RESEARCH ARTICLE

Bioavailability Enhancement of Poorly Water-soluble Nano Diosgenin by Encapsulation using Chitosan/Bovine Serum Albumin Bilayers

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Abstract

Aim: To enhance the bioavailability of poorly water-soluble diosgenin (DG) by chitosan/BSA encapsulated nanoparticle formulation. **Material and Methods:** 0.1% chitosan aqueous solution was mixed with freshly prepared saturated DG solution followed by probe-sonication for 30 min., thereafter 0.1% BSA solution was added drop-wise and sonicated for another layer formation. Procedure was repeated for bilayer formation. After each layer formation nanoparticles were centrifuged and resuspended in fresh DI water. **Results and Discussion:** The DLS of nanosized DG shown to have a radius of 2 nm to 40 nm. Surface morphology of nanoDG has been observed using SEM. Non-significant changes under the UV/Vis and IR, suggest the retention of drug properties at nano level. Chitosan/BSA coatings generated a higher surface charge (ca. +28 mV and -30mV) resulting in good colloidal stability. Dissolution technique was used for release profiling of drug from layer-by-layer assembly. **Conclusion:** The combination of biocompatible ionic polymers chitosan/BSA bilayer encapsulation around nanoDG, allowed sustained release of drug molecule up to 30 hours.

Key words: Bovine serum albumin, chitosan, diosgenin, hydrophobic drugs, nanocolloids, release mechanism

INTRODUCTION

Encapsulation of hydrophobic drug molecules has gained much attention in the past one decade,[1-5] and biodegradable nanoparticle coatings have been studied more often as drug delivery system due to their enhanced bioavailability, effective encapsulation, sustained release, and less toxic properties. Formulations of various nanoparticle systems utilizing poly(lactide-co-glycolic acid), polylactic acid, chitosan, gelatin, polybutyl(cyano)acrylate, and polyglycolic acid have been reported.[6,7] However, these formulations commonly have the size of drug carriers within the range of 200–300 nm, which is not the right particle size distribution for the most efficient medical applications.[6-8] Furthermore, nanoscale drug delivery vehicle for the continuous slow release of hydrophobic drugs has not yet been established except micelles, although loading of desired drugs found to be very less in micelles.[9-11]

In this paper, diosgenin (DG) has been taken as a drug molecule for nanosizing. Structurally, DG [(25R)-spirost-5-en-3b-ol] is a spirostan consisting of a hydrophilic sugar moiety linked to a hydrophobic phytosteroid sapogenin and has structural similarity with cholesterol and other steroids [Figure 1]. DG has been used as precursor in the manufacturing of synthetic steroids and for several hormones in the pharmaceutical industry.[12] It has been reported that DG inhibits early events of azoxymethane-induced aberrant crypt foci formation in F344 rats when given during either initiation/post-initiation or promotion stage.[13] It exhibits anticancer effects by

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Received: 18-01-2018
Revised: 06-05-2018
Accepted: 15-05-2018

blocking the proliferation of HT-29 human colon cancer cells and induces antiproliferative and proapoptotic effects in part by modulating bcl-2 and caspase-3 expression *in vitro*.[14-16] However, the pharmaceutical applications of DG are very limited because of its poor pharmacokinetics (log *P* = 5.7).[17]It has very low water solubility, roughly 0.02 mg/L, being highly hydrophobic.[19-21] Bioavailability of ***DG*** may be low owing to it being metabolized before adequate plasma concentrations are reached and/or its water solubility being extremely poor with slow absorption. Solubility is one of the essential parameters to achieve therapeutic drug concentration in systemic circulation for optimal pharmacological response. Enhancing the dissolution and bioavailability of hydrophobic drugs is a major challenge. Although it can be observed that DG is a promising drug candidate for colon cancer, it has not made much impact due to extremely low bioavailability.[18]

Potential advantages of drug delivery are (1) continuous maintenance of drug levels in a therapeutically desirable range or in an environmentally responsive manner; (2) decrease in drug side effects due to targeted delivery to particular cell type or tissue; (3) potentially decreased amount of medication needed; (4) reduced number of dosages and possibly minimally invasive dosing, leading to increased patient compliance with the prescribed medication regimen; and (5) facilitation of drug administration for medications with short *in vivo* half-lives (for example, peptides and proteins).

A useful methodology has been described for the preparation of stable drug nanocolloids with diameters of <50 nm that is based on the probe sonication-assisted drug encapsulation from their solutions in aqueous/organic solvent mixture. Nanoparticle aggregation was controlled by polycationic adsorption onto particles where chitosan has been involved for the same, followed by layer-by-layer (LbL) polyelectrolyte nanoencapsulation, using alternate bovine serum albumin (BSA) and polyanion. The aim of the present work is to enhance the dissolution of hydrophobic drug by implementing solid dispersion technique.

EXPERIMENTAL

The reagents used were purchased of highest purity available, chitosan (low MW) extra pure was purchased from Sisco Research Laboratories Pvt. Ltd. (SRL) with 99.9% purity, BSA was procured from Central Drug House Pvt. Ltd. (CDH), and DG from Acros Organics.

The present approach for drug nanoformulation includes the probe sonication assistance of drug particle and drug loading. The chitosan and BSA are being loaded onto the drug so as to enhance the stability and solubility of the drug. The preparation of stable DG nanocolloids was carried out by following procedure, initiated by reducing the solubility by adding aqueous media. Initially, 0.1% aqueous chitosan solution was prepared. To make the chitosan surface positive, pH 5.4 was maintained by adding acetic acid. DG solution was made by dissolving 50 mg of DG in a minimum amount of ethanol, whereas a separate solution of 0.1% BSA was prepared in DI water.

In a small beaker, 15 mL of chitosan solution was taken, then freshly prepared DG solution was added to the solution dropwise while stirring, then the mixture was probe sonicated (using Ultrasonic Atomizer Probe Sonicator, Athena Technology) for 30 min, as depicted in Figure 2. The DG was homogeneously dispersed into the chitosan solution. Autoaggregation of the prepared nanoparticles was prevented by the cationic chitosan polymer encapsulation onto drug particles, followed by LbL electrostatic self-assembly of polyelectrolyte nanoshells when 15 mL of BSA solution was added dropwise to the above solution, and it was sonicated for 15 min.

In a similar manner, 15 ml of chitosan solution and 15 mL of BSA solution were added to the above-prepared solution consecutively and were sonicated for 15 min each. The encapsulation of polyelectrolyte onto drug nanoparticles creates a barrier for their further growth and aggregation. The obtained crystal particles were stable and did not aggregate

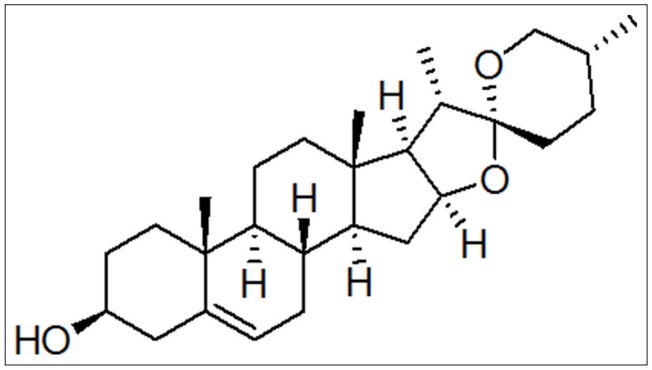

Figure 1: Diosgenin: A phytosteroidsapogenin

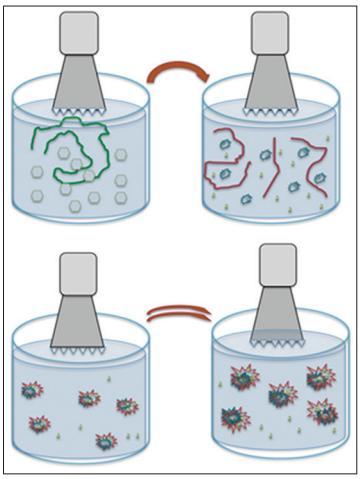

Figure 2: Schematic diagram of Layer-by-layer encapsulated nanodiosgeninpreparation

after sonication was stopped. The reason for this can be an increase in surface charge provided by the adsorbed charged polymeric layer. After 60 min of sonication, DG nanoparticles were separated from solution by centrifugation and again suspended in DI water. Further, polyelectrolyte multilayer was built on DG nanoformulation by alternate adsorption of BSA-polyanions and chitosan-polycations forming LbL polymeric assembly. To minimize the DG particle size, a series of samples were processed under various conditions (different alcohol-to-water ratios, DG concentrations, and time and speed of water addition for crystallization). The main factor that affected the size of the particles was the rate of water addition and DG concentration.

The formed nanocolloid of DG was then analyzed by various techniques such as measurement of translation diffusion by dynamic light scattering (DLS), ultraviolet spectroscopy (UV), infrared spectroscopy, etc. The dissolution of the formed nano DG was studied in comparison to the DG, and its release was studied by dissolution technique.

RESULTS AND DISCUSSION

DLS is a famous technique used to determine the particles size in suspension, polymers, micelles, and proteins in solution. This method determines the particle size by measuring the temporal fluctuations of the intensity of light scattered from a suspension or polymeric solution. The useful size range for the DLS technique is quite large from below 5 nm (0.005 μ) to several microns.

The DLS of nanosized DG was done using *Rigaku* Ultima X-ray diffractometer (made in Japan). The technique of DLS is also known as quasielastic light scattering or photon correlation spectroscopy.

From the above Figures 3a and b, it can be observed that the frequency of occurrence, that is, a larger number of particles at a given time have been shown to have a radius of around 2–40 nm. This shows that particles are being formed at nanoscale particles

Surface morphology of prepared nano diosgenin has been observed using scanning electron microscopy (SEM) [Figure 4] with a secondary electron source. In encapsulated nano DG, formation of cubes can be observed easily, whereas some amount of agglomeration is also present. There is also some appearance of hierarchical granular microstructures with an average size ranges in 40 nm. The formation of well compact properly dispersed and distributed flakes of nano diosgenin take place. Some amount of particles agglomeration is also present due to which appearance of irregular shaped particle with an average size of 2 nm–40 nm.

However, peak broadening was observed in encapsulated drug due to the presence of chitosan and BSA.

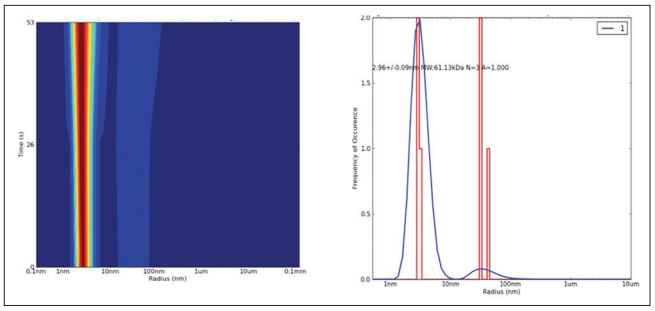

Figure 3: (a) Distribution of nanosized encapsulated diosgenin and (b) Histogram of encapsulated nanodiosgenin derived from dynamic light scattering technique

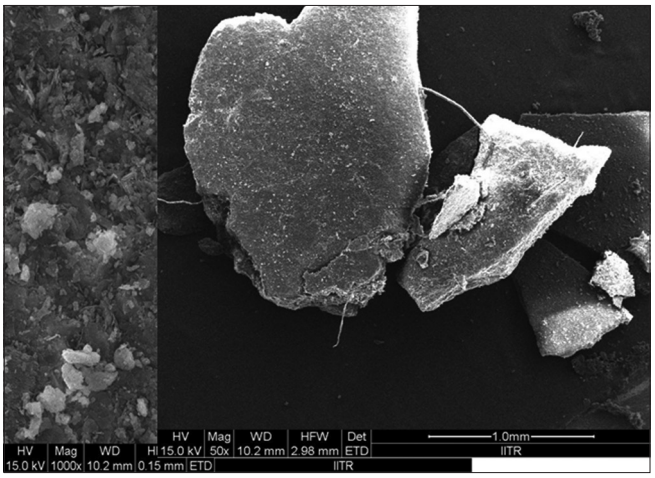

Figure 4: Scanning electron microscopy images (FEI) of (a) initial DG and (b) encapsulated nano diosgenin

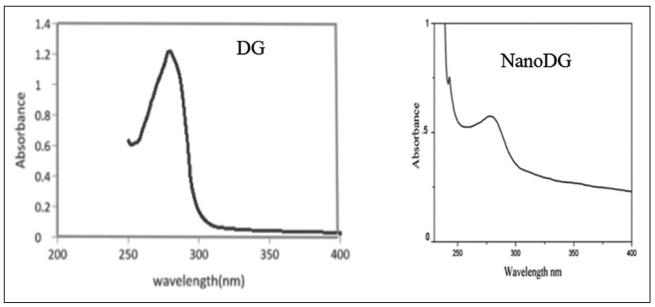

Figure 5: Ultraviolet-vis spectra of (a) diosgenin [$\lambda_{max}(\varepsilon)$=279 nm (12500 $mol^{-1}dm^3cm^{-1}$)], (b) nanodiosgenin [$\lambda_{max}(\varepsilon)$=280 nm (5000 $mol^{-1}dm^3cm^{-1}$)]there is no change in maxima which indicate the retention of drug properties after nanosization of diosgenin up to 2 nm. However, peak broadening was observedin encapsulated drug due to presence of Chitosan and BSA

Encapsulated nanoparticles were obtained with edges from 2 to 37 nm, and an estimated average particle size (22 nm) was with DLS imaging (Hitachi) and light-scattering experiments. However, non-significant changes were observed under the UV/Vis [Figure 5] and IR [Figure 6] spectroscopies suggest the retention of drug properties while nanosizing of DG drug molecule. Due to the adsorption of cationic chitosan,

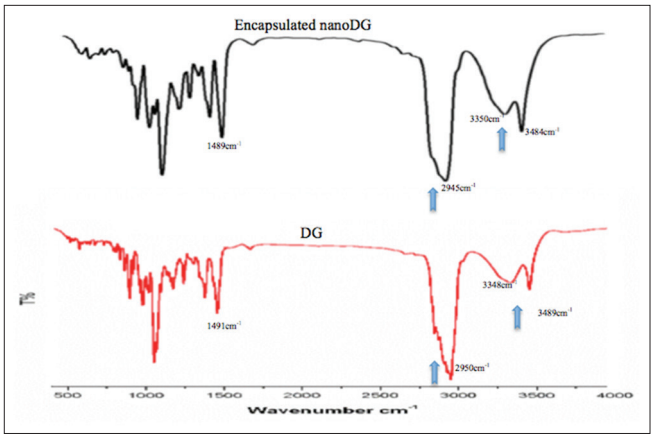

Figure 6: Infared spectra of both DG and encapsulated nano diosgenin (DG) found to be similar, that means the properties of drug molecule has been retained, only minor changes were observed (indicated by arrows) in encapsulated nano DG due to the presence of chitosan and BSA

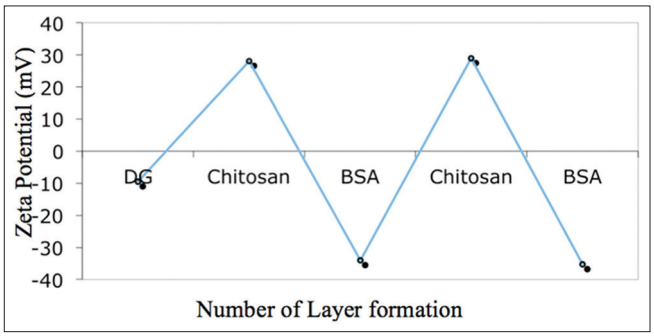

Figure 7: (Zeta) ξ potential during diosgenin nanoparticle Layer-by-layer coating via the alternate adsorption of polyelectrolytes (pH 5.6, chitosan and BSA, BVK TEC instrument)

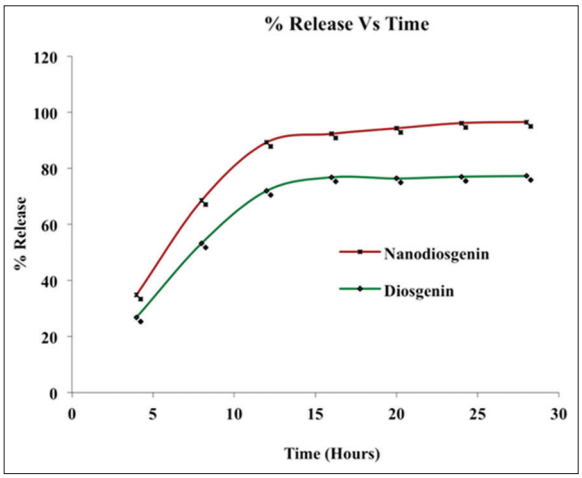

Figure 8: Nanodiosgenin release curves measured with ultraviolet (UV) absorption at a wavelength of 400 nm (UV-vis SPECORD 250 - 222P136 instrument)

the surface ξ potential of these nanoparticles was positive, ca. +28 mV and after the adsorption of anionic BSA, the ξ potential value alternatively switched to -30 mV, while chitosan/BSA bilayer formation (BVK TEC. Zeta analyzer) [Figure 7]. A high surface charge increases the colloidal stability for this formulation. For example, a DG nanocolloid sample of a 0.5 mg/mL drug concentration was preserved for 2 months as a stable dispersion.

Release profile

The dissolution or drug release testing is an important tool in drug development and quality control as it measures the *in vitro* release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment. It includes exposing the dosage form under certain circumstances so as to achieve the release of drug.

Oral dosage dissolution testing

In vitro dissolution testing of oral dosage forms measures the dissolution rate of an amount of drug substance that goes from the solid state into solution per unit time under conditions that simulate those that occur *in vivo*. This test has the ability to predict accurately and precisely expected bioavailability (crucial for therapeutic efficacy), indicates the robustness of the dosage form (drug product safety), and suggests the variations in the manufacturing process (which may have a critical influence on performance). Various apparatus can be used for dissolution testing which includes USP Apparatus 1 (baskets), USP Apparatus 2 (paddles), USP Apparatus 3 (reciprocating cylinders), USP Apparatus 4 (flow-through cell), USP Apparatus 5 (paddle over disk), USP Apparatus 6 (cylinders), and USP Apparatus 7 (reciprocating holders).

$$\%\ \text{Drug dissolved} = \left(\frac{\text{Atest}}{\text{Astd}}\right) \times \left(\frac{\text{Dtest}}{\text{Dstd}}\right) \times \left(\frac{\text{P}}{100}\right) \times \left(\frac{1}{\text{Label claim}}\right) \times 100$$

The percentage corrected drug dissolved was found out by adding the factor to the consecutive values of the percentage drug dissolved which calculated using the formula given below:

$$\text{Factor} = \frac{\%\ \text{Drug dissolved} \times \text{Sampling volume}}{\text{Total volume}}$$

Percentage drug release of pure drug and nanosized encapsulated formulations estimated. The drug Warfarin was taken as a standard; subsequently, percentage corrected drug dissolved for DG was calculated (detailed experiment has been mentioned in supporting document). Afterward, the observed values were plotted for mean percentage corrected drug dissolved versus time for both DG and encapsulated nano DG which followed Fickian diffusion [Figure 8].

The dissolution profile of 15 mg encapsulated nano DG with (Chitosan/BSA) coating was analyzed by dissolution method.

Using pH = 7.5 by using potassium phosphate buffer at 37 ± 0.5°C as dissolution medium and paddle apparatus at 100 rpm.

CONCLUSION

A new combination of carrier molecules has been used to synthesize nanoparticle formulation for poorly water-soluble drug molecule, DG. The drug sample was initially dissolved in ethanol, a miscible solvent with water. Gradually, the drug solubility was decreased on addition of aqueous positively charged chitosan-polymer solution assisted by probe sonication. Chitosan coatings generated a higher surface charge resulting in good colloidal stability. Further, bilayer encapsulation was achieved through the alternate coating of negatively charged polymer bovine serum albumin (BSA). Smaller size up to 2 nm–≈40 nm of bilayer encapsulated DG molecules was obtained. Furthermore, ξ potential of +30 or −28 mV resulted into a stable nanocolloids for months when kept in a saturated solution. The combination of biocompatible ionic polymers chitosan/BSA bilayer encapsulation around nano DG allowed sustained release of drug molecule up to 30 h.

ACKNOWLEDGMENT

We are thankful to STAR College Scheme (DBT) running at ARSD College, University of Delhi, for supporting the work. We are grateful to Prof. A. P. Gupta, Department of Polymer Technology, Amity University Haryana, for fruitful discussion.

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Source of Support: Nil. **Conflict of Interest:** None declared.