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ENHANCEMENT OF TENOXICAM SOLUBILITY BY HP-BETA-CYCLODEXTRIN BASED NANOSPONGE

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ABSTRACT

Cyclodextrin-based nanosponges are hyper cross-linked polymeric networks formed by cyclodextrins and cyclodextrin derivatives; they obtained by reacting cyclodextrin with cross-linker agents such as (carbonyl diimidazole, organic carbonates, and carboxylic dianhydride) and have many applications as drug delivery systems for pollutants removal in environmental issues and industrial fields. Several β-CD, sulfobutylether-β-CD and hydroxypropyl-β-CD nanosponges were prepared using suitable substance as cross-linker. They have been used to increase the solubility and stability of poorly soluble pharmacological active substances, as they combine the complex forming properties of CDs and properties of polymers. The cyclodextrin capacity to form inclusion complexes is enhanced when the CD molecules form aggregates or cross-linked together with other compounds (cross-linker), we have synthesized cyclodextrin based nanosponges (from HP-b-cyclodextrin). The complexing properties of the nanosponges were investigated against tenoxicam (non-steroidal anti-inflammatory drug, practically insoluble in water) and compared with native HP-b-CD monomer. Solubility studies were performed according to the method reported by Higuchi and Connors and the phase solubility diagrams were plotted and used to calculate the values of apparent stability constant and complexation efficiency. The results showed the significant enhancement in aqueous solubility of tenoxicam by 3.17 folds higher.

KEYWORDS: Tenoxicam, Beta cyclodextrin nanosponge, Solubilization enhancement, Complexation efficiency.

INTRODUCTION

Cyclodextrins are especially useful in the pharmaceutical industry as they can increase the apparent solubility of drugs and can modify the dissolution rate and sometimes even the dissolution profile. Moreover, cyclodextrins improve the stability of several labile drugs or mask some of the organoleptic characteristics leading to an enhanced compliance.[1]

The most common cyclodextrins are alpha (α), beta (b) and gamma (γ), and they consist of six, seven and eight glucopyranose related units respectively in a toroidal shape. The interior is not hydrophobic, but less hydrophilic than the aqueous environment. So, they are capable to host other hydrophobic molecules, as many lipophilic drugs. To overcome some of their disadvantages (i.e., renal toxicity), rapid dissociation of the inclusion complexes and to improve the inclusion capacity of native cyclodextrins, one major way are prominent, the synthesis of cyclodextrin-based polymeric materials called cyclodextrin nanosponges.[2]

Cyclodextrin nanosponges (CDNS) represent a very promising class of cross-linked polymers showing a unique three-dimensional architecture that consists of both hydrophilic and hydrophobic nanosized pores, where a large variety of guest compounds can be effectively encapsulated.[3][4]

They are reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior.[5] They have been found to be safe for oral and invasive routes and thus could serve as a potential carrier for drug delivery.[6]

Nanosponges are prepared by the cross-linking of between cyclodextrins (CD), with suitable cross-linking agents (CL) such as carbonyl diimidazole (CDI), pyromellitic anhydride (PMA), hexamethylene diisocyanate, toluene diisocyanate, dianhydride or carbonate. The cyclodextrin to cross-linker ratio can be varied during their preparation to improve the drug loading and solubilization efficiency.[7]

Tenoxicam is a poorly water-soluble drug; for such drugs, dissolution plays an important role in their absorption.[8] Although it has excellent oral bioavailability, its poor aqueous solubility limits its absorption dissolution rate and thus delays its onset of action. To enhance drug solubility in water and biological fluids, many approaches have recently been devised and include salt formation, solubilization, particle size reduction, solid dispersion (SD), self-

dispersing liquid preparations[9], and the use of inclusion compounds based on cyclodextrin.[10]

The purpose of this work was to enhance the solubilization efficiency of a poorly water-soluble drug tenoxicam (TNX) (Fig. 1a), by complexation with new class modified HP-b-cyclodextrin- based nanosponges (HP-b-CDNS) crosslinked by new type of cross-linker named benzophenone tetracarboxylic dianhydride (BTDA) (Fig. 1b) in distilled water. The study included four types of HP-b-CD- based nanosponges were purposely designed by varying the molar ratio of HP-b-cyclodextrin and benzophenone tetracarboxylic dianhydride (BTDA), and investigate their potentiality for enhancement solubilization of tenoxicam.

The effects of 2-HP-β-CDNS on equilibrium solubility of tenoxicam were assessed via phase-solubility analysis. The value of the apparent stability constant (K) is used to compare the affinity of drugs for different CDs.[11] A more precise method for evaluation of the solubilizing effects of 2-HP-β-CDNS is to determine their complexation efficiency (CE).

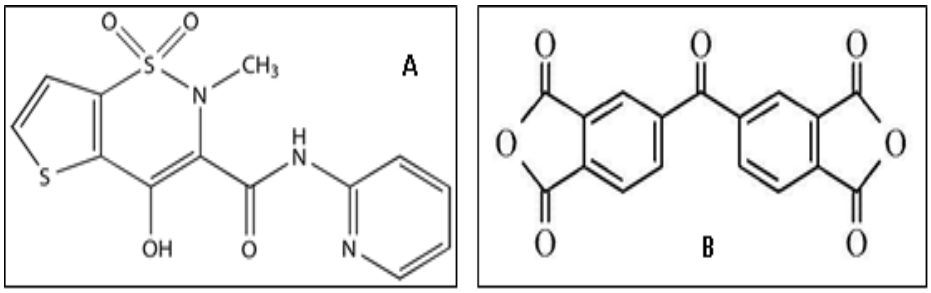

Figure 1. Chemical structure of a) tenoxicam, b) benzophenone tetracarboxylic dianhydride.

MATERIALS AND METHODS

HP-b-Cyclodextrin was purchased from Hyper-chem Co. (China). The BTDA was purchased from Sigma–Aldrich, and TNX was purchased from HiMedia Lab Pvt. Ltd, Mumbai, India. All other chemicals and reagents were of analytical grade.

Synthesis of NS

The NS were obtained by following the synthetic procedure reported in the Italian patent.[12] Briefly, HP- b-CD and BTDA in the molar ratios (1:2, 1:4, 1:6 and 1:8) were dissolved in DMSO containing triethylamine were allowed to react at room temperature for three hours. 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, 1:6 and 1:8) named the formulations plain

HP-b-CDNS$_2$, HP-b-CDNS$_4$, HP-b-CDNS$_6$ and HP-b-CDNS$_8$, respectively.[13] The ratio of HP-b-CD and cross-linker can be varied during their preparation to study the effect of crosslinking degree on solubilization efficiency of tenoxicam.

Solubilization efficiency of the prepared nanosponges

The solubilization efficiency of all prepared nanosponges (HP-b-CDNS$_2$, HP-b-CDNS$_4$, HP-b-CDNS$_6$ and HP-b-CDNS$_8$) and its native HP-b-CD (displayed in a table (1) was investigated for their solubilization enhancement capacity. An excess quantity of tenoxicam (10 mg) was suspended in 20 ml of HPLC distilled water with fixed quantity (20 mg) of NS under investigation; the vials were placed on a mechanical shaker at ambient temperature. After equilibrium for 48 h, the suspensions were then centrifuged for 10 min at 2500 rpm to separate the free tenoxicam and the colloidal supernatant was collected and then analyzed using a calibration curve for tenoxicam concentration by UV spectrophotometer at 375 nm. Finally, the solubility enhancement factor (S_{NS}/S_d) in the presence of βCDNS was evaluated.

Phase solubility studies

The most commonly used approach to study inclusion complexation and degree of complexation is the phase solubility method carried out according to Higuchi and Connors method[14][15], which examines the effect of nanosponges on the solubility of the drug. An excess amount of tenoxicam (approx. 10 mg) was added to rising amounts of blank nanosponges prepared 2-HP-βCD (20–300 mg) and ten mL of distilled water and shake at room temperature for two days until equilibrium was established.[16][17]

The suspensions were equilibrated one hour. Then, filtered through a 0.45 um nylon filter membrane (Whatman _ PuradiscTM), the absorbance at 375 nm was measured, and the concentration of the dissolved tenoxicam was determined.[18]

Solubility measurements and the determination of saturation concentrations of TNX with plain HP-b-CD were carried out by same described procedure where the concentrations of the native cyclodextrins were selected based on its solubility in water.

Determination of apparent stability constant (K)

The value of the apparent stability constant (*K*) is used to compare the affinity of drugs for different cyclodextrins or cyclodextrin derivatives. Each experiment was carried out in triplicate.

The obtained stability constant values give an idea of the extent of interaction between cyclodextrin-based nanosponges and the drug.[19]

The apparent binding constants of tenoxicam with all prepared cyclodextrin-based nanosponge and cyclodextrin monomer (HP-b-CD) were estimated from the phase-solubility diagrams that constructed by plotting the total molar concentration of cyclodextrin nanosponge in terms of the monomer unit as a function of the total molar concentration of ligand (tenoxicam) (Fig. 2) and calculated from the slope of ascending linear part and the intrinsic solubility (S_0) of the tenoxicam in water according to Equation 1.

$$K_{app} = \frac{\text{Slope}}{So(1+Slope)} \qquad \text{..... equation 1}$$

where S_0 intrinsic solubility of tenoxicam at 25ºC in the absence of cyclodextrin or cyclodextrin-based nanosponge, fairly approximated from the y-intercept.

Determination of complexation efficiency *CE*

A more precise technique for assessing of the solubilizing effects of cyclodextrins or their derivatives is to determine their complexation efficiency. The complexation efficiency is calculated by the slope of the phase-solubility profile using equation 2, which is referred to as the complexation efficiency (CE).[20]

$$CE = S_0 K_{1:1} = \frac{[D/CD]}{[CD]} = \frac{\text{Slope}}{1 - \text{Slope}} \qquad \text{..... equation } \mathbf{2}$$

Since the numerical value of CE is only dependent on the slope of the phase-solubility profile, less variation is usually observed in the CE values compared to the stability constant K value.

RESULTS AND DISCUSSION

Solubilization efficiency of the prepared nanosponges

All the HP-b-CD based nanosponges enhanced the solubility of tenoxicam, as shown in (Table 1). The results ranging from (**0.093** ± 0.01 mg/ml to **0.17** ± 0.02 mg/ml), comparing with free drug solubility (**0.054** ± 0.011 mg/ml).

In particular, the HP-B-CD NS_2 reached the highest solubilization efficiency (**0.17** ± 0.02 mg/ml) and enhanced the solubility of TNX by **3.15** folds significantly ($P<0.01$) greater than those of plain tenoxicam.

It is undoubtedly, higher crosslinking make more dense nanocavities and network within the matrix of nanosponges which might cause difficulty for the drug to enter freely into the matrix; consequently, solubility efficiency decreased as crosslinking increased and reached to lower solubility enhancement at ratio (1:8) of (HP-b-CD: CL). Further, the presence of hydroxypropyl substituted group might further hinder the interaction of drug molecule with nanosponge; consequently, a part of TNX interaction with HP-b-CD cavities might be entangled.[21]

On the other hand, the solubilization of TNX by equivalent quantities of the corresponding native HP-b-CD monomers (physical mixture), was estimated against the pure drug and the results revealed that there is significant ($P<0.05$) enhancement in solubilization capability around 2.56 folds higher and recorded the solubility to about **0.138** ± 0.022 mg/ml, as illustrated in Fig. (2).

Table 1: Solubilization Efficiency Parameters for 2-HP-βCD based Nanosponge at various ratios (with crosslinker) and its native 2-HP-b-CD unit.

Sr. No.	Code of formulation	Solubilization Efficiency Studies	
		Solubility mg/ml mean ± S.D	Enhancement factor S_{NS}/S_d
1	Tenoxicam plain	**0.054** ± 0.01	0
2	Hp-b-CD NS_2	**0.170** ± 0.02	3.17
3	Hp-b-CD NS_4	**0.149** ± 0.02	2.78
4	Hp-b-CD NS_6	**0.097** ± 0.02	1.8
5	Hp-b-CD NS_8	**0.093** ± 0.01	1.72
6	HP-b-CD complex	**0.138** ± 0.022	2.56

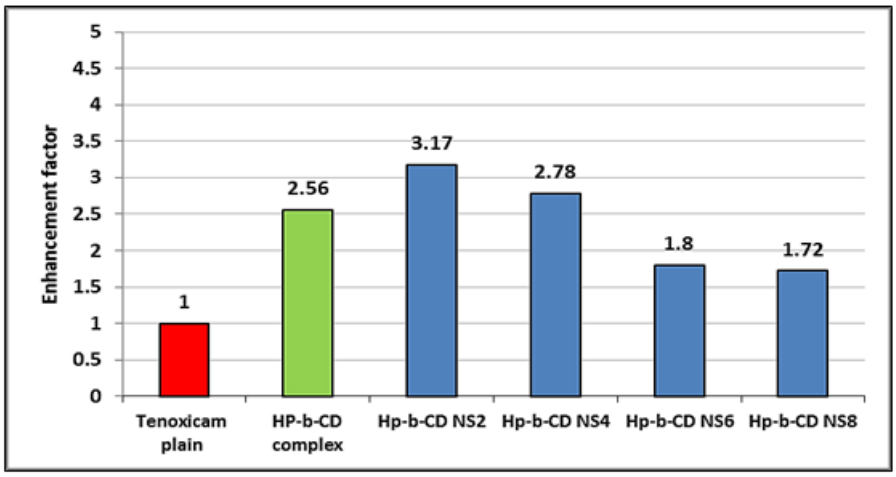

Figure 2. Enhancement solubility factors of various hypercross-linked 2-HP-b-CDNSs (■) and physical mixture (■) compared with plain TNX (■).

Phase solubility studies

Determination of the apparent stability constant and complexation efficiency

From data obtained in Table 2, nanosponge synthesized by HP-b-CD at ratio 1:2 (HP-b-CD: crosslinker) records insignificantly (P>0.05) higher stability constant and CE among other related NS whose ranged from (**57.14** ± 3.1 M^{-} to **79.23** ± 3.8 M^{-}) and (0.0105 to 0.0158) for stability constant and complexation efficiency respectively.

As there is an increase in drug solubilization, more of drug will interact with nanosponge, so, the obtained apparent stability constant values in each case give an idea of the extent of interaction of NS with drug. It has been stated previously, that a higher degree of crosslinking, lower affinity of nanosponge to interact with the drug, consequently lower estimated values related to apparent stability constants and complexation efficiency will be obtained. Even more, the steric hindrance due to the substitution of β-CD plays a significant role in both the cross-linking reaction and interactions facilities between tenoxicam and HP-b-CD, which seems to be hindered by the hydroxyl propyl-substituent in the b-CD molecule during the inclusion process.[22]

The estimated values of CE for all HP-b-CD-NS and physical mixture of drug with plain HP-beta-CD unit obtained (Fig. 3), with higher values belong to Hp-b-$CDNS_2$ (0.0158) indicating higher solubilizing and complexation effect of such formulation on tenoxicam as discussed before, and other Hp-b-CDNSs which has lower in trend of Hp-b-$CDNS_2$ > Hp-b-$CDNS_4$> Hp-b-$CDNS_8$ > Hp-b-$CDNS_6$ > physical mixture (TNX: HP-b-CD).

Table 2: The *CE*, Apparent Stability Constant, Slope and Intercept of Nanosponge Systems as a result of effect of the 2-HP-b-CDNS on Tenoxicam Solubility in Distilled Water, at 25°C.

Sr. No.	Code of Formulation	Phase-solubility studies				
		Slope (M^{-})	Intercept S_o(M)	R^2	apparent stability constant K_{app} (M^{-}) mean ± S.D.*	Complexation efficiency (CE)**
1	**Hp-ß-CD NS 1:2**	0.0156	0.0002	0.88	**79.23** ± 3.8	0.0158
2	**Hp-ß-CD NS 1:4**	0.0138	0.0002	0.98	**69.96** ± 2.9	0.0140
3	**Hp-ß-CD NS 1:6**	0.0113	0.0002	0.90	**57.14** ± 3.1	0.0114
4	**Hp-ß-CD NS 1:8**	0.0131	0.0002	0.94	**66.36** ± 1.02	0.0133
5	**HP-ß-CD complex**	0.0104	0.0002	0.98	**52.5** ± 1.45	0.0105

* K_{app} =Slope/(S_o (1+Slope)), ** CE = slope /(1-slope).

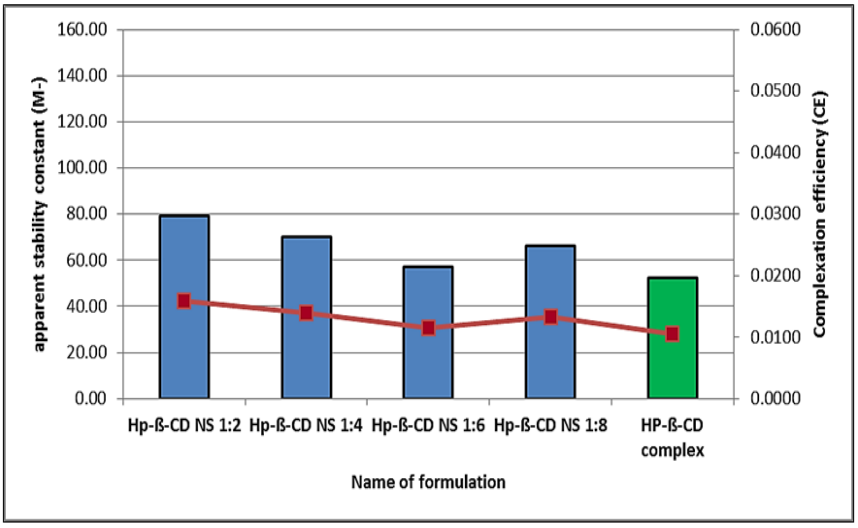

Figure 3: Apparent stability constants and complexation efficiency of various HP-b-CDNS (■) and physical mixture ((■) (TNX: HP-b-CD).

CONCLUSIONS

- The aqueous solubility of tenoxicam was linearly increased as a function of the concentration of HP-b-CDNS and HP-b-CD.
- The increase in aqueous solubility attributed to the formation of a soluble complex in solution. The complexes formed between tenoxicam and HP-b-CDNS were quite stable.
- The addition of HP-b-CDNS has markedly enhanced the solubilizing efficiency of native HP-b-CD, and the HP-b-$CDNS_2$ exhibited higher solubilizing efficiency when compared to another ratio of (HP-b-CDNS: CL) given highest enhancement (1.72-3.17 folds) in the solubilizing efficiency of HP-b-CDNS for tenoxicam.
- Hence, HP-b-$CDNS_2$ is recommended for enhancing the complexation and solubilizing efficiencies and to improve the solubility of tenoxicam, a BCS class II drug.

RERERENCES

1. Peeters, J., et al.: Characterization of the interaction of 2- hydroxypropyl-bcyclodextrin with itraconazole at pH 2, 4, and 7. J. Pharm. Sci., 2002; 91(6): 1414–1422.
2. Ajuha A., Baboota S., Ali J., Mustafa G.: Cyclodextrins as potential excipients in pharmaceutical formulations: solubilizing and stabilizing effects, in Cyclodextrin in Pharmaceutics. Cosmetics and Biomedicine, Edited by Erem Bilensoy, John Wiley & Sons, 2011; Hoboken, New Jersey: 42-68.
3. F. Trotta, W. Tumiatti, R. Cavalli, O. Zerbinati, C. M. Roggero, R. Vallero, Patent number WO 06/002814 (2006).

4. F. Trotta, W. Tumiatti, Patent number WO 03/085002 (2003).
5. Swaminathan, S., et al.: Cyclodextrin-based nanosponges encapsulating camptothecin: physicochemical characterization, stability and cytotoxicity. Eur. J. Pharm. Biopharm., 2010; 74: 193–201.
6. Swaminathan, S.: Studies on novel dosage forms. Masters of pharmaceutical Sciences Dissertation submitted to the University of Mumbai., 2006.
7. Vyas A., Saraf S., Saraf S., Cyclodextrin based novel drug delivery systems. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 2008; 62: 23-42.
8. Amidon GL, Lennemas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res., 2005; 12: 413-420.
9. Gershanik T, Benita S.: Self dispersing liquid formulations for improving oral absorption of lipophilic drugs. Eur J pharm., 2000; 50: 179-188.
10. Dixit RP, Nagaresenker MS.: In vitro and in vivo advantage of celecoxib surface solid dispersion and dosage form development. Ind J Pharm Sci., 2007; 69: 370-377.
11. T. Higuchi and A. K. Connors. Phase Solubility Techniques. In: C.N. Reilly, Ed., Advances in Analytical Chemistry and Instrumentation., 1965; 4: Wiley-Interscience, New York., 117-212.
12. Trotta, F., Tumiatti, W., Vallero, R.: Italian Patent No. MI2004A000614.
13. 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 b-CD nanosponges. J. Incl. Phenom. Macrocycl. Chem., 2011; 69: 403–409.
14. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instrum., 1965; 4: 117–212.
15. Singh R, Bharti N, Madan J, Hiremath SN.: Characterization of cyclodextrin inclusion complexes-a review. J Pharm Sci Technol., 2010; 2(3): 171-183.
16. GURSALKAR TEJASHRI1, BAJAJ AMRITA, JAIN DARSHANA: Cyclodextrin based nanosponges for pharmaceutical use: A review; Acta Pharm., 2013; 63: 335–358.
17. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an update review. AAPS Pharm Sci Tech., 2005; 6(2): E329-E357.
18. I Monica Rao • Amrita Bajaj: In vitro and in vivo evaluation of b-cyclodextrinbased nanosponges of telmisartan. Incl Phenom Macrocycl Chem., 2013; 77: 135–145.

19. S. Swaminathan, P. Vavia, F. Trotta and S. Torne: Formulation of betacyclodextrin based nanosponges of Itraconazole. J. Incl. Phenom. Macrocycl. Chem., 2007; 57: 89–94.
20. Loftsson, T., M´asson, M., Sigurj´onsd´ottir, J.F.: Methods to enhance the complexation efficiency of cyclodextrins. S.T.P. Pharma Sci., 1999; 9: 237–242.
21. Singireddy Anandam, Subramanian Selvamuthukumar: Fabrication of cyclodextrin nanosponges for quercetin delivery: physicochemical characterization, photostability and antioxidant effects. J Mater Sci., 2014; 49: 8140–8153.
22. Francesco Trotta: Cyclodextrin-based nanosponges as drug carriers. Beilstein J. Org. Chem., 2012; 8: 2091–2099.