopy (AFM) studies: Atomic Force Microscopy (AFM) studies were carried out to characterize the surface morphology of prepared drug loaded nanoparticles. The nanoparticle suspension was prepared with milli-Q water and dried overnight in air on a clean glass surface and observation was performed with AFM (JPK Nano Wizard II, JPK instrument, Berlin, Germany) with silicon probes with pyramidal cantilever having force constant of 0.2 N/m. To avoid damage of the sample surface, all measurements were conducted in intermittent contact mode and the tip to sample distance was kept constant using the amplitude feedback function in attractive forces regimen. The scan speed of 2 Hz and 312 kHz resonant frequency was used for displaying amplitude, signal of the cantilever in the trace direction and to obtained images [10].

Determination of percentage of drug entrapment efficiency: Prepared nanoparticle suspensions were centrifuged at 2000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free drug in the supernatant was determined by the UV-Visible Spectrophotometer [11].

Drug Entrapment (%)=Amount of drug added-Amount of free drug/Amount of drug added × 100

Determination of drug entrapment efficiency and loading capacity by RP-HPLC method: The Entrapment Efficiency (EE) and Loading Capacity (LC) were estimated by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method [12]. In brief, 1 mg/ml of the drug loaded nano particulate solution in methanol was prepared and 20 µ L of the sample was injected manually to HPLC equipped with Shimadzu LC-20 AD PLC pump and SPD-M_{20} A PDA detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP_1 software. The chromatographic separation was achieved by using Phenomenex C_{18} (150 mm × 4.6 mm, 5 µm) analytical column. The mobile phase used consisting of methanol and water (75:25, v/v) was passed through 0.45 µm membrane filter and degassed by ultra-sonication. The flow rate was maintained at 1.0 mL/min and the measurements were made at 282 nm. The column was maintained in ambient condition using thermostat. The amount of the drug in the sample was determined from the peak area correlated with the standard curve. The standard curve was prepared under the same identical condition.

X-ray diffraction study: X-ray diffraction analysis was conducted using a XRD-6000 diffractometer. X-ray diffraction analysis was used to detect the crystallinity of the pure drug and the formulation. The powder was placed in an aluminium sample holder. Cu radiation was generated at 30 mA and 40 kV. Samples were scanned at a range of 10° to 90° with scan speed of 10° min^{-1}, as previously explained [13].

Determination of zeta potential: The zeta potential of the drug-loaded chitosan nanoparticles was measured on a zetasizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell [14,15]. All the samples were measured in water at 25 $^{\circ}$C in triplicate.

Fourier Transforms Infrared (FTIR) spectroscopy analysis: The chemical integrity and possible chemical interaction between the drug and polymer can be determined by FTIR analysis (Perkin Elmer, FT-IR Spectrometer, SPECTRUM RX I, and USA). Samples were mixed separately with potassium bromide (200–400 mg) and compressed by applying pressure of 200 kg/cm^2 for 2 min in hydraulic press to prepare the pellets. The pellets of the native drug, polymer and the drug loaded nanoparticles were analysed by placing it on the light path. All samples were scanned by aver-aging 32 interfero grams with resolution of 2 cm^{-1} in the range of 4000–400 cm^{-1}.

***In vitro* drug diffusion studies using EGG membranes:** Permeation study with egg membrane was done according to the method reported by Ansari [16]. The egg shell was kept in concentrated HCl for 2 h. The separated membrane was attached to diffusion cell. 20 mg

of the drug was placed in the diffusion cell with 10 ml of phosphate buffer (pH 7.4). 50 ml of phosphate buffer (pH 7.4) was placed in the receptor compartment in 100 ml beaker. The assembly was then attached to magnetic stirrer. Samples were withdrawn at specific time interval for 6 h and analysed using UV-visible spectrophotometer at given wavelength.

***In vitro* release study using Franz diffusion cell:** A Franz diffusion cell was used to monitor drug release from the nanoparticles [17]. The receptor phase was phosphate buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrane with molecular weight cut off 12,000 to 14000 Daltons was used to separate receptor and donor phases. The latter consisted of a 2 ml suspension of nanoparticles containing 10 mg of drug, mixed for 5 seconds to aid re-suspension, in a 1% w/v Tween 80 solution in PBS. Samples (1 ml) from the receptor phase were taken at time intervals and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of drug into the receptor phase was evaluated spectrophotometrically [18].

***In vitro* release of Nanoparticles by simple diffusion cell:** The *in vitro* release of nanoparticles was studied by using simple diffusion cell apparatus which is opened at both ends, One end tied with sigma dialysis membrane which serves as a donor compartment. The dissolution medium used was freshly prepared phosphate buffer saline pH 7.4. Sigma membrane was soaked overnight in the dissolution medium. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37 °C ± 0.5 °C. Periodically 5 ml of sample was withdrawn and analysed spectrophotometrically [19, 20].

***In vitro* drug release using dialysis tube:** *In vitro* release studies were carried out by using dialysis tubes with an artificial membrane. The prepared nanoparticles and 10 ml of phosphate buffer pH 7.4 was added to the dialysis tube and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer a temperature was maintained at 37 ± 1°C. 5 ml of sample of receptor compartment were taken at various intervals of time over a period of 24 h and each time fresh buffer was replaced. The amount of drug released was determined spectrometrically.

***In vitro* release kinetics study:** In order to analyse the drug release mechanism, *in vitro* release data were fitted into a zero-order, first order, Higuchi, and Korsmeyer-peppas model.

Zero order kinetics: The zero order rate equation describes the systems where the drug release rate is independent of its concentration.

$$Q_1=Q_0+K_0t$$

First order kinetics: The first order equation describes the release from a system where the release rate is concentration dependent. Kinetic equation for the first order release is as follows

$$\text{Log } Q_t=\log Q_0+K_1\, t/2.303$$

Higuchi model: Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent.

$$Q_t=KH\, t_{1/2}$$

Korsmeyer–Peppas model: To find out the drug release mechanism, first 60% drug release data can be fitted in Korsmeyer–Peppas model which is often used to describe the drug release behaviour from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

$$\text{Log } (M_t/M_\infty)=\text{Log KKP} + n \text{ Log } t$$

***In vivo* bio distribution of nanoparticles containing drug:** *In vivo* bio distribution studies were carried out in wistar rat weighing 100 to 150 gms and were divided into 3 groups containing 3 animals. The 3 groups namely group 1 treated with free drug, group 2 treated with drug loaded nanoparticles and group 3 treated with solvent control. The mice of each group were injected by IV route free drug and drug loaded nanoparticles with a dose of 3.6 mg per body weight and the solvent control that is pH 7.4. After the 18 hrs of drug injection, animals were sacrificed, then blood was taken and plasma separated out, also different organs like liver, lungs, kidney and spleen were extracted out and homogenised in phosphate buffer saline (pH 7.4) followed by centrifugation. Supernatant of tissue homogenised were analysed by HPLC to estimate the bio distribution [21].

Accelerated stability study: The nanoparticles were packed in borosilicate glass vials and these samples were stored in environmental simulation chambers for constant climatic conditions. The storage conditions used in the stability study, as well as the times when the samples were tested according to the protocol of International Conference on Harmonization (ICH) guidelines. Physicochemical characterization of the drug loaded Eudragit®RS_{100} nanoparticles was carried out over 6 months at regular intervals by dispersing 1 mg of drug loadedEudragit®RS_{100} nanoparticles in 10 ml of distilled water to observe any degradation. The studies were carried out in triplicates from time to time. Particle size and zeta potential was measured using Zeta sizer, based on quasi-elastic light scattering, at a given wavelength at 25°C. The chemical stability (drug content) of the formulation was determined by RP-HPLC at 282 nm [22].

CONCLUSION

Because of the novel features that nanoparticles bring to materials, they have become one of the most important research areas of our day. When compared to a material with greater grain size, the same material created in nano–scale has several innovations due to its significantly distinct and superior qualities. After their initial emergence in science, high costs have become more common, the cost has come down to reasonable levels, and new nanomaterials are being developed on a daily basis. Nanoparticles' use and potential have recently expanded, particularly in some industries. Drug delivery, cancer treatment, and the manufacture of high strength materials has all been the subject of extensive research and development in recent years. It appears that nanoparticle research will be pursued in earnest.

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