NARINGENIN LOADED CYCLODEXTRIN NANOPARTICLES FOR IMPROVED DRUG DELIVERY

ABSTRACT

Herbal drug naringenin is well known for its anti-cancer, anti-oxidant, anti-inflammatory activities, as carbohydrate metabolism promoter, immunity system modulator, BCRP/ABCG2 inhibitor, hormone substitute and hormone antagonist since decades. However, use of naringenin as pharmaceutical aid is hindered due to its low aqueous solubility. In the present study, β-cyclodextrin and hydroxy propyl -β- cyclodextrin based biocompatible nanoforms were developed by the exploitation of self-assembly properties of cyclodextrins in aqueous media using sodium dodecyl sulphate and Pluronic F108 as co-surfactants. Prepared formulations were evaluated for various parameters such as particle size, zeta potential, polydispersity index, percent encapsulation efficiency, drug solubility and *in vitro* permeation studies. Particles present in all the formulations were not greater than the 161.2 nm with surface charge up to -24.8 mV and highest polydispersity index of 0.739. Aqueous solubility of drugs in prepared formulations was increased by 4 fold. Drug permeability was increased twice of free drug naringenin. It was concluded that the cyclodextrin based biocompatible nanosystems can be developed in the improvement of aqueous solubility and hence the bioavailability of herbal drugs.

Keywords: Cyclodextrin nanoparticles, DLS, nanoparticles, naringenin, cyclodextrin, permeation

INTRODUCTION

National Nanotechnology Initiative (NNI) defined the nanoparticles as the structures that are in the range of 1 to 100 nm in at least one dimension[1]. However, most of the literature described the nanoparticles as the particles with size up to 1000 nm[2]. The smaller size and larger surface area of nanoparticle systems help to improve bioavailability by significant increase in solubility and give additional capability to cross organ barriers[3]. Movement of drug from its site of administration to its site of action, increased resistance time in the body, sustained release and reduced drug toxicity can be obtained through modern approach[4]. For *in vivo* delivery, nanoparticles with size range 10-100 nm have ideal pharmacokinetic properties. Smaller nanoparticles are subject to tissue extravasations and renal clearance while bigger particles are rapidly opsonised and expelled from the circulation system through the macrophages of the reticulo-endothelial framework.

Plants are the natural sources of various molecules have distinct biological activities, which are well recognized since long times[5-6]. But these phytomedicines show low therapeutic or *in vivo* efficacy mostly due to poor aqueous/lipid solubility, unstable structure in biological environment, drug loss via metabolism or rapid clearance, and drug degradation in the presence of gastric juice[7].

Cyclodextrins, cyclic oligosaccharides, are the bacterial degradation products of starch by enzyme CD glucosyltransferase comprising α-(1,4) glucopyranose units. Mainly three types of cyclodextrins are produced: α, β, and γ having 6, 7 and 8 glucopyranose units, respectively. Cyclodextrin possesses distinctive advantages due to its ability to form host-guest inclusion complex with various organic and inorganic lipophilic drugs[8,9]. Recently, cyclodextrin has been explored as a promising approach for nanoparticle-based delivery systems. CD amalgamated nanoparticles are highly advantageous because it increases drug aqueous solubility, instability, enhances drug encapsulation efficiency/drug loading and permeation through biological membrane and carries drug to its site of action[10,11].

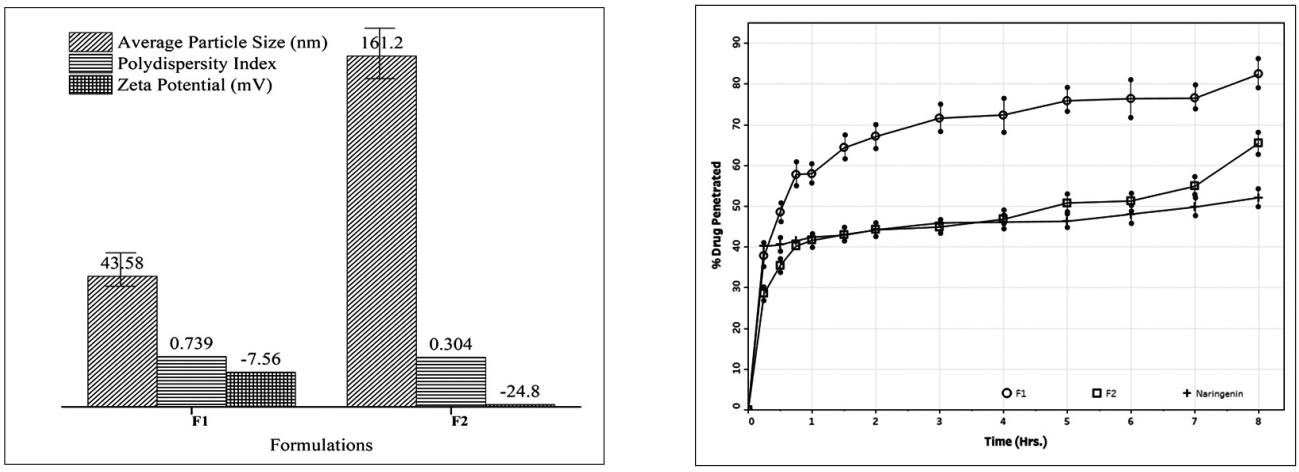
Naringenin is the aglycone form of naringin from the class flavonoids, having different physiological properties such as antioxidant, anti-inflammatory, carbohydrate metabolism promoter, immunity system modulator, BCRP/ABCG2 inhibitors, hormone substitutes and hormone antagonists. Naringenin is commonly found in citrus fruits, cocoa, cherries, grapefruits and tomatoes. However, due to the low water solubility, use of naringenin in pharmaceutical preparations is limited. Entrapment of naringenin into the cyclodextrins based nanoparticles has the potential to improve the drug bioavailability in various dimensions.

In the present study, β-cyclodextrin and hydroxy propyl -β- cyclodextrin nanoparticles of naringenin were developed using sodium dodecyl sulphate and Pluronic F108 as co-surfactants. Prepared formulations were evaluated for particle size, zeta potential, polydispersity index, percent encapsulation efficiency, drug solubility, and *in vitro* permeation studies.

Table I: Composition of naringenin loaded nanoparticles of β-CD and HPβ-CD

Formulation	Naringenin (mM)	β-CD (mM)	HPβ-CD (mM)	SDS (mM)	Pluronic F108 (mM)
F1	1	-	4	-	2
F2	1	4	-	5	-

β-CD: β cyclodextrin; HPβ-CD Hydroxypropyl β cyclodextrin



(A) (B)

Fig. 1: (A) DLS data of formulations; (B) Comparative *in vitro* permeation study plot for free drug and formulations

MATERIALS AND METHODS

Materials

Naringenin was obtained from TCI Chemicals (India) Pvt. Ltd. Pluronic F108 was procured from Sigma Aldrich, US. Sodium dodecyl sulphate (SDS), hydroxypropyl-beta cyclodextrin (HPβCD) and β-cyclodextrin were procured from HiMedia Laboratories, India.

Methodology

β-Cyclodextrin and HPβCD nanoparticles were prepared using slightly modified methods which have been reported in some previous studies[12,13].

Direct preparation method of β-cyclodextrin nanoparticles: Drug, β-cyclodextrin and sodium dodecyl sulphate (SDS) were accurately weighed in the molar ratio of 1:10:5 and dissolved in 100 mL of distilled water with constant stirring (Table I). Heat was provided to the solution until isotropic solution was produced. Prepared isotropic solution was then concentrated with constant stirring up to 5 % w/V of total concentration of β-cyclodextrin and SDS. All the solutions were then incubated for a period of 24 h at 25 °C for self-assembly of β-CD/SDS nanoparticles. Produced samples were packed and stored at 4 °C for further studies.

Dilution method of HPβCD nanoparticles: In the preparation of nanoparticles drug and Poloxamer 188 were dissolved in 50 mL of distilled water in the ratio of 1:2 under constant stirring with magnetic stirrer. HPβCD equivalent to drug HPβCD molar ratio 1:4 added dissolved in 30 mL of distilled water. Then prepared HPβCD solution was slowly added into drug/Poloxamer solution with constant stirring and heating. Produced solutions were then left overnight to cool down and for ageing. Samples were stored in refrigerated conditions for further studies.

Characterization of nanoparticles was done for following parameters

Sizing and PDI measurement: Prepared samples were analyzed for average particle size and polydispersity index at room temperature with angle of detection of 90° by the use of photo correlation spectroscopy or particle size analyzer by Zetasizer Nano ZS90 (Malvern Panalytical Ltd., Malvern, UK.).

Zeta potential: Surface charge or the zeta potential of all the samples were determined in zeta cuvette at 25°C by Zetasizer.

Solubility: All the nanoparticles dispersed samples were diluted with distilled water and analyzed for drug concentration by UV-Visible spectrophotometer.

Encapsulation efficiency: Encapsulation efficiency of samples was determined by the use of simple

ultracentrifugation technique. For this, initial amount of drug was determined by exposing the diluted samples to UV-visible spectrophotometer. Then, 10 mL of each sample was centrifuged at 10000 rpm at room temperature for 120 minutes to settle the particles at the bottom. Aliquot of supernatant was taken and diluted with distilled water for determination of free drug in the samples.

Encapsulation Efficiency (%) = [(Initial concentration-Final concentration)/ Final concentration] x 100

Permeation studies: Six glass tubes were taken, and cellophane membrane was tied over one end of each glass tube with thread. Six beakers filled with 80 mL of phosphate buffer pH 7.4 were placed over magnetic stirrer and membrane attached end of glass tubes were dipped into each beaker. Each of the pure drugs and formulations in known concentration of drug was placed inside each glass tube over the membrane. Magnetic beads were dropped down into beakers the stirrers switched on and run at constant speed. Small amount of sample was taken out from the beaker and replaced with fresh buffer in fixed time interval for 8 h. All the samples were diluted with buffer and observed for drug extent with UV-Visible spectrophotometer[14].

RESULTS AND DISCUSSION

Sizing and PDI measurement: Average particle size of both formulations was found to be 43.58 ± 8.02 and 161.2 ± 14.16 nm for F1 and F2 (Fig. 1 A). Particle size of nanoparticles play vital role in release of drug as smaller the particle size, larger the surface area and faster the drug release from the particles. Hence, it has been suggested that formulations F1 will show faster and greater drug release/permeation[15]. Polydispersity index of nanoparticles indicates the uniformity in size of nanoparticles. Polydispersity indices for formulations F1 and F2 were 0.739 and 0.304. F2 showed the smaller polydispersity index, indicating that in this formulation, large number of particles was in the same size range. Rest of the formulations had larger PDI value, showing that they contained the particles having broad size range[16].

Zeta potential: Formulations F1 and F2 were having the zeta potential value of -7.56 and -24.8mV (Fig. 1 A). Greater the value of zeta potential, higher the repulsion forces between the particles and less chances of particle agglomeration. Since formulation F2 had higher zeta potential value, therefore this formulation could be more stable than F1[17].

Solubility: Naringenin solubility (0.214±0.08 mg mL^{-1}) in water was increased up to 4 and 3.9 fold in formulation F1 (1.042±0.40 mg mL^{-1}) and F2 (0.849±0.24 mg mL^{-1}). Increase in solubility of drugs may be due to the formation of inclusion complexes of drugs with cyclodextrins and wettability properties of contained surfactants. Formulation F1 showed greater drug solubility and may be due to high solubility of HPβCD than β-CD and presence of smaller particles[18].

Encapsulation efficiency: Formulations F1 and F2 had percent encapsulation efficiency of 80.9 ± 2.4 % and 43.1±1.67 %, respectively. F1 had higher encapsulation efficiency, which suggested that HPβCD in formulations, increased the encapsulation efficiency of drug by forming effective host-guest complexes and it was well supported by the previous studies[19,20].

Permeation studies: Rate and extent of drug permeation is shown in Fig. 1 (B), which showed that it was almost twice in formulations as compared to that of free drug. Permeation of drug in formulations was found to be improved due to smaller particle size, improvement in solubility by cyclodextrin complexation, and wetting properties of surfactants[8].

Various previous studies have reported the improved drug delivery through binary composite of naringenin with lipids and cyclodextrins in the form of inclusion complexes and nanoparticles[19-22]. This preliminary study indicates the potential of naringenin cylodextrin nanoparticles.

CONCLUSION

As evidenced by the study, the biodegradable cyclodextrin based nanoparticles can be designed and exploited in the delivery of herbal components having challenging bioavailability. Furthermore, it was reported that the addition of SDS and Pluronic F108 as co-surfactant into the systems can modify the size of particles by maintaining it in nano range and provide a greater surface charge which it turn may lead to better stability. Cyclodextrin based biocompatible nano systems may prove to be cost effective and of vital importance for research in drug delivery.

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Lokesh Adhikari[a], Nitin Kumar[a], Abhijit Saha[b], Ajay Semalty[a] and Mona Semalty[a]*

[a] Department of Pharmaceutical Sciences, H.N.B. Garhwal University (A Central University) Srinagar (Garhwal)- 2461 74, Uttarakhand, India

[b] UGC-DAE Consortium for Scientific Research, Kolkata – 700 098, West Bengal, India

***For Correspondence: E-mail: monasemalty@gmail.com**

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