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Solubility and dissolution rate enhancement of ibuprofen by cyclodextrin based carbonate nanosponges

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Abstract: In the present study nanotechnology approach, i.e., a cyclodextrin (CD) based carbonate nanosponge was used to improve the solubility and dissolution of ibuprofen. Solvent and ultrasound assisted methods were used to prepare nanosponges using two CDs (β-CD and 2-hydroxypropyl-β-CD (2HP-β-CD)) and a cross-linker (CL) diphenyl carbonate (DPC) in varying molar ratios. Nanosponges were investigated for their solubilizing efficiency and phase solubility studies. Structural analysis by Fourier transform infrared (FTIR) and powder X-ray diffraction (PXRD), thermo-analytical characterization by differential scanning calorimetry (DCS), morphology by scanning electron microscopy (SEM). *In-vitro* drug release followed by *in-vivo* analgesic and anti-inflammatory studies were performed. 2HP-β-CD based nanosponges (molar ratio 0.01:0.04) prepared by ultrasound assisted method showed the highest solubilizing efficiency (i.e., 4.28 folds). Stability constant values showed that all complexes were stable. Inclusion complexes of drug was confirmed by PXRD and DSC. SEM images showed porous structures confirming the formation of cross-linked network. Particle size was in the range of 296.8±64 to 611.7±32nm. *In-vitro* release studies showed enhanced dissolution profile from nanosponge formulation (~94% from I11) as compared to the pure drug (~45% Ibuprofen) in 120min. Significant ($p<0.05$) extent of pain inhibition and anti-inflammatory activity was observed for nanosponge formulation when compared with the pure drug. CD based carbonate nanosponges with better solubility, enhanced release profile, improved analgesic and anti-inflammatory activity were successfully formulated for ibuprofen.

Keywords: Carbonate nanosponge, cyclodextrin, ibuprofen, nanotechnology, solubility enhancement.

INTRODUCTION

Ibuprofen is a potent non-steroidal anti-inflammatory drug (NSAID) belonging to BCS class II (low solubility, high permeability) drugs and have been used in the management of rheumatoid arthritis, osteoarthritis and traumatic contusions (Reis *et al*., 2014). The limited aqueous solubility (0.021mg/ml at 25°C), dissolution rate, and high lipophilicity ($LogP_{o/w}$=3.97) of ibuprofen restrict its oral bioavailability and consequently pose a delay in its analgesic effect. Low solubility also enhances the residence time of ibuprofen in the mucosa thereby increasing its irritating side effect on the gastrointestinal tract (GIT) (Irvine *et al*., 2018). Many conventional tablets, capsules and oral suspensions of ibuprofen are available in the market. The marketed formulations are mostly in the form of salt conjugates of ibuprofen prepared by crystallization processes (Ragab and Rohani, 2009). Techniques which were used in the past in attempt to enhance the solubility of ibuprofen are solid dispersion (Hasnain and Nayak, 2012), Co-milling (Hussain *et al*., 2018), block copolymers (Dugar *et al*., 2016), and dendrimers (Milhem *et al*., 2000). Nowadays formulation scientists are exploring the use of nanotechnology to develop nanocarriers to address the solubility issue (Zaman, 2016).

Amongst the new nano formulation techniques, 'nanosponges' have earned popularity as potential nanocarriers due to it being a small sponge-like structure having the capability of incorporating a wide variety of substances / drugs within its core (Trotta *et al*., 2012). At the same time, nanosponges have been found to be free of the drawbacks associated with other carrier systems e.g., sterilization problem of nano lipid carriers, polymer depended biocompatibility of dendrimers, chemical instability and cost of transferosome, premature and less entrapment of active ingredients in microspheres (Kumar *et al*., 2019). Nanosponges, which are hyper crosslinked, solid, porous, colloidal sized structures, can be formed by polymerization reaction of cyclodextrin (CD) with active carbonyl compounds as a cross linkers (CLs) (such as carbonyldiimidazole (CDI), diphenyl carbonate (DPC), and trifosgene) which leads to the formation of nanosponges having carbonate bonds between two monomers of CDs and known as CD based carbonate

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nanosponges. Nanosponges form supramolecular inclusion and non-inclusion complexes with many drugs (Sherje *et al*., 2017). A number of physicochemical properties like taste, solubility and dissolution rate are significantly improved as a result of this complexation (Khalid *et al*., 2021). Further advantages include reduced side effects of drugs and improved stability (Tejashri *et al*., 2013). These smart nanocarriers can be formulated for oral, parenteral and topical route of administration as they have been considered safe for oral and invasive routes. Also, it is possible to formulate a targeted drug delivery (Swaminathan *et al*., 2013b) by using these nano-carriers.

The aim of the current study was the development and characterization of CD based carbonate nanosponges of ibuprofen to enhance its solubility and dissolution rate. In the current study, we formulated nanosponges using two types of CDs (β-CD and 2-hydroxypropyl-β-CD (2HP-βCD) and DPC as a cross linker. The cross-linking was achieved by using solvent method and ultrasound assisted method. By changing the polymer and CL: polymer, different nanosponges were produced having different entrapment efficiency and fashioning a tailored release profile. Synthesized nanosponges were evaluated for structural, thermo-analytical and morphological characteristics. It is evident from literature (Ansari *et al*., 2011; Pushpalatha *et al*., 2018; Zidan *et al*., 2018) that generally enhanced solubility leads to the improved therapeutic effect. Hence in this study, pharmacodynamics studies were performed as a proof of concept of better therapeutic activity with the improvement in drug solubility. In future we will use these nanosponges in formulating the targeted drug delivery.

MATERIALS AND METHODS

Ibuprofen was obtained as gift sample from Mass Pharma Pvt. Ltd. Lahore (Pakistan). β-CD (Merck, Germany), 2HP-βCD (Carbosynth, San Diego), DPC (Oakwood chemicals, Columbia), dimethyl formamide (DMF) (Merck, Germany) were used.

Preparation of nanosponges

CD based carbonate nanosponges were prepared in two stages. Firstly plain CD based carbonate nanosponges were developed which were loaded with the ibuprofen in the second step.

Step 1: Preparation of plain CD based carbonate nanosponges

Twelve types of carbonate nanosponges were prepared by reacting CDs (β-CD and / or 2HP-βCD) with a CL (DPC) in three molar ratios, i.e., 0.01:0.02, 0.01:0.04 and 0.01:0.06 (table 1). Two methods were used to achieve hyper crosslinking of CD with the CL, i.e., solvent method and ultrasound assisted method (Six carbonate nanosponges were prepared by each of the method).

In solvent method, required amounts of each of the CD was dissolved separately in 100 ml of DMF followed by addition of DPC. The reaction was carried out at 90°C for 6h. After the reaction was completed, the solution was cooled at room temperature. This solution was then added to excess of distilled water to obtain the solid product which was recovered by vacuum filtration. Product was purified by soxhlet extraction using ethanol. Subsequently, it was dried overnight in an oven at 60°C, ground and stored at 25°C in a tight container (Anandam and Selvamuthukumar, 2014a).

In ultrasound assisted method cross linking of each of the CD was done in anhydrous form with an excess of melted DPC. The reaction was carried out under sonication in an ultrasound bath at 90°C for 5h. After cooling, the product was ground and washed repeatedly with distilled water and acetone to remove unreacted CD, DPC and the phenol crystals generated as by-product of the reaction. The resultant product was stored at 25°C (Trotta *et al*., 2008).

STEP 2: Loading of drug in nanosponges

Ibuprofen loading in all types of nanosponges was achieved using incubation process followed by lyophilization. Drug was dispersed in an aqueous suspension (50ml) of each type of plain nanosponges in 1:1 weight ratio and was stirred. After 24h, the suspension was centrifuged at 2000rpm for 10min to separate the un-complexed drug as a residue below the colloidal supernatant. The colloidal supernatant was lyophilized using freeze dryer (Scanvac coolsafe 110-4, Labogene) operated at -20°C and 13.33mbar pressure to obtain drug loaded nanosponges formulations which were given the codes I1-I12 as shown in table 1. The obtained formulations were kept in a vacuum desiccator at 25°C till further use (Swaminathan *et al*., 2013a).

Characterization of plain nanosponges

Plain nanosponges were investigated for their potential to enhance the solubility of ibuprofen via solubilizing efficiency and phase solubility studies and for structural analysis by Fourier transform infrared (FTIR).

Solubility studies (solubilizing efficiency and phase solubility studies)

Solubilizing efficiency of nanosponges was investigated. An excess amount (100mg) of the drug and fixed amount (20mg) of β-CD, 2HP-βCD and all types of nanosponges in a separate volumetric flasks was suspended in water (20ml) and shaken overnight at room temperature. After appropriate dilution, the filtrate absorbance was measured using the calibration curve (concentration range: 1-10µg/ml) of the drug by UV spectrophotometer at λ_{max} 280nm (Olteanu *et al*., 2015).

Higuchi and Connors method was used to perform phase solubility studies on selected nanosponges showing the highest solubilizing efficiency. In this method, solubility enhancement of drug was assessed as a function of host (CD) concentration (Cho and Jung, 2015). An excess amount of drug was added to separate glass containers, having increasing concentrations (0.001 to 0.006 moles) of nanosponges in distilled water i.e. molar solutions. The glass containers were sealed and rotated for 48h at 30rpm at ambient temperature. After 48h, sample solutions were filtered, suitably diluted and drug content was determined spectrophotometrically. Phase solubility diagram was plotted using the dissolved drug concentration against respective concentration of nanosponges. According to the hypothesis of 1:1 stoichiometric ratio of complex, apparent stability constant (K_c) was calculated using the equation (1).

$$K_c = \frac{\text{slope}}{s_0(1-\text{slope})} \quad (1)$$

Where S_0 is the intrinsic solubility of the drug (Rao and Bhingole, 2015).

Structural analysis by FTIR

On the basis of highest solubilizing efficiency, one type of nanosponge representing each CD and each method was selected for FTIR analysis. FTIR spectra of pure CD and selected plain nanosponges were obtained by ATR-FTIR (Bruker, Alpha-P) over a range of 4000 to 400cm^{-1} (Shringirishi *et al*., 2014).

Characterization of drug loaded nanosponge formulations

Drug loaded nanosponge formulations were initially characterized for entrapment efficacy, particle size (PS), polydispersity index (PDI) and zeta potential (ZP). Considering the initial parameters of plain and drug loaded nanosponges, best formulation was selected for further structural, thermo-analytical and morphological investigations.

Entrapment efficiency (%)

Entrapment efficiency (%) was calculated by equation (2). Each formulation (20mg) was dissolved separately in 100ml phosphate buffer (pH 7.2) under sonication for 20min. Drug content was analyzed spectrophotometrically from the calibration curves at λ_{max} 222nm (Abbas *et al*., 2018).

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual amount of drug in nanosponges}}{\text{Total amount of drug used in nanosponges}} \times 100 \quad (2)$$

Particle size (PS), polydispersity index (PDI) and Zeta potential (ZP)

PS, PDI and ZP of formulations was obtained by zeta sizer (Malvern, ZSP Nano). Aqueous dispersions of formulations were diluted to suitable scattering intensity at 25°C and the mean hydrodynamic diameter was recorded using dynamic light scattering measurements.

Structural analysis

Structural analysis was performed using FTIR and PXRD techniques. FTIR spectra of selected nanosponge formulations were scanned by ATR-FTIR (Bruker, Alpha-P) from 4000 to 400 cm^{-1}. PXRD analysis of the pure drug, Physical mixture (PM) of drug with polymer ((β-CD or 2HP-βCD) in 1:1 weight ratio) and selected formulation was performed by X-ray diffractometer (D8 Advance, Bruker) using Cu Kα radiation (λ=1.54060 A°) at a scanning rate of 100/min. Diffraction angle 2θ range was 10-80º (Zidan *et al*., 2018).

Thermo-analytical investigation

Thermo-analytical investigation of pure drug, PM of the drug with polymer and selected formulations was performed by DSC and thermal gravimetric analysis (TGA). Thermal behavior was assessed by using differential scanning calorimeter (TA instruments, SDT Q600), heating from room temperature to 300°C at the rate of 10°C/min under N_2 (Zainuddin *et al*., 2017).

Table 1: Composition of CD based carbonate nanosponges

Method Used	Code for empty nanosponges	Type of CD	CD to CL molar ratio	solvent	Formulation code after drug loading
Solvent method	B-CD NS1	β-CD	0.01:0.02	DMF	I1
	B-CD NS2	β-CD	0.01:0.04	DMF	I2
	B-CD NS3	β-CD	0.01:0.06	DMF	I3
Ultrasound assisted method	B-CD NS4	β-CD	0.01:0.02	-	I4
	B-CD NS5	β-CD	0.01:0.04	-	I5
	B-CD NS6	β-CD	0.01:0.06	-	I6
Solvent method	HP-BCD NS1	2HP-βCD	0.01:0.02	DMF	I7
	HP-BCD NS2	2HP-βCD	0.01:0.04	DMF	I8
	HP-BCD NS3	2HP-βCD	0.01:0.06	DMF	I9
Ultrasound assisted method	HP-BCD NS4	2HP-βCD	0.01:0.02	-	I10
	HP-BCD NS5	2HP-βCD	0.01:0.04	-	I11
	HP-BCD NS6	2HP-βCD	0.01:0.06	-	I12

Morphological analysis

SEM was used to examine the morphology of selected formulation. Sample of nanosponges were fixed on aluminum stub and for better contrast samples were coated with gold, using sputter coater (Denton, Desk V HP) functioning at 40mA for 25sec under vacuum. Morphology of nanosponges was observed with field emission scanning electron microscope (FE-SEM, Nova Nano SEM 450) operating at 15kV (Yaşayan *et al*., 2020).

In-vitro drug release & kinetic studies

In-vitro release of pure drug and the selected nanosponge formulation (Rao *et al*., 2018) was carried out in 900ml of phosphate buffer (pH 7.2) at 37±0.5°C. A sample of nanosponge formulation (n=6) containing 100mg of the ibuprofen was enclosed in hard gelatin capsule and was placed in Type-II dissolution apparatus (ErwekaDT700) which was run at a paddle speed of 50rpm for 2h. Aliquots (5ml) were withdrawn at fixed time intervals (5, 15, 30, 60, 90, and 120min) and filtered. The withdrawn aliquots were reinstated with fresh dissolution media (5ml) to maintain the sink conditions. Drug content was analyzed spectrophotometrically at λ_{max} 222nm. Kinetic analysis was performed by zero-order, first-order, Higuchi, Korsmeyer-Peppas, Hixson- Crowell & Baker Lonsdale kinetic models (Omar *et al*., 2020). Best fit of the release data was determined based on the coefficient of correlation (R^2).

In-vivo activity studies

Selected drug loaded nanosponge formulation was subjected to peripheral anti-nociceptive activity employing Swiss albino mice (weighing 25 to 30g) and anti-inflammatory activity using Wistar rats (weighing 180 to 200g) of either sex. For each activity, animals were randomly divided into 3 groups namely control group, standard group and test group with six animals (n=6) in each group. Control group was administered with normal saline at the dose of 10ml/kg. A dose of 25mg/kg body weight of pure ibuprofen and equivalent nanosponge formulation was administered orally to standard group and test group respectively. The study was conducted according to research protocols approved by animal ethical committee, university college of pharmacy, university of the Punjab, Lahore (Ref no: AEC/PUCP/1073A).

Peripheral anti-nociceptive activity was determined by acetic acid induced abdominal writhing in mice. Injected 0.6% acetic acid (10 mL/kg) intraperitoneally into the lower right quadrant of the abdomen of mice at an angle of 30° and a depth of 5mm. Normal saline, pure drug and nanosponge formulation was given to their respective groups and was administered 30min before acetic acid injection. Immediately after the injection, no. of writhes in animals for 90mins were observed. Analgesic effect was measured as the % inhibition in the number of writhes (Suthakaran and Senthil, 2018) and was calculated using equation 3:

$$\% \text{ inhibition} = \frac{Wc - Wt}{Wc} \times 100 \qquad (3)$$

Where, Wc = No. of writhes in control group, Wt = No. of writhes in drug treated groups

Anti-inflammatory activity was investigated by Carrageenan induced paw edema method (Ashraf *et al*., 2019). In this method, lambda carrageenan solution (1% w/v in normal saline) was used to produce acute inflammation in wistar rats. Solution (0.1ml) was injected into the sub-plantar region of the rat's left paw. The paw volume of rats was measured by digital plethysmometer (Panlab HA, Cornella spain) before the injection of carrageen, 2.5h after the injection of carrageenan solution (just before the administration of the test drugs) and then again two hours after test drug administration (Mumuni A *et al*., 2014). The % inhibition was calculated by using equation (4).

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100 \qquad (4)$$

Where, Vc is the average increase in paw volume of the control group, Vt is the average volume of the paw of drug treated groups**.**

STATISTICAL ANALYSIS

SPSS version 20.0 was used for the statistical analysis. All data were expressed as mean ± SD. To compare the means, one-way analysis of variance (ANOVA) was used followed by post-hoc Tukey HSD. The criterion for statistical significance was fixed at $p<0.05$.

RESULTS

CD based carbonate nanosponges were successfully prepared by solvent method and ultrasound assisted method. Plain nanosponges were initially investigated for solubilizing efficiency of nanosponges and phase solubility studies and for confirmation of nanosponge formation by structural analysis using FTIR technique.

Solubility Studies

Maximum increase (4.2 folds) in solubility was observed with HP-BCD NS5 (CD-CL ratio 0.01:0.04, prepared by ultrasound assisted method) (fig. 1).

One nanosponge formulation based on each CD showing the highest solubilizing efficiency i.e. B-CD NS5 and HP-BCD NS5 (after which there was no increase in solubility) was selected for phase solubility analysis. The phase solubility diagrams of ibuprofen (fig. 2) showed that the solubility of ibuprofen linearly increased with B-CD NS5 concentration from 0.001 to 0.004mol/l. However, the solubility of ibuprofen slightly decreased with an increase of B-CD NS5 concentration from 0.005 to 0.006mol/l. This diagram could be categorized as Bs

type which denoted complexes with limited solubility (Ghorab and Adeyeye, 2001; Salústio *et al*., 2009). The calculation of K_c and assumption of 1:1 complex formation was based on the linear portion of the plot. On the other hand phase solubility diagrams of ibuprofen with HP-BCD NS5 showed AL type behavior which means linear increases of drug solubility as a function of nanosponge concentration (Felton *et al*., 2014).

Experimental value of intrinsic solubility (S_0) of ibuprofen was 0.000054 moles (reported value 0.0001017 moles). The K_c values are given in table 2.

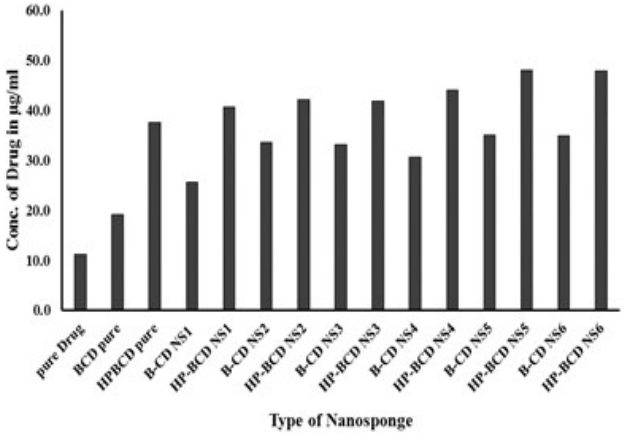

Fig. 1: Solubilizing efficiency of the nanosponges

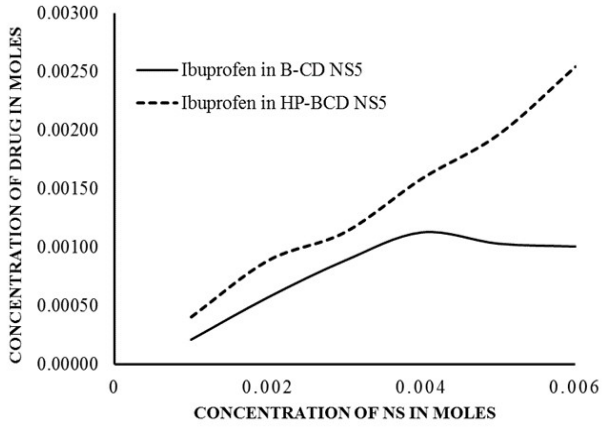

Fig. 2: Phase solubility diagram of ibuprofen in increasing concentrations of nanosponges.

Table 2: K_c values for nanosponges with ibuprofen

Nanosponge	B-CD NS5	HP-BCD NS5
Linear equation	y = 0.3065x - 7E-05	y = 0.4113x - 2E-05
R2 value	R^2 = 0.9923	R^2 = 0.9898
K_c value(L.Mol^{-1})	3906.42	4449.95

Structural analysis

Fig. 3 has shown the comparison of the FTIR spectra of formed nanosponges with that of the pure CD. FTIR spectra of β-CD showed characteristic peaks at 3295, 2930 and 1647 cm^{-1} corresponding to symmetric and stretching antisymmetric of OH group, C-H absorption and C-O stretch. Intense band at 1021 cm^{-1} is present which is produced by the C–O bond's vibration (Rojas-Mena *et al*., 2015). The FTIR spectra of HP-β-CD showed a broad peak at 3335cm^{-1} due to the O–H stretching vibrations. The alkyl region (for C-H stretching vibrations) is pointed out by a band at 2930cm^{-1} wavelength. Strong band at 1018cm^{-1} formed by the C-O bond's vibration. Peaks at 1107, 1080cm^{-1} due to C-H and C-O stretching vibration (Sbora *et al*., 2015; Su *et al*., 2012). The FTIR spectra of selected β-CD and 2HP-βCD based plain nanosponges has shown a peak, characteristic of the carbonate ester group, in the range 1750-1770 cm^{-1}, endorsing that carbonate linkage was added to the primary hydroxyl groups of the pure CD unit (Swaminathan *et al*., 2010) and confirming the formation of CD based carbonate nanosponges. This peak was absent in the FTIR spectrum of respective CD.

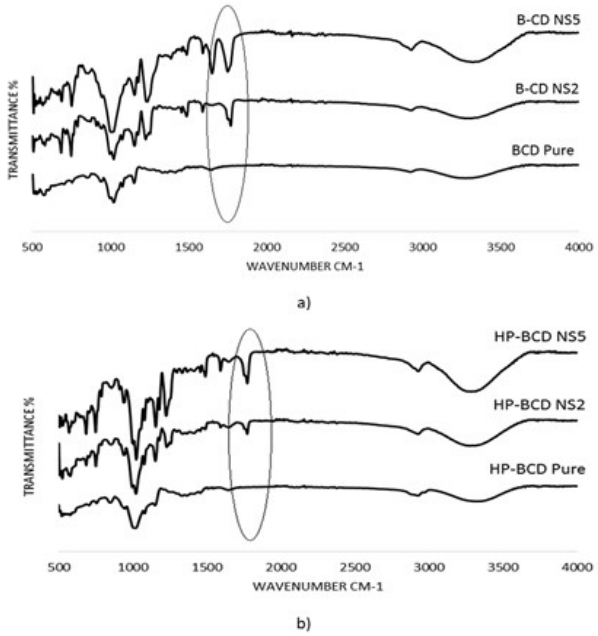

Fig. 3: FTIR spectra of selected plain a) β-CD based nanosponges compared with pure β-CD, b) 2HP-βCD based nanosponges compared with pure 2HP-βCD

Characterization of drug loaded nanosponges

Entrapment Efficiency

Entrapment efficiency of ibuprofen in nanosponge formulations was found to be in the range of 47.59% to 88.13%. Highest amount (88.13%) of ibuprofen was appeared to be loaded in the nanosponge formulation (I11) prepared by ultrasound assisted method and having 0.01:0.04 CD-CL ratio (fig. 4).

Particle size (PS), polydispersity index (PDI) and Zeta potential (ZP)

Particle size of ibuprofen formulations was approxi-mately in the range of 296.8±64 to 611.7±32nm with low

PDI indicating that nanosponges have monodispersed particles (table 3). Nanosponge formulations showed sufficiently high ZP (-17 to -32 mV).

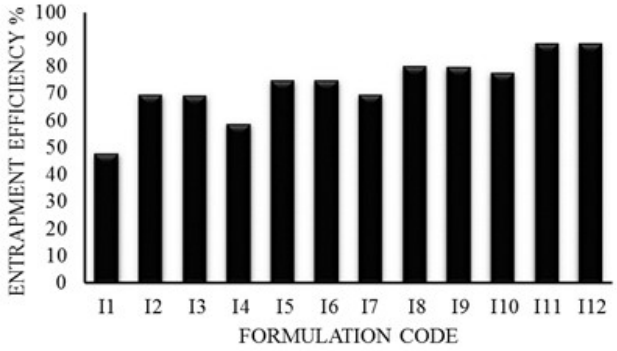

Fig. 4: Entrapment efficiency of nanosponge formulations

Table 3: Particle size, zeta potential & polydispersity index of the nanosponge formulations

Formulation code	PS (nm)	ZP (mV)	PDI
I1	521.2±30	-17	0.4
I2	453.2±46	-25	0.172
I3	611.7±32	-27	0.2
I4	501.1±38	-21	0.33
I5	431.2±34	-27	0.21
I6	583.4±57	-29	0.32
I7	463.1±51	-24	0.325
I8	398.2±32	-27	0.1
I9	520±63	-29	0.165
I10	400±59	-25	0.198
I11	296.8±64	-30	0.122
I12	484.2±31	-32	0.14

Our results showed that I11 formulation had the highest solubility, desired particle size (296.8±64nm), least PDI (0.122), highest ZP (-30) and highest drug entrapment efficiency (88.13%). So it was selected for further structural, thermos-analytical and morphological investigations.

Structural analysis

Comparative FTIR analysis of the nanosponge formulation and pure drug has been given in fig. 5. FTIR spectra of pure ibuprofen presented characteristic peaks at 1705.29cm^{-1} and at 2871.83 cm-^{1} due to carbonyl and hydroxyl stretching. C-H stretch is present at 2955.16cm^{-1} (Nokhodchi *et al*., 2010). FTIR spectra of I11 nanosponge formulation has shown either disappearance and/or broadenings of peaks which might be because of the weak interactions between nanosponges and candidate drug. The existence of the carbonate bond at 1700-1750cm^{-1} has been found in I11 formulation. Major change was observed in the fingerprint region, moreover higher peak in the region of the carbonyl group between 1600-1800 cm^{-1} and an increase in C=O stretching due to the addition of carboxylic groups was observed (Rao *et al*., 2013; Rao and Shirsath, 2017).

PXRD pattern of nanosponge formulation (fig. 6) exhibited broad peaks as compared to pattern of pure drug which showed many sharp and intense peaks.

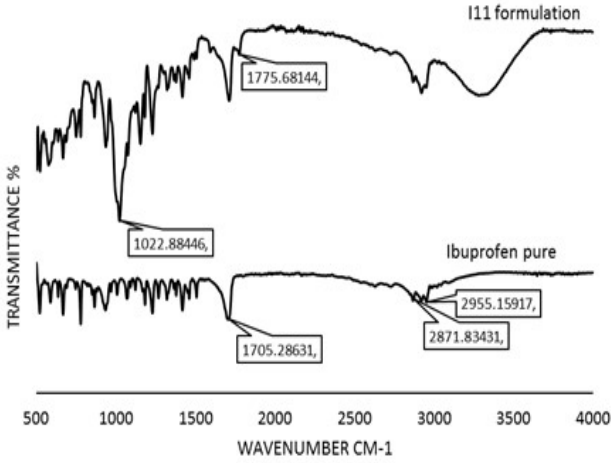

Fig. 5: FTIR spectra of I11 and pure ibuprofen

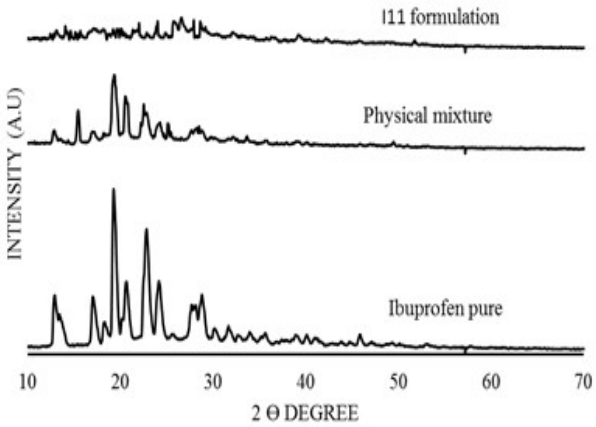

Fig. 6: PXRD of ibuprofen nanosponge formulation (I11)

Thermo-analytical investigation

DSC thermograms of pure drug, PM of drug with polymer and nanosponge formulation (I11) have been shown in fig. 7a. Pure ibuprofen showed sharp endothermic peak at 78˚C representing the melting point (Mello and Ricci-Júnior, 2011; Paul *et al*., 2013). PM showed broad and shallow melting endotherm indicating the displaced peak of drug. However, the DSC thermograms of I11 displayed no melting peak for ibuprofen indicating complete entrapment of drug within the nanosponge in the amorphous form. TGA revealed the stability of selected nanosponge formulation up to 300°C (fig. 7b) (Anandam and Selvamuthukumar, 2014b).

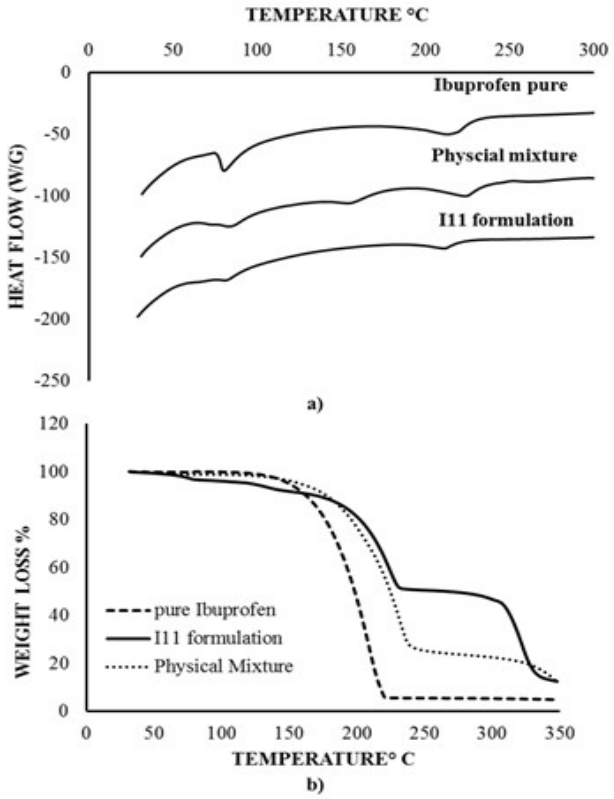

Fig. 7: a) DSC of I11. b) TGA of I11 formulation

Morphology

SEM images of nanosponge formulation (fig. 8) demonstrates that nanosponge formulation possess highly porous structures having rough surfaces thus confirming the formation of a cross linked network.

In-vitro drug release and kinetics

A comparison of the release profile of I11 formulation to that of the pure drug has been presented in fig. 9. A significant enhancement in the rate of release of drug was appeared from nanosponge formulation (~94%) as compared to pure drug (~45%) in 120min. The initial burst effect observed in the release profiles of the nanosponge formulation might be due to the drug encapsulation as non-inclusion complex on the surface of nanosponges. After the initial burst release, approximately linear profile from the nanosponge formulation was observed (Swaminathan *et al*., 2010). Release kinetics results (table 4) showed that ibuprofen follow first order and demonstrated a Fickian release of the drug (n<0.5).

In-vivo activity

Peripheral analgesic and anti-inflammatory property of ibuprofen loaded nanosponge formulation (I11) was evaluated and compared with the pure ibuprofen. Nanosponge formulation showed the highest extent of pain inhibition and anti-inflammatory property as compared to the pure drug. The percentage inhibition of writhing was 33.8% with pure ibuprofen whereas ibuprofen nanosponge formulation showed percentage inhibition of 74.43% (table 5). The Maximum edema inhibition of 52.63 % was recorded for ibuprofen nanosponge formulation (I11) whereas pure ibuprofen showed edema inhibition of 25.9% (table 6).

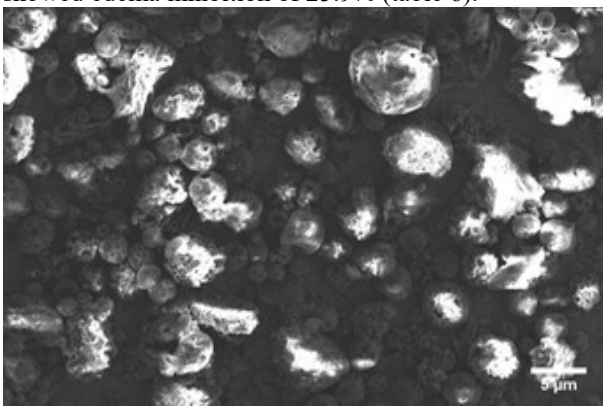

Fig. 8: SEM image of nanosponge formulation

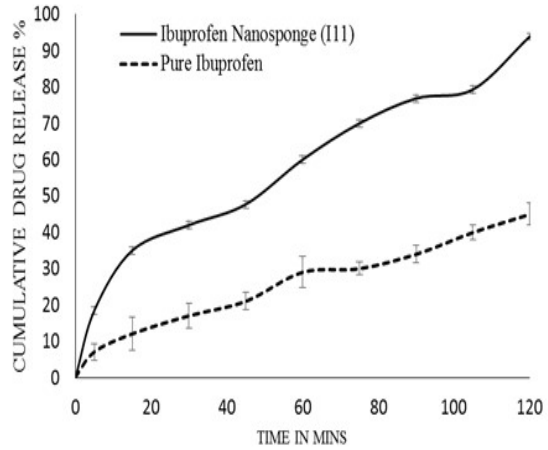

Fig. 9: *In-vitro* cumulative drug release % versus time (mins) for I11 formulation

Table 4: Release Kinetics of ibuprofen (I11) nanosponge formulations

S. No.	Kinetic Model	Parameter
1	Zero order	K_0 = 0.858
		R^2 = 0.8268
2	First order	K1 = 0.017
		R^2 = 0.9876
3	Higuchi	KH = 8.016
		R^2 = 0.9562
4	Korsmeyer Peppas	KKP = 9.941
		R^2 = 0.9724
		n = 0.430
5	Hixson Crowell	KHC = 0.005
		R^2 = 0.9355
6	Baker Lonsdale	KBL = 0.002
		R^2 = 0.9615

To compare means, one way ANOVA was applied and results showed that there is a significant difference

Table 5: Percentage pain inhibition of selected nanosponge formulation

Group	Drug	Number of writhes (mean ± SD)	% Inhibition
Control	Normal saline	36.5±3.20	
Standard	Pure ibuprofen	24.16±2.40	33.80
Test	ibuprofen nanosponges (I11)	*#9.33±1.50	74.43

*P <0.001 as compared to control group, # P <0.001 as compared to standard group

Table 6: Percentage inflammation inhibition of selected nanosponge formulations

Group	Drug	Paw volume (ml) Mean ± SD			% inhibition
		carrageenan		2 hrs after the test drug	
		0 min	after 2.5 hrs		
Control	Normal saline	1.37±0.045	3.32±0.050	3.32±0.048	
Standard	Pure Ibuprofen	1.42±0.031	3.43±0.034	2.54±0.032	25.95
Test	Ibuprofen nanosponge (I11)*#	1.39±0.037	3.42±0.029	*#1.62±0.041	52.63

*P <0.05 as compared to control group, # P <0.05 as compared to standard group

between groups. Post hoc TUKEY HSD test results showed that ibuprofen nanosponge formulation significantly reduced the number of writhes and showed promising anti-inflammatory activity as compared with their control and standard group.

DISCUSSION

In the present study, solvent method and ultrasound assisted method were used to prepare CD based carbonate nanosponges of ibuprofen employing two types of CD (β-CD and 2HP-βCD) and various CD-CL ratios. Degree of cross-linking (CD-CL ratio), type of CD and method of preparation appeared to be the contributing factors that affected the solubilizing efficiency of nanosponges. All types of nanosponges prepared by either method exhibited a greater solubilizing efficiency as compared to their monomer i.e. β-CD and / or 2HP-βCD (Torne *et al*., 2013). However, nanosponges prepared by ultrasound assisted method exhibited more solubilizing efficiency than those prepared by solvent method possibly due to the difference in the nature of nanosponges obtained by two different methods. As the type of CD is concerned, ibuprofen's solubility increased significantly with the side chain-substituted 2HP-βCD based nanosponges in comparison to β-CD based nanosponges (Felton *et al*., 2014). Various ratios of CD-CL (0.01:0.02, 0.01:0.04 and 0.01:0.06) were used. Solubilizing efficiency of nanosponges was increased with the increasing CD-CL ratio from 0.01:0.02 to 0.01:0.04. Nanosponges with 0.01:0.04 and 0.01:0.06 ratio of CD-CL showed similar potential to solubilize the drug crediting to the drug saturation solubility in the nanosponges. CDs (host molecules) have been reported to form inclusion complexes with the drugs (guest molecules) by including part of a guest / drug molecule into the central cavity of CD with a consequent change in the physiochemical properties of the included drug. Hence, the enhancement of solubility of ibuprofen by nanosponges might be explained as a result of entrapment of drug in the nanosponge matrix as well as formation of inclusion complexes (Ansari *et al*., 2011).

Kc values give an estimation of the stability of the formed nanosponges. We have reported Kc values for cyclodextrin based carbonate nanosponges of ibuprofen for the first time. A higher value of Kc (4449.95 L.Mol-1) for the complexes formed between ibuprofen and 2HP-βCD revealed them more stable than β-CD based nanosponges (Kc value: 3906.42 L.Mol-1). Literature also describes different values of the stability constant for the same type of polymer and drug owing to different methods used in complex formation, experimental variability, pH, method of analysis and most importantly, in the polymeric network the degree of substitution determines the interaction between host and guest (Martin *et al*., 2006; Cirri *et al*., 2006). Another reason in disparity of stability constant values for CD based carbonate nanosponges may be a consequence of the fact that β-CD or / and its derivatives can solubilize the drugs primarily because of the formation of inclusion complexes, but sometimes non-inclusion complexation can also be established which can influence the complexation (Woldum *et al*., 2008).

FTIR analysis of the plain nanosponges confirmed the formation of carbonate linkage in pure CD unit. It is suggested that due to crosslinking, nanocavities were formed in the polymer where drug (guest) molecules became entrapped. Thus, this structural arrangement could lead to improved solubilizing ability of nanosponges compared to the pure CD (Swaminathan *et al*., 2013a).

For drug loaded nanosponges, our study disclosed that the degree of cross-linking (CD-CL ratio) affected the respective drug loading in nanocavities. In nanosponge formulations (I1, I4, I7 and I10) having CD-CL ratio 0.01:0.02, the lesser quantity of cross-linker formed an incomplete network with decreased sites for the drug complexation whereas in nanosponges (I3, I6, I9 and I12) with CD-CL ratio 0.01:0.06, the greater quantity of CL offered a denser network by cross-linking of CD and therefore drug interaction with CD cavities hindered. However, the drug loading in nanosponges prepared by solvent method was lesser compared to those prepared by ultrasound assisted method, proposing the detrimental role of method of preparation of nanosponges in complexation of drug with CD (Swaminathan *et al*., 2010). Nanosponge formulation (I11) prepared by solvent assisted method with CD-CL ratio 0.01:0.04 was reported to entrap highest amount of the drug by virtue of the formation of maximum sites for the drug complexation by using above mentioned CD-CL ratio. Sufficiently higher stability of I11 may be favored by the presence of more cations on the surface of the particles resulting in higher zeta potential (-30mv) It was also found that increase in the charge on the nanosponges is directly proportional to the CL ratio (Ansari *et al*., 2011; Swaminathan *et al*., 2013a).

Structural analysis of drug loaded nanosponges by FTIR confirmed the drug loading in plain nanosponges. In PXRD analysis absence of any considerable peak in nanosponge formulation can be suggestive of a complexation of drug and nanosponges resulting in loss of crystallinity of the drug and indicating the amorphous nature of the entrapped drug (Javadzadeh *et al*., 2010; Torne *et al*., 2013). Further more thermo-analytical investigation indicated complete entrapment of drug within the nanosponge in the amorphous form.

The binary complexes of ibuprofen with nanosponges were found to enhance the solubility and rate of drug release most probably because of reduction of particle size (nanometer range) and the inclusion complex formation of drug into the nano-channels within the colloidal matrix of nanosponges. CD based nanosponges showed significant ($p<0.05$) analgesic and anti-inflammatory activity that may be the result of enhanced solubility and dissolution rate.

CONCLUSION

The present study revealed the improvement in solubility as well as release of poorly soluble BCS Class II NSAID (ibuprofen) by using CD based carbonate nanosponges. Maximum solubility enhancement (4.28 folds) was achieved by using 2HP-BCD based nanosponges having CL ratio 0.01:0.04 and prepared by ultrasound assisted method. It was found that the degree of cross-linking (CD-CL ratio), type of CD and method of preparation are the factors that may affect the characteristics of nanosponges for example solubilizing efficiency, stability constant values, entrapment efficiency, zeta size, zeta potential. Further due to improvement in drug solubility nanosponge formulation of ibuprofen demonstrated better analgesic and anti-inflammatory activity when compared with the pure ibuprofen. Enhancement of *in vivo* activities may suggest for the dose reduction and avoidance of adverse effects. The novel cyclodextrin based nanosponges of ibuprofen prepared in this study can be evaluated for its pharmacokinetics and bioavailability in animal models. Further targeted drug delivery for arthritis patients can be developed by using these nanosponges.

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