Acute and Repeated Dose Toxicity Studies of Different β-Cyclodextrin-Based Nanosponge Formulations

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ABSTRACT: Nanosponges (NS) show promising results in different fields such as medicine, agriculture, water purification, fire engineering and so on. The present study was designed to evaluate toxicity of different NS formulations (namely, S1–S6) synthesized with different cross-linking agents such as carbonyl diimidazole, pyromellitic dianhydride and hexamethylene diisocynate; and preparation methods in experimental animals. Acute and repeated dose toxicity studies of formulations were carried out as per OECD guidelines 423 and 407, respectively. For acute toxicity study, formulations were administered to female rats at doses of 300 and 2000 mg/kg orally. The general behaviour of the rats was continuously monitored for 1 h after dosing, periodically during the first 24 h and daily thereafter for a total of 14 days. On day 14, animals were fasted overnight, weighed, and sacrificed. After sacrification, animals were subjected to necropsy. For repeated dose toxicity study, rats of either sex were orally administered with formulations at the dose of 300 mg/kg per day for a period of 28 days. The maximally tolerated dose of all formulations was found to be 2000 mg/kg. Repeated administration of formulations for 28 days did not show any significant changes in haematological and biochemical parameters in experimental animals. These results indicate that the formulations are safe, when tested in experimental animals. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association
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INTRODUCTION

Cyclodextrin-based nanosponges (NS) are spherical, regular shape, cross-linked nanoparticles. NS emerge with promising results in different fields such as medicine as drug carrier, drug solubiliser, controlled-release matrix system; agriculture as longevity enhancing agent for fruits and flowers; water purification as adsorbing agent, fire engineering as smoke adsorbent and flame retardant and so on.[1] NS are condensed, complexed and/or polymerised β-cyclodextrin (β-CD) with different cross-linkers such as hexamethylene diisocynate (HMDI),[2] diphenyl carbonate,[3] pyromellitic dianhydride (PMDA),[4] carbonyl diimidazole (CDI)[5] and so on. NS are mostly prepared by polymer condensation and interfacial condensation methods.[2] NS, a nanoparticle-based drug delivery system have numerous applications in pharmaceuticals such as enhancing the dissolution rate, solubility and stability of drugs, to mask unpleasant flavours, to convert liquid substances to solids and to prolong the release of drug.[5] NS showed superior complexing ability than natural cyclodextrins towards many molecules leads to increased encapsulation efficiency and stability.

This behaviour has been exploited to improve the solubility and bioavailability of poorly water-soluble drugs. Particularly, paclitaxel and tamoxifen encapsulated in NS showed an increased bioavaibility compared with the free drug after their oral administration to rats.[6,7] To develop therapeutic formulations, safety requirements are mandatory.

In acute previous toxicity study, the oral administration of PMDA cross-linked β-NS in rats at the dose of 2000 mg/kg does not show any signs of toxicity up to 14 days and no animals died. This indicates that β-NS was nontoxic in rat to an oral dose of 2000 mg/kg of body weight.[4] The present study was designed to evaluate toxicity of different NS formulations (namely, S1–S6) with different cross-linking agents such as CDI, PMDA and HMDI; and preparation methods in experimental animals.

MATERIALS AND METHODS

Materials

β-Cyclodextrin was gifted from Roquette Italia SpA (Cassano Spinola, Italy). CDI, PMDA, HMDI and potassium hydroxide were obtained from Sigma–Aldrich (Munich, Germany). Dichloromethane, dimethyl formamide (DMF), acetone, dimethyl sulfoxide (DMSO), triethyl amine (TEA) and ethanol were purchased from Sigma–Aldrich (Munich, Germany). All other chemicals and reagents were of analytical grade.

Methods

Preparation of Cross-Linked NS

The cross-linked NS were prepared in 1:8 molar ratio of β-CD and CDI or HMDI or PMDA (Table 1) by the methods

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Table 1. Test Compounds

Serial Number	Formulation Code	Test Compound
1	S1	BNS–CDI (1:8)–CM
2	S2	BNS–CDI (1:8)–NM
3	S3	BNS–HMDI (1:8)–CM
4	S4	BNS–HMDI (1:8)–NM
5	S5	BNS–PMDA (1:8)–CM
6	S6	BNS–PMDA (1:8)–NM

as described below.

- *(1)* Bulk condensation method (CM)

- *(i)* CDI cross-linked NS

17.42 g of anhydrous β-CD (15.35 mmol) was added to 100 mL DMF in round bottom flask to attain complete dissolution by monitoring clear solution. Then, 19.91 g of CDI (122.8 mmol) was added as a cross-linking agent and the solution allowed to react at 100°C. After the completion of condensation reaction, the solid block of cross-linked cyclodextrin was roughly ground in mill or unreacted reagents were completely removed by Soxhlet extraction with ethanol.[8]

- *(ii)* HMDI cross-linked NS

3.91 g of anhydrous β-CD (3.44 mmol) was added to 16 mL DMSO in round bottom flask to achieve complete dissolution by observing clear solution. Then, 4.64 g of HMDI (27.5 mmol) was added as a cross-linking agent and the solution allowed to react at 70°C for 4 h. After the completion of reaction, the solid block of cross-linked cyclodextrin was roughly ground in mill or unreacted reagents were completely removed by Soxhlet extraction with ethanol.

- *(iii)* PMDA cross-linked NS

11.35 g of anhydrous β-CD (10.00 mmol) and 17.45 g of PMDA (80.0 mmol) were dissolved in 100 mL of DMSO containing 2.7 mL TEA (19.4 mmol) and were allowed to react at room temperature for 3 h. Once the reaction was over, the solid obtained was ground in a mortar and Soxhlet extracted with acetone for 24 h.

- *(2)* Interfacial condensation method (NM)

1.135 g of β-CD (1.000 mmol) was completely dissolved in 20 mL of 0.1 M aqueous solution of potassium hydroxide under magnetic agitation or by means of sonicator. 1.297 g of CDI (8.00 mmol) or 1.346 g of HMDI (8.00 mmol) or 1.745 g of PMDA (8.00 mmol) was dissolved in methylene chloride to obtain an organic CDI or HMDI or PMDA solution.

The alkaline β-CD solution was added to the organic CDI or HMDI or PMDA solution under continuous agitation. After 30 min of reaction, the precipitate was washed with deionized water and centrifuged at 3000 rpm for 10 min. The filtrate was collected and dried in vacuum dessicator to obtain the NS.[9]

The schematic representation of the structure of NS is shown in Figure 1.

Suspensions of test compounds were prepared by using 1% sodium carboxy methyl cellulose (CMC) for oral administration.

Particle Size

The sizes of NS were measured by dynamic light scattering using a Brookhaven particle sizer (New York, USA) equipped with particle sizing software. NS were suitably diluted with distilled water for each measurement. The measurements were made at a fixed angle of 90° for all NS samples and 25°C.

Experimental Animals

Wistar albino rats were purchased from Bharat Serum (Thane, India). All animals were housed in animal facility with temperature 25 ± 2°C, relative humidity of 75 ± 5%, and a 12-h light–dark cycle. Standard basal diet (Nutrimix Laboratory Animal Feed, Maharashtra, India) and purified water were provided *ad libitum* to the animals. Rats were assigned to each dose group by stratified random sampling based on body weight. The animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Acute Toxicity Study

The acute toxicity of samples was carried out in rats weighing 150–170 g, using the OECD guideline no. 423.[10] The animals were divided into 13 groups containing three female rats in each. The NS samples were prepared in 0.1% sodium CMC in water before oral administration and were administered at doses of 300 and 2000 mg/kg body weight, after a short fasting period. The control group received 0.1% CMC solution.

The general behaviour of the rats was continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter for a total of 14 days. Various cage side observations were performed, such as behaviour patterns, somatomotor activity, changes in eyes and mucous membranes, skin and fur and also respiratory, circulatory, autonomic and central nervous systems.

Animals were strictly observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The body weight, food and water intake for the treatment and control group were recorded weekly. On day 14, animals were fasted overnight, weighed and sacrificed. After scarification, the animals were subjected to necropsy.

Repeated Dose Toxicity Study

Repeated dose toxicity study was carried out on Wistar rats of either sex (150–170 g) as per OECD guidelines 407. Animals were divided in six groups, each group containing five males and five females. Group I was control group that received vehicle. Group II, III, IV, V, VI and VII received test compounds S1, S2, S3, S4, S5 and S6 NS formulation, respectively, at dose 300 mg/kg. Test compounds were administered orally in morning using gavage for 28 days.

All rats were observed daily for morbidity and mortality. Body weight, food intake and water intake were measured at 7-day interval starting from 0 day till 28 days. On 28th

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