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Colloidal stability and physicochemical characterization of bombesin conjugated biodegradable nanoparticles

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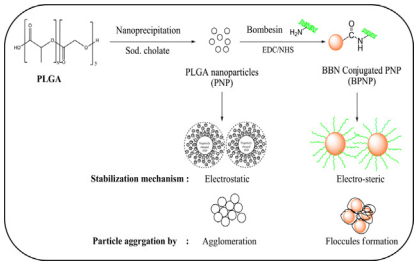
HIGHLIGHTS

- Bombesin grafted biodegradable nanoparticles (BPNP) were prepared and optimized.
- Effect of time, medium and electrolytes concentration on stability was evaluated.
- Changes in optical density and particle size were used as stability indicators.
- Critical flocculation concentration was determined by calculating Fuchs factor.
- BPNP were found to be more stable than unconjugated nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this investigation was to explore the potential of bombesin (BBN) peptide conjugated biodegradable polymeric nanoparticles as cancer targeting system. Poly (lactic-co-glycolic) acid nanoparticles were prepared using different concentration of surfactants. Nanoparticles, prepared with 0.25% sodium cholate (PNP), showed the smallest size (86.15 nm) and good zeta potential value (−34.2 mV). Bombesin was covalently conjugated to the nanoparticle surface by amide bonding. The surface conjugation was confirmed by change in surface potential and Fourier transform infrared analysis. Slight change in pH and buffer medium did not show significant effect on conjugation efficiency. Colloidal stability of nanoparticles was evaluated with respect to time, salt induced aggregation, storage medium and release of BBN from nanoparticle surface. The stability factor was determined by change in optical density and particle size with time. Nanoparticles were found to be more stable in phosphate buffer saline and serum when compared to that in normal saline and distilled water. Bombesin conjugated nanoparticles (BPNP) showed more stability in both PBS and serum than PNP which may be due to steric stabilization of nanoparticles by the peptide. Similarly, in aggregation resistance studies, BPNP were stable up to 0.7 M concentration of sodium sulphate. Bombesin was released only 12.76% in 24 h under acceleration conditions, showing the stability of amide bond formed between nanoparticles and peptide. Conclusively, the bombesin peptide conjugated PLGA nanoparticle could be a promising drug delivery system for targeting of anticancer drugs.

1. Introduction

Nanoparticles are promising carriers for several biomedical applications such as imaging, diagnostics and drug delivery [1–3]. In drug delivery, their applications are mostly explored in development of targeted drug delivery systems especially for anticancer

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drugs. Because of small size, nanoparticles can easily enter into the cancer cells through the leaky vasculature (passive targeting). In addition, the surface of nanoparticles can be easily manipulated to give receptor-specific targeted system [4].

The efficacy of the nanoparticulate systems is very much dependant on three basic surface properties such as size, surface charge and hydrophobicity [5,6]. These parameters affect stability as well as in vivo performance like translocation to target site, binding and cellular uptake [7]. Nanoparticles are colloidal systems that range in size range 10–1000 nm and their stability is mainly determined by the surface chemistry. The physical stability of colloidal systems is explained by their aggregation and coagulation behaviour. According to DLVO theory, the stability of nanoparticles depends on the balance between attractive Van der Waals and repulsive electrostatic forces due to double layer of counter ions [8,9]. Among three different types of Van der Waals forces (Keesom, Debije and London), London forces i.e. induced dipole – induced dipole, are mainly responsible for attraction and aggregation of nanoparticles. The strength of electrostatic repulsion forces depends on the distance from the nanoparticle surface. Electrostatic forces are expressed in terms of Nernst potential and zeta potential (ζ). The Nernst potential is the potential difference between the actual nanoparticle surface and electroneutral region. The zeta potential or electrokinetic potential is the potential difference between shear plane and electroneutral region. Nernst potential has little effect in colloidal stabilization and is not practically assessable. Thus zeta potential is an important parameter of electrostatic repulsion. When the electrostatic repulsive forces dominate the attractive Van der Waal forces, the system remains stable and in dispersed state. Thus high zeta potential value (more negative or positive) indicates more the colloidal stability of nanoparticles. But, practically attractive forces are found to be stronger compared to repulsive forces and nanoparticles tend to form aggregates within seconds.

Surface hydrophobicity is another major cause of the instability of nanoparticles. It also affects the behaviour of nanoparticles in aqueous environment and decides the fate and transport of nanoparticles [5]. The hydrophobicity plays an important role in the interaction of nanoparticles with the bio-membranes [10–12].

Several approaches can be used to increase the stability of nanoparticles [13]. First, by forming an electric double layer around nanoparticles i.e. charge or electrostatic stabilization. Second, by adsorption or chemically attachment of polymeric molecule on the nanoparticle surface (steric stabilizer) i.e. steric stabilization [14]. Third, combination of both electrostatic and steric stabilization i.e. electrosteric stabilization. Generally, steric stabilization or electrosteric stabilization approaches are used in nanoparticle stabilization because of its several advantages – electrolyte insensitivity, redispersibility of nanoparticles, can accommodate high concentration and suitability to multiphase systems [15–17].

The primary objective of this investigation was to develop a sterically stabilized bombesin (BBN) peptide conjugated biodegradable polymeric nanoparticles based system for cancer targeting. Several targeting ligands have been conjugated to nanoparticles for the delivery of anticancer drugs [18–20]. However, surface conjugation changes the dimension and other physicochemical parameters such as surface charge, which are directly related to the stability of nanoparticle. Hence, the colloidal stability of unconjugated polymeric nanoparticles (PNP) and BBN conjugated nanoparticles (BPNP) was studied in the presence of electrolytes and physiological conditions. The nanoparticle stabilization and aggregation mechanisms were investigated in detail. This study also provides insight about the selection of method for the determination of stability factor.

2. Materials and methods

2.1. Materials

Poly (D,L-lactic-co-glycolic acid) (PLGA) containing a free carboxyl end group (uncapped) with *L*/*G* molar ratio of 50:50, bombesin, sodium cholate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(n-morpholino)ethanesulfonic acid (MES) and N-[tris(hydroxymethyl) methyl]-2-aminoethane sulfonic acid (TES) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Bradford reagent was purchased from Biomatik (Hyderabad, India). Rose bengal dye and other chemicals were of analytical grade and were purchased from s.d. fine-chem Ltd. (Hyderabad, India).

2.2. Preparation of PLGA nanoparticles

PLGA nanoparticles (PNP) were prepared by a modified solvent evaporation (nano-precipitation) method. PLGA (20 mg) was dissolved in 2 ml acetone and then quickly poured in 20 ml of deionised water containing different concentrations (0.1%, 0.25% and 0.5%, w/v) of surfactants (polyvinyl alcohol, Tween® 20 and sodium cholate). The dispersion was sonicated for 2 min in an ice bath. The organic phase was evaporated off by magnetic stirring at 1000 rpm for 3 h. Finally, the dispersion was centrifuged at 15,000 rpm for 20 min at 4 °C. Nanoparticles were washed thrice with deionised water, lyophilized and stored at 2–8 °C.

2.3. Bioconjugation of BBN to PLGA nanoparticle

Ten milligrams of PNP were dispersed in 5 ml of MES buffer (0.1 M) and incubated with 497.95 μM of EDC and NHS each. The dispersion was kept on gentle stirring for 1 h at room temperature. To this, 100, 250 and 500 μl of BBN solution (1 mg/ml) was added, mixed well and kept for further stirring of 6 h. BBN conjugated nanoparticles (BPNP) were collected after centrifugation at 10,000 rpm for 10 min and washed thrice with distilled water. The conjugation efficiency was determined by measuring BBN in supernatant by Bradford protein assay. The absorbance was measured at 595 nm using microplate reader (Synergy 4, Biotek, USA).

2.4. Effect of pH and buffer on conjugation efficiency

The effect of pH and buffer medium on the peptide conjugation efficiency was studied using 0.1 M MES buffer pH 6.2, phosphate buffer saline pH 7.4 (PBS) and 0.1 M TES buffer pH 8.2.

2.5. Nanoparticle characterizations

Both BBN conjugated and unconjugated nanoparticles were characterized by particle size, zeta potential, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) analysis. Mean particle diameter (PD), polydispersity index (PDI) and zeta potential of both PNP and BPNP were measured by proton correlation spectroscopy using Malvern Zetasizer Nano-ZS (Malvern instrument Ltd., Malvern, UK). Samples were diluted 10 times with distilled water and analyzed at 25 °C with a backscattering angle of 173°.

Surface morphology of nanoparticles was determined by SEM analysis. The samples were sputtered on thin film of gold and scanned using SEM machine (SEM-LEOS 1430VP, LEO Electron Microscopy Ltd., UK) equipped with tungsten filament.

Fourier transform infrared (FTIR) analysis of unconjugated and conjugated nanoparticles are carried by potassium bromide (KBr)