J Incl Phenom Macrocycl Chem (2012) 74:447–454
DOI 10.1007/s10847-012-0140-x

ORIGINAL ARTICLE

Influence of different techniques on formulation and comparative characterization of inclusion complexes of ASA with β-cyclodextrin and inclusion complexes of ASA with PMDA cross-linked β-cyclodextrin nanosponges

Pravin K. Shende · Francesco Trotta · R. S. Gaud · Kiran Deshmukh · Roberta Cavalli · Miriam Biasizzo

Received: 23 December 2011 / Accepted: 1 March 2012 / Published online: 13 April 2012

Abstract Acetyl salicylic acid (ASA), a non-steroidal anti-inflammatory drug, was formulated into inclusion complexes by grinding and precipitation with β-cyclodextrin and freeze drying with pyromellitic dianhydride (PMDA) cross-linked β-cyclodextrin nanosponges. Particle size, zeta potential, encapsulation efficiency, accelerated stability study, *in vitro* and *in vivo* release studies were used as characterization parameters. TEM studies showed that the particle sizes of different inclusion complexes of ASA have diameters ranging from 40.12 ± 8.79 to 59.53 ± 15.55 nm. It also revealed the regular spherical shape and sizes of complexes that are even unaffected after drug encapsulation. Zeta potential was sufficiently high to obtain a stable colloidal formulation. The *in vitro* and *in vivo* studies indicated a slow and prolonged ASA release from PMDA cross-linked β-cyclodextrin nanosponges over a long period. XRPD, DSC and FTIR studies confirmed the interactions of ASA with nanosponges. XRPD showed the crystalline nature of ASA decreased after encapsulation. These results indicate that ASA nanosponges formulation can be used for oral delivery.

P. K. Shende (✉) · F. Trotta · M. Biasizzo
Department of Chemistry IFM, University of Torino, Via P. Giuria 7, Turin, Italy
e-mail: shendepravin94@yahoo.co.in

P. K. Shende · R. S. Gaud
School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

P. K. Shende · K. Deshmukh · R. Cavalli
Department of Science and Technology of Pharmaceutics, University of Torino, Via P. Giuria 9, Turin, Italy

Keywords ASA · Inclusion complex · Nanosponges · β-Cyclodextrin

Introduction

The formation of inclusion complexes with wide variety of guest molecules is one of the most interesting properties of cyclodextrins (CDs). A controlled release nanoparticle system containing nanosponges (NS) shows the promising results in anticancer drug delivery system [1], proteins delivery system [2], anti-inflammatory drugs delivery system [3, 4] etc. CD based NS are biocompatible nanoporous nanoparticles, obtained by the cross-linking of CD with carbonyl diimidazole, hexamethylene diisocynate, toluelyl diisocynate, dianhydride or carbonate. They are spherical, solid particles and have been reported to form inclusion and non-inclusion complexes with different drugs and to improve the solubility of poorly soluble molecules [5].

Acetyl salicylic acid (ASA) is a non-steroidal anti-inflammatory and antipyretic drug which acts by inhibition of prostaglandin synthesis and used to relief the pain. It is a COX-2 inhibitor and is amply used as analgesic drug also. ASA is rapidly hydrolyzed in plasma to salicylic acid, with a half-life of 15–20 min. It has a direct irritant effect on gastric mucosa due to inhibition of prostaglandins and prostacyclins and thus causes ulceration, epigastric distress and hemorrhage. Controlled release formulation of ASA would reduce frequency of administration, reduce the undesired side effects and improves the patient compliance [6, 7].

The objective of the present study was to develop inclusion complexes of ASA with β-cyclodextrin (β-CD) and pyromellitic dianhydride (PMDA) cross-linked β-CD NS (β-NS), as a carrier for controlled release in inflammatory conditions and for prolonging the shelf-life.

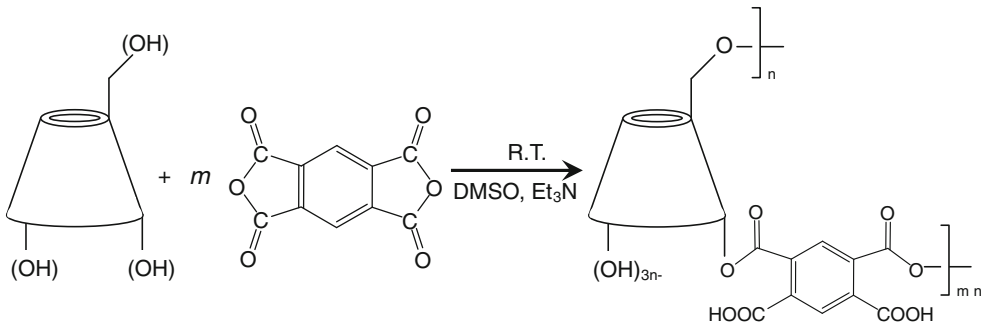

Fig. 1 The schematic presentation of formation of PMDA cross-linked β-CD nanosponges (β-NS)

Materials and methods

β-Cyclodextrin was purchased from Wacker Chemie GmbH (Munchen, Germany). PMDA and ASA were purchased from Sigma–Aldrich. All other chemicals and reagents were of analytical grade.

Methods

Preparation of ground mixture [8, 9]

An equimolar mixture of ASA with β-CD in the ratio (1:1) was ground in a vibrational rod mill (Heiko Seisakusho) at ambient temperature for 1 h. This formulation was abbreviated as F1.

Precipitation method [10]

Accurately weighed ASA and β-CD in the molar ratio (1:1) was dispersed in distilled water. Then the solution was heated at 60 °C to obtain viscous, concentrated and translucent liquid. The solution was kept at room temperature to give a precipitation of inclusion complex. The obtained precipitate was centrifuged, filtered and dried to get inclusion complex of ASA and β-CD (F2).

Synthesis of NS

The NS were obtained by following the synthetic procedure reported in the Italian patent [11]. Briefly, β-CD and PMDA in the molar ratios (1:2, 1:4 and 1:8) were dissolved in DMSO containing triethylamine 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. The molar ratios (1:2, 1:4 and 1:8) named the formulations plain F3, F4 and F5, respectively [12]. The ratio of β-CD and cross-linker can be varied during their preparation to improve the drug encapsulation and to obtain a modified release profile. The schematic presentation shown in Fig. 1.

Preparation of ASA-loaded NS

Acetyl salicylic acid loaded NS were prepared by freeze drying. ASA was dispersed in aqueous suspensions of the various types of NS in a ratio of 1:1 (w/w) and was stirred for 24 h. Then suspensions were centrifuged at 1,500 rpm for 15 min to separate the uncomplexed drug as a residue below the colloidal supernatant. The filtrate was then lyophilized to get ASA-loaded NS namely F3, F4 and F5, depending upon the ratios of β-CD: cross-linker. The ASA-loaded NS formulations were milled and stored in a covered vacuum desiccator at ambient temperature until further use.

Characterization

Particle size

The particle size and shape of various inclusion complexes were assessed by transmission electron microscopy (TEM) analyses (Philips CM10 instrument) at 92, 000x magnification.

Zeta potential

Zeta potential of formulations of inclusion complex of ASA was determined by Malvern Zetasizer (Aimili Ltd.).

Encapsulation efficiency

Formulations of inclusion complexes equivalent to 10 mg of ASA were dissolved in 10 ml of phosphate buffer (pH 7.4) in presence of methanol. The drug content was analyzed by UV-Spectrophotometer (Perkin Elmer) at 234 nm. The drug encapsulation efficiency was calculated by the following formula:

$$\% \text{ Drug entrapment efficiency} = \text{Drug}_{\text{encapsulated}} \times 100/\text{Drug}_{\text{total}}.$$

In vitro release study [13]

In vitro release of ASA from different formulations of inclusion complex was analyzed by membrane dialysis method against phosphate buffer (pH 7.4) at 37 °C. Briefly, a 10 mg of formulations of ASA suspended in 2 ml of phosphate buffer (pH 7.4) was placed in the dialysis tube (Sartorius, cut off 12,000 Da) and then suspended in a temperature controlled, jacketed flask containing 20 ml of phosphate buffer pH 7.4. At various time intervals, aliquot samples were withdrawn and analyzed by UV spectrophotometer (Perkin Elmer) at 234 nm.

Accelerated stability study

The accelerated stability study was carried out according to ICH guidelines (1993) [14]. Sealed vial of freshly prepared formulation (F4) were placed in stability chamber maintained at 25 °C, 60 % RH. The formulation (F4) subjected to stability tests were analyzed for 3 months period for its physical appearance, size and nature of drug with a frequency of 1 month sampling.

In vivo evaluation

Albino rats of Wistar strain (120–150 g) of either sex were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C; relative humidity 60–70 %) in a 12 h light dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experiment.

Acute toxicity

The acute toxicity of the aqueous extract of *Gmelina arborea* (AE) was evaluated in mice using the OECD Guidelines 423 (OECD, 2001) [15]. Female Wistar Albino rats (150–180 g) were used to assess the toxicity level. The rats were fasted over-night prior to dosing (food was withheld for 12 h but not water). During the period of fasting, rats were weighed and β-CD cross-linked NS (plain β-NS) and ASA loaded β-NS (i.e. F4) were administered. The β-NS and formulation (F4) at dose of 2,000 mg/kg was administered to three female rats in a single dose by oral gavage. Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.

Analgesic activity

In this method, rats in groups of five each were treated with distilled water, plain ASA and formulation (F4). Analgesic activity was assessed by counting the number of writhes induced by 0.6 % acetic acid (10 ml/kg, *i. p.*). The number of muscular contractions was counted over a period of 20 min after acetic acid injection. The data represent the total number of writhes observed during 20 min and is expressed as writhing numbers.

$$\%\text{Inhibition} = \frac{\text{Mean number of writhes(Control)} - \text{Mean number of writhes(Test)} \times 100}{\text{Mean number of writhes(Test)}}$$

Anti-inflammatory activity

Anti-inflammatory activity was assessed by carrageenan induced paw edema method in rats. Rats in groups of five each were treated with vehicle, ASA and formulation (F4) (equivalent to 100 mg of ASA) one prior to carrageenan injection. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1 % suspension of carrageenan in normal saline, in the right hind paw of the rats [16]. The paw volume was measured plethysmographically at a time interval of 0, 1, 2, 4, 6 h. The difference between the two readings was taken after carrageenan injection as the volume of edema and percentage anti-inflammatory activity was calculated.

FTIR

FT-IR spectra of pure ASA, β-NS formulation plain (F4) and formulation (F4) were obtained by FT-IR Spectrophotometer (Perkin–Elmer) using potassium bromide (KBr) pellets.

X-ray powder diffraction

Acetyl salicylic acid and formulation (F4) complex were subjected to X-ray powder diffraction (XRPD) studies using Huber Guinier camera G670 (sequential collection between 2.5° and 100° at 2θ).

Differential scanning calorimetry

Thermal analysis of pure ASA, formulation plain (F4), and the formulation (F4) were carried out using DSC (Perkin–Elmer) method with a heating rate of 10 °C/min.

Results and discussion

Particle size, zeta potential and encapsulation efficiency

Transmission electron microscopy studies showed that the particle sizes of different inclusion complexes of ASA have diameters ranging from 40.12 ± 8.79 to 59.53 ± 15.55 nm. It also exposed the regular spherical shape and sizes of complexes that are even unaffected after drug encapsulation. The size of different formulations of inclusion complex was found to be F1 < F2 < F5 < F4 < F3 (Figs. 2, 3, 4, 5 and 6). The entire formulations prepared were found to be fine and free flowing powders.

Zeta potential of different formulations was determined, as a measure of surface charge. The results of zeta potential determination are presented in Table 1. The β-CD possessed a negative zeta potential (−23.9 mV ± 0.71) and different NS without drug possessed in the range −28.98 mV ± 3.02 to −31.06 mV ± 1.31. Zeta potentials of the different formulations of ASA-NS were compared to the zeta potential of ground mixture (F1) and zeta potential of precipitation method particles (F2). This reduction in

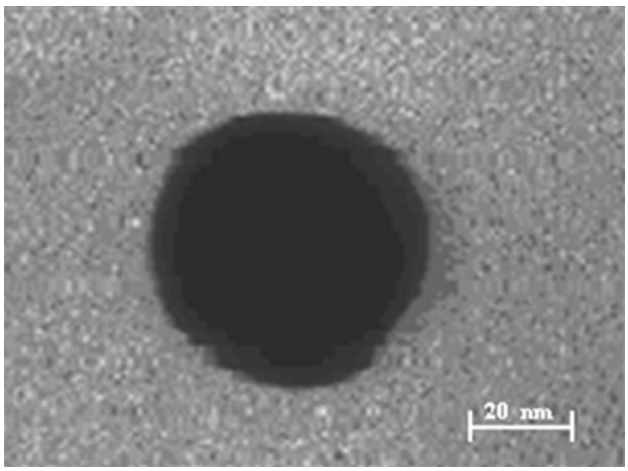

Fig. 3 TEM of formulation F2

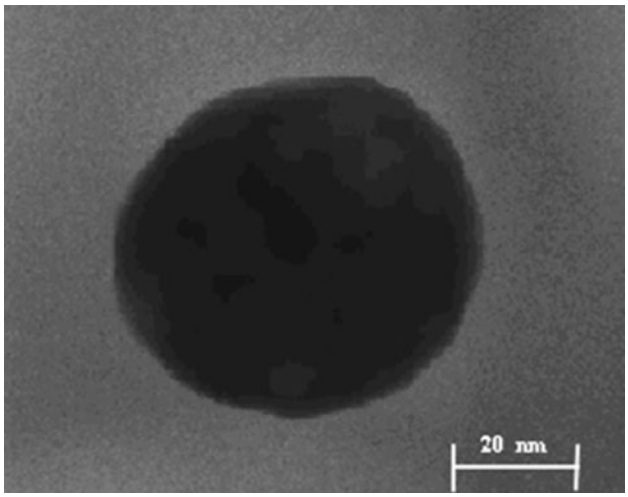

Fig. 4 TEM of formulation F3

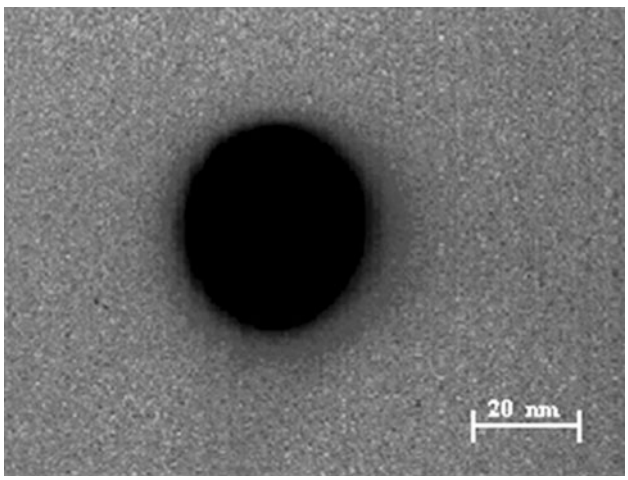

Fig. 2 TEM of formulation F1

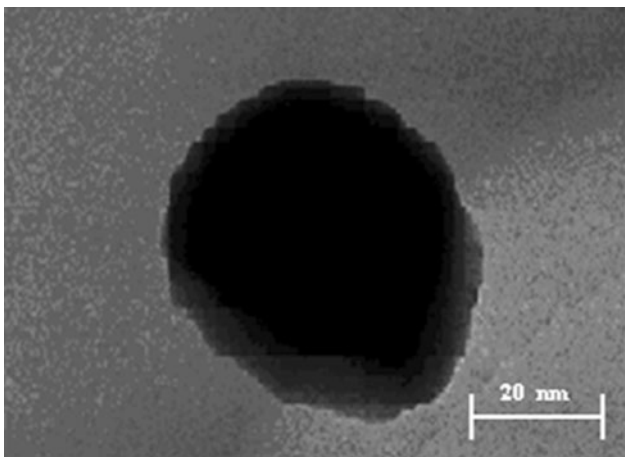

Fig. 5 TEM of formulation F4

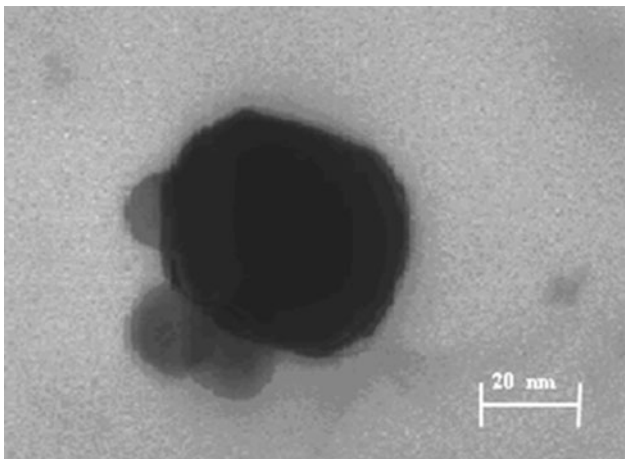

Fig. 6 TEM of formulation F5

Table 1 Particle size and zeta potential of ASA loaded different formulations

Si. No.	Formulation	Particle size (nm) ± SD	Zeta potential (mV) ± SD
1	F1	40.12 ± 8.79	−40.07 ± 0.98
2	F2	41.74 ± 10.34	−38.72 ± 2.06
3	F3	59.53 ± 15.55	−35.45 ± 2.73
4	F4	51.61 ± 13.47	−34.51 ± 0.95
5	F5	49.32 ± 11.23	−36.40 ± 1.80

zeta potential provides further evidence that negatively charged ASA has been adsorbed, which increased a portion of the negative surface charges on the particles. The value obtained was about −40 mV, which means that the particles have poor tendency to aggregate [17].

The % encapsulation efficiency of ASA in prepared inclusion complexes was found to be in the range of 84.21 ± 2.73–91.87 ± 3.62 %. The encapsulation efficiency of entire formulations was in order of F4 > F2 > F3 > F1 > F5 as shown in Fig. 7. It was found that for the NS ASA was loaded in the highest amount in F4 as much as 91 % (w/w), while 88 and 73 % (w/w) in F3 and F5, respectively. The encapsulation efficiency of complex between β-CD and ASA formulations was more in precipitation method compared to ground mixture [18] which might be depend on four different ways of inclusion complex formation between ASA and β-CD: 1. it could go into the CD cavity from the wider side of the cone shaped β-CD molecule with the lipophilic benzene ring going in first and the acetyl group partly standing out of the cavity; 2. it could go into CD cavity from wider side with the acetyl group going in first and benzene ring partly standing out; 3. it could go into CD cavity from the narrow side with benzene ring going in first and acetyl group partly standing out; and 4. it could go into the CD cavity from narrow side with the acetyl group going in first and benzene ring partly standing out [19, 20].

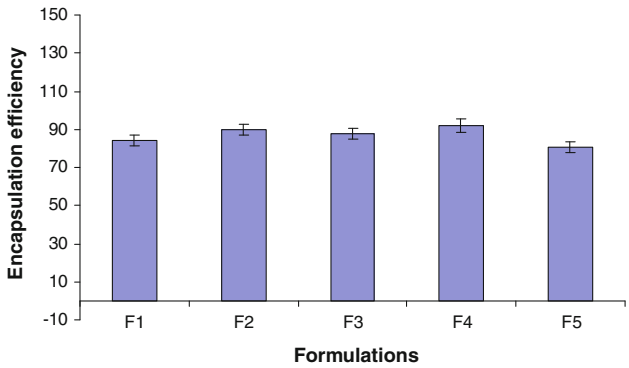

Fig. 7 Encapsulation efficiency of different formulations

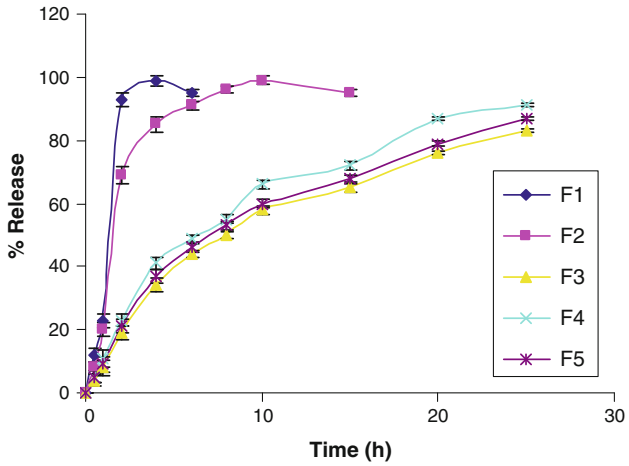

Fig. 8 *In vitro* release of ASA from different formulations

Table 2 Stability characteristics of formulation F4

Table	Particle size	Encapsulation efficiency	% release
1 month	52.34 ± 13.21	91.11 ± 2.25	90.18 ± 3.22
2 month	52.89 ± 13.28	91.05 ± 2.31	89.86 ± 3.18
3 month	53.17 ± 13.28	90.98 ± 2.33	89.77 ± 3.20

The different ASA loading showed that the degree of cross-linking affected the complexation capacity of NS. It might be supposed that in F3, the lower amount of cross-linker formed a network with an uncompleted cyclodextrin cross-linking and with decreased sites for the drug complexation; thus, ASA might not be included in higher amount in this types of NS. While in F5, the higher amount of cross-linker might provide a high cross-linking of β-CD, and consequently a part of ASA interaction with β-CD cavities might be entangled.

Table 3 Effect of formulation (F4) on acetic acid induced writhing in mice

Treatment	Number of writhing	% inhibition
Control	80.7 ± 3.5	–
ASA	39.21 ± 2.7*	51.41
Formulation (F4)	17.19 ± 2.1*	78.70

$n = 5$. The observations are mean ±S.E.M. *$P < 0.05$, as compared to control

In vitro release studies

The *in vitro* release profile of all formulations is shown in Fig. 8. It was found that the ASA ground mixtures and inclusion complex by precipitation method released faster than the ASA and its inclusion complex with NS. The inclusion complexes of ASA with NS showed the controlled release in the manner F4 > F5 > F3. *In vitro* release and dissolution (IVRAD) enhanced may be due to the decrease in crystallinity and the increase in solubility of the drug [8].

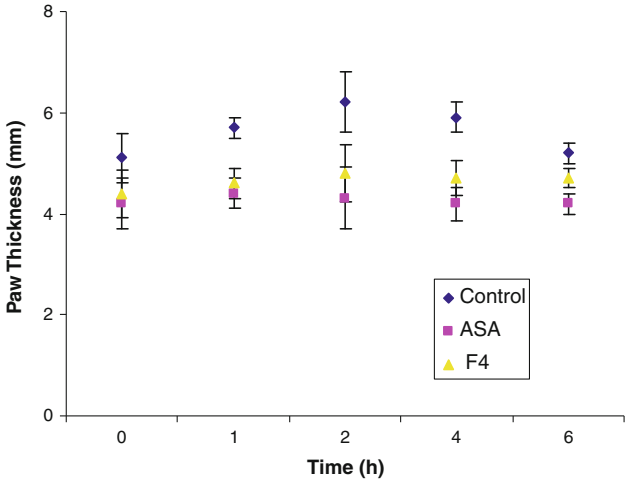

Fig. 9 Effect of ASA on carrageenan induced paw edema in rats

The % of ASA released from β-NS formulations after 24 h ranged between 83 to about 91 % showing a strong interaction of the drug with the three different ratios of NS. A slow ASA release might decrease the toxic side effects

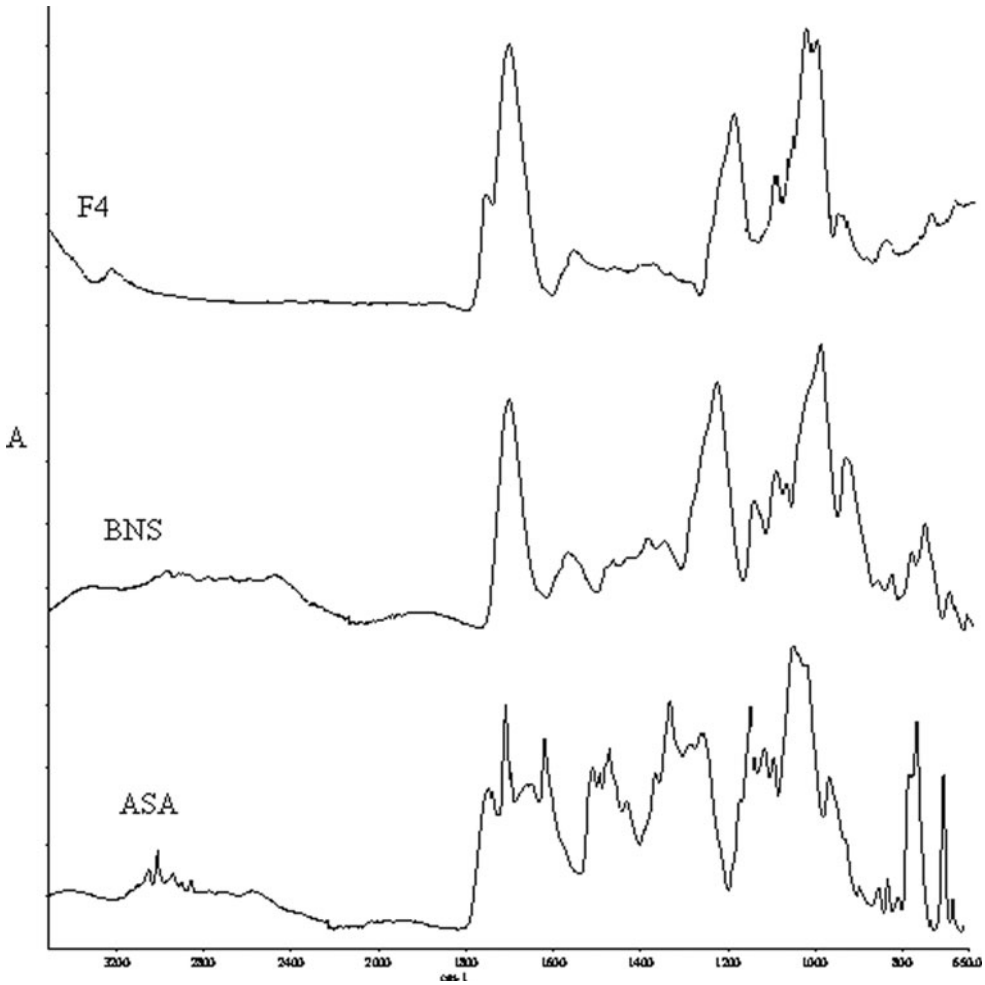

Fig. 10 FTIR spectra of ASA, PMDA cross-linked β-CD and formulation F4

of the drug to the tissues. The slow release of ASA from F3 than from other formulations might be due to the lowest extent of cross-linking which might permit the encapsulation of ASA mainly as inclusion complex in the NS structure. These ASA NS showed comparatively fast controlled release pattern to camptothecin NS [5].

Considering the ASA-encapsulation and *in vitro* release studies, we selected only the formulation (F4) to determine the *in vivo* release study, stability study and further instrumental studies.

Accelerated stability study

The measurement of size, ASA content and *in vitro* release of ASA of formulation (F4) demonstrated the preservation of nanoparticles during the stress testing as shown in Table 2.

In vivo evaluation

Acute toxicity The acute oral toxicity of ASA in rat is 1,000 mg/kg [21]. The oral administration of β-NS in rats at the dose of 2,000 mg/kg does not exhibit 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 2,000 mg/kg of body weight.

Analgesic activity The intraperitoneal administration of acetic acid produced writhing responses in experimental animals. Maximum percentage of inhibition of writhing response was observed with formulation (F4). ASA showed a maximum inhibition of writhing response as 51.41 %. The results are given in Table 3.

Anti-inflammatory activity In carrageenan induced rat paw edema, administration of formulation (F4) reduced inflammation significantly ($P < 0.01$ and $P < 0.05$) compared to plain ASA and control group (Fig. 9). The carrageenan induced inflammation was used as a standard model of screening for anti-inflammatory activity in various experimental compounds. It is commonly used due to absence of apparent systemic effects, antigenic nature of carrageenan and highly reproducible model. The formulation (F4) treated animals showed good anti-inflammatory activity in carrageenan induced inflammation compared with control animals. This action may be due to inhibition of the inflammatory mediators release by formulation (F4).

FTIR The spectrum of ASA showed two absorption bands, one of them at 1,756 cm^{-1} is due to acetoxyl carbonyl stretching and the other at 1685 cm^{-1} is due to hydrogen-bonded carboxyl carbonyl stretching. There is no change in the above two absorption bands in inclusion complex but change in frequency by complexing with PMDA cross-linked β-CD NS (β-NS) as shown in Fig. 10.

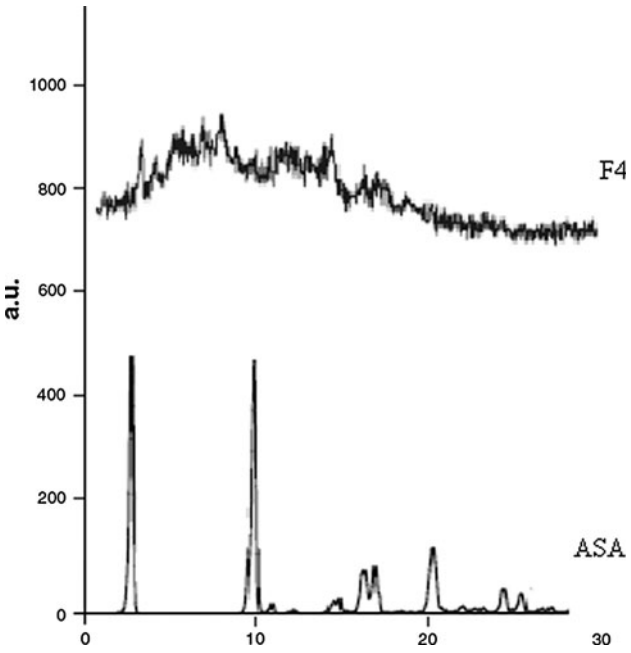

Fig. 11 XRPD pattern of ASA and formulation F4

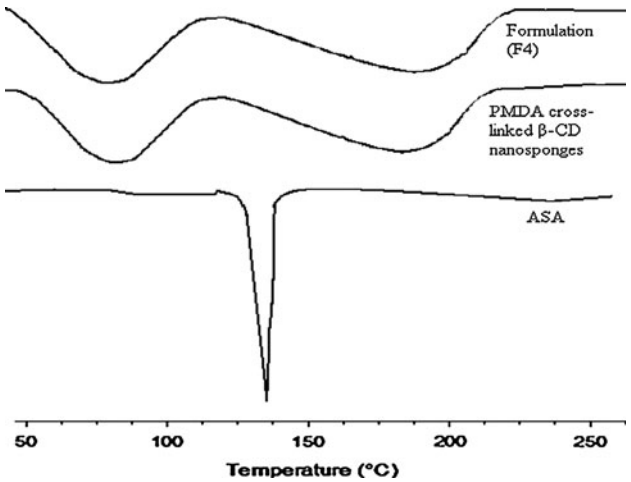

Fig. 12 DSC thermograms of ASA, β-NS and formulation F4

X-ray powder diffraction The comparison of complex and ASA showed that there was significant change in the intensities of powder, 2θ values (Fig. 11). Formulation (F4) gives rise to fluffy mass powder showing highly porous structure losing all its crystallinity which was confirmed by XRPD study.

Differential scanning calorimetry Differential scanning calorimetry thermograms of ASA, β-NS (formulation plain

F4) and their complex (F4) as showed in Fig. 12. The endothermic peak at around 138 °C was due to the fusion of ASA. However, in the complex this endotherm was suppressed indicating the partial protection due to the encapsulation of ASA with NS. The percentage of crystalline nature was drastically changed in NS complex indicating ASA was dispersed in NS loosing most of its crystallinity. This might be because of inclusion as well as non-inclusion phenomenon of β-NS with ASA.

Conclusion

β-Cyclodextrin based NS were able to complex efficiently with ASA and to release it slowly in physiological media. This nanosponge based formulation possesses nanosize, spherical shape and display significantly better encapsulation and stability. NS showed as promising carriers for ASA acting as a reservoir for the controlled release of the active pharmaceutical ingredient.

References

1. Torne, S., Ansari, A., Vavia, R., Trotta, F., Cavalli, R.: Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded nanosponges. Drug Deliv. **17**, 419–442 (2010)
2. Swaminathan, S., Cavalli, R., Trotta, F., Ferruti, P., Ranucci, E., Gerges, I., Manfredi, A., Marinotto, D., Vavia, P.: In vitro release modulation and conformational stabilization of a model protein using swellable polyamidoamine nanosponges of β-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem **68**, 183–191 (2010)
3. Ansari, K., Vavia, P., Trotta, F., Cavalli, R.: Cyclodextrin-based nanosponges for delivery of resveratrol: In vitro characterisation, stability, cytotoxicity and permeation study. AAPS PharmSciTech **12**, 1 (2011)
4. Cavalli, R., Trotta, F., Tumiatti, W.: Cyclodextrin-based nanosponges for drug delivery. J. Incl. Phenom. Macrocycl. Chem. **56**, 209–213 (2006)
5. Swaminathan, S., Pastero, L., Serpe, L., Trotta, F., Vavia, P., Aquilano, D., Trotta, M., Zara, G., Cavalli, R.: Cyclodextrin-based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity. Eur. J. Pharm. Biopharm. **74**, 193–201 (2010)
6. Patel, D., Hesse, A., Ogunbona, A., Notarianni, L., Bennett, P.: Metabolism of aspirin after therapeutic and toxic doses. Hum. Exp. Toxicol. **9**, 131–136 (1990)
7. Vane, J., Bottin, R.: The mechanism of action of aspirin. Thromb. Res. **110**, 255–258 (2003)
8. El-Gendy, G., Terada, K., Yamamoto, K., Nakai, Y.: Molecular behavior, dissolution characteristics 'and chemical stability of aspirin in the ground mixture and in the inclusion complex with di-0-methyl-/3-cyclodextrin. Int. J. Pharm. **31**, 25–31 (1986)
9. Szejtli, J.: Cyclodextrin technology, p. 280. Kluwer, Dordrecht (1988)
10. Mannila, J., Jarvinen, T., Jarvinen, K., Jarho, P.: Precipitation complexation method produces cannabidiol/b-cyclodextrin inclusion complex suitable for sublingual administration of cannabidio. J. Pharm. Sci. **96**, 312–319 (2007)
11. Trotta, F., Tumiatti, W., Vallero, R.: Italian Patent No. MI2004 A000614
12. Mele, A., Castiglione, F., Malpezzi, L., Ganazzoli, F., Raffaini, G., Trotta, F., Rossi, B., Fontana, A., Giunchi, G.: HR MAS NMR, powder XRD and Raman spectroscopy study of inclusion phenomena in β-CD nanosponges. J. Incl. Phenom. Macrocycl. Chem. **69**, 403–409 (2011)
13. Shende, P., Gaud, R.: Formulation and comparative characterization of chitosan, gelatin, and chitosan–gelatin-Coated Liposomes of CPT-11–HCl. Drug Dev. Ind. Pharm. **35**, 612–618 (2009)
14. http://www.ich.org/
15. Organization of Economic Co-operation Development: The OECD guidelines for testing of chemicals, 423. Acute oral toxicity test, Paris (2001)
16. Kulkarni, Y., Gokhale, S., Veeranjaneyulu, A., Surana, S., Tatiya, A.: Effect of *Persea macrantha* against acute inflammation and adjuvant-induced arthritis in rats. Pharm Biol. **47**, 304–308 (2009)
17. Das, S., Banerjee, R., Bellar, J.: Aspirin loaded albumin nanoparticles by coacervation: implications in drug delivery. Trends Biomater. Artif. Organs **18**, 203–212 (2005)
18. Shekh, I., Gupta, V., Jain, A., Gupta, N.: Preparation and characterisation of B cyclodextrin aspirin inclusion complex. Int. J. Pharm. Life Sci. **2**, 704–710 (2011)
19. Nishioka, F., Nakanishi, I., Fujiwara, T., Tomita, K.: The crystal and molecular structure of the β-cyclodextrin inclusion complex with aspirin and salicylic acid. J. Incl. Phenom. Macrocycl. Chem. **2**, 701–714 (1984)
20. Loftsson, T., Ólafsdóttir, B., Friðriksdóttir, H., Jónsdóttir, S.: Cyclodextrin complexation of NSAIDSs: physicochemical characteristics. Eur. J. Pharm. Sci. **1**, 95–101 (1993)
21. Boyd, E.: The acute oral toxicity of acetylsalicylic acid. Toxicol. Appl. Pharmacol. **1**, 229–239 (1959)