Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 3, 2014

Original Article

PREPARATION AND SPECTROSCOPIC CHARACTERIZATION OF INCLUSION COMPLEX OF 2-PHENYL-4H-BENZO[d][1,3]OXAZIN-4-ONE AND β-CYCLODEXTRIN

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Received: 10 Feb 2014, Revised and Accepted: 28 Apr 2014

ABSTRACT

Objective: Quinazoline-4-(3H)-one derived drugs are emerging as new generation drugs, which motivated us to study some special features of the molecules. The major predicament is their low water solubility, which often gives unreliable results in biological experiments. In the present study, we have tried to solubilize the molecule making a host-guest complex with β-cyclodextrins with the expectation that it may develop new formulation of the stated molecule. An optimized method for the complexion of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one with β – cyclodextrin is reported herein.

Methods: 2-phenyl-4H-benzo[d][1,3]oxazin-4-one was synthesized following standard method. Different methods were tried to encapsulate the molecule into the cavity of cyclodextrin. The compound and its complexation with β- cyclodextrin were analyzed by spectral methods.

Results: The result showed that the encapsulated 2-phenyl-4H-benzo[d][1,3]oxazin-4-one into the cavity of β-CD fitted better and thus improved its solubility. It was also observed that kneaded method is the best way to prepare the host guest complex.

Conclusion: 2-phenyl-4H-benzo[d][1,3]oxazin-4-one fits better in β- cyclodextrin and an optimized method for the preparation of 1:1 complex is derived.

Keywords: Inclusion complex, β- cyclodextrins, 2-phenyl-benzo[d] 1,3-oxazine-4-one, Benzoxazine.

INTRODUCTION

Quinazoline-4-(3H)-one derivatives have gained extensive research interest due to their wide range of biological activity. It is reported that they exhibit antitubercular, antihypertensive, anticancer, anti-HIV, antiviral, anti-inflammatory and antifungal activities [1,2]. Despite their great medicinal value to emerge as successful drugs, aqueous solubility is one of the key determinants [3]. Cyclodextrins (CDs) are water-soluble, homochiral, cyclic oligosaccharides containing six, seven, or eight α-1, 4-linked D-glucopyranose units (α, β, and γ cyclodextrins), and have pore sizes ranging from 4.9 to 7.9 Å [4-7]. The hydrophobic nature inside its cavity with an outside hydrophilic part enables β-CD to encapsulate hydrophobic molecules to form thermodynamically favored molecular microcapsules, namely inclusion complexes or host–guest complexes. This binding between the guest molecules and host β -CDs is not permanent, but rather it remained in a dynamic equilibrium. The strength of binding mainly depends on specific local interactions between the surface atoms and the extent of how "host–guest" complex fits together.

In recent times, this approach of complexion with β-Cyclodextrins has been frequently used to increase oral bioavailability [8-10]. In this approach some drugs gain shelf life [11] to a certain extent, and additionally it contributes to controlled drug release rate, improved organoleptic properties and maximized gastrointestinal tolerance [12]. Thus, increased solubility of a drug plays a very important role in absorption, which ultimately affects its bioavailability [13].

A number of quinazoline products based upon cyclodextrins complex have been reported. Most of the studies, however remained confined to established drugs while very few emphasized to increase the solubility of potentially biologically active benzoxazine derivatives which may help developing new drugs in the future. Recently, a protocol for synthesis of 2, 3-dihydroquinazoline-4(1H)-one derivatives, where a prior formation of an inclusion complex of isatoic anhydride with β -cyclodextrin was reported [14]. The host–guest complexation between 5-aminoisoquinoline and β-CD was also studied [15]. It is therefore important to develop methods which can be applied to enhance the efficiency of drug–Cyclodextrins complexation [16].

Our laboratory has been active for years in studying the antibacterial activity of some 2- substituted Quinazoline-4-(3H)-ones compound [17]. We have reported the vibrational spectra of the said compound [18]. In this paper, the complex is prepared by the kneaded method and is characterized by UV and IR spectroscopy. It is observed that the effects of complexation by β-CD showing enhancement of the aqueous solubility of the compound. We prepared the complex in different methods and screened the most suitable method of its preparation.

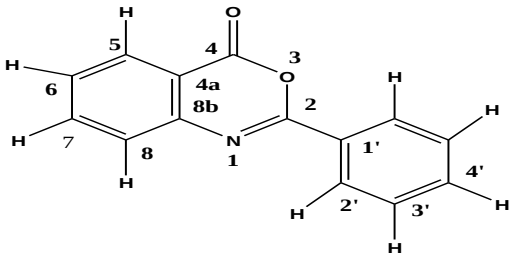
MATERIALS AND METHODS

All the chemicals were purchased from Merck India and used without further purification. β- cyclodextrin (HiMedia) was used for this study. All other reagents were of analytical grade. UV-1700 Spectrometer, Jasco, Tokyo, Japan and Shimadzu FT-IR spectrophotometer were used for characterization of complexes.

Synthesis of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one

To a solution of anthranilic acid (0.01mol) dissolved in pyridine (60 ml), benzoyl chloride (0.02 mol) was added. The mixture was stirred for 30 min. at room temperature. This was poured into a beaker containing 5% NaHCO3 (50 ml) solution. The solid obtained was filtered and recrystallized from ethanol. Purity of the compound was checked by TLC, using benzene and ethyl acetate as mobile phase in the ratio of 7:3. Iodine vapour was used as a developer. Yield was found to be 80%, Melting point was determined in open capillary tubes on a Thomas Hoover apparatus and was uncorrected, m.p. 120°C. The electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadruple mass spectrometer. NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 spectrophotometer, solvent CDCl3, TMS internal standard, the peak assignments were done on the basis of TOCSY, COSY and HSQC(HETCORR) spectra in addition to 13C-spectra; proton shifts ä (ppm): H5, 8.3, H7 7.8, H8 7.7, H6 7.6, H1' 8.3, H3' 7.6,, H2' 7.5; 13C shifts δ (ppm): C5 128.6, C6 128.8, C7 136.6, C1 132.6, C2' 128.3,, C4' 128.6, C3' 128.6, C4 159.6, C4a 117.0, C8b 146.2, C2 157.1, C1' 130.2; Proton coupling values in Hz: J5,6 8.1, J5,7 1.2, J5,8 0, J6,7 7.5, J8,6 1.2, J8,7 7.8, J3',1' 8.7, J1,3' 8.7, J3',2' 6.9, J1,2' 1.5, J2',1' 1.5. MS(m/z)223 (M+); Anal. Calculated/found: C 75.33/75.37; H

4.03/3.99; N 6.27/6.3. The FT-IR spectrum was recorded on a FTIR-8300 SHIMADZU spectrophotometer with KBr pellets and nujol.



2-phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one

Formation of complexes

Preparation of Physical mixture (PM)

The physical mixtures of the compound 2-phenyl-4H-benzo[d][1,3]oxazin-4-one and β-CD [1:1 molar ratio] were made by mixing together in a mortar and pestle.

Preparation of the complex by Kneading method (KN)

The physical mixture was triturated in a mortar with a small volume of water-ethanol solution. The thick slurry was kneaded for 45 min and then dried at 40 ^{0}C. Dried mass was pulverized and sieved through a 100 micron mesh.

Preparation of the complex by Co-evaporation method (COE)

The aqueous solution of β-CD was added to an alcoholic solution of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one. The resulting mixture was stirred for 1 hr and was evaporated at a temp of 45^{o}C until dry. The dried mass was pulverized and sieved through a 100 micron mesh.

Preparation of the complex by Freeze–Drying Method (FD)

The physical mixtures in 500 ml double distilled water stirred for 5 days. The suspension was freeze-dried, and the freeze-dried complex, thus produced was pulverized and sieved through a mesh.

Characterization of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one: β-CD (1:1) complex

Thin Layer Chromatography

The compound, CD and complex dissolved in distilled water. TLC was done using the solvent system ethyl acetate: butanol (5:4) in F549 TLC plates. The spots were identified in UV.

UV spectroscopic study

All spectra were recorded in the wavelength range 200–500 nm at room temperature (UV-1700 Spectrometer, Jasco, Tokyo, Japan). The complex of the compound was solubilized in distilled water by stirring for ten minutes and thereafter the total solution was filtered. UV spectra were studied with this filtered solution without delay.

Fourier Transform Infrared spectrophotometry [FT-IR]

IR spectra of stated compound, β-CD, physical mixture of compounds and the inclusion complex were monitored by mulling in nujol. All the samples were scanned in the region 4000-400 cm^{-1}.

Optimization of the complex formation

The standard curve was prepared by dissolving the 2-phenyl-4H-benzo[d][1,3]oxazin-4-one in the water. The complexes formed in different methods were quantified in solution by comparing OD at 280nm from this standard curve.

Molecular modeling

The geometry optimization of the compound and β-CD inclusion complexes was performed in gas phase and in the Cosmo-solvation sphere using MM2 and PM3 semi empirical quantum methods and the minimized energy molecular models were found to be docked properly when water was set as a solvent as a cosmo-solvation sphere (in different methods).

RESULT AND DISCUSSION

The inclusion complexes formed by different methods were primarily characterized by the degree of transparency of the solution made in water. In 5.0 ml of distilled water, β- CD [34 mg (6 mM)] solubilized to clear solution, the physical mixture [17 mg β- CD + 3.34 mg stated compound (3 mM : 3 mM)] formed a turbid suspension, and the complex [17 mg β- CD + 3.34 mg stated compound (3 mM : 3 mM)] was found to be faintly turbid as shown in Fig. 1A, B, & C respectively. A comparative TLC study was done on pre-coated silica-gel plates using the solvent mixture [ethyl acetate: butanol (5:4 v/v)]. The compound showed Rf. value of 0.8 while β-CD did not move with the solvent system. The spots corresponding to β- CD and the compound was visible with little trailing of the compound spot in the physical mixture (PM) while in the complex a large trailing was observed with faint spot of the free compound. The occurrence of the faint spot along with the trailing is the indication of the slow diffusion of the compound in the eluting solvent mixture used in TLC study.

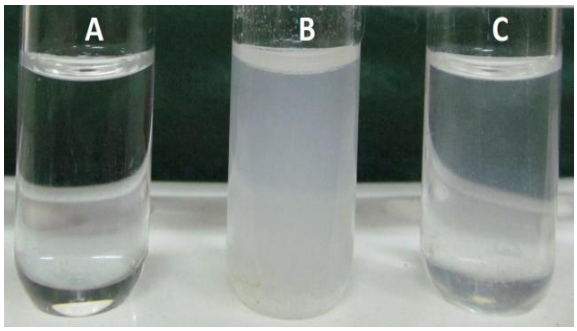


Fig. 1: Solution in water (A) β-CD (B) Physical mixture (C) Complex

In the UV spectra, the relative absorbance of the compound was changed due to the complex formation as shown in Fig. 2. It was found that the complex produced in the PM without the addition of the water was very low in comparison to the complex produced by the KN method which involved the addition of water during crushing. The study shows that the Dissolution rate of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one (Quinazolone) was enhanced to a great extent by complex formation using the kneading method as compared to other methods.

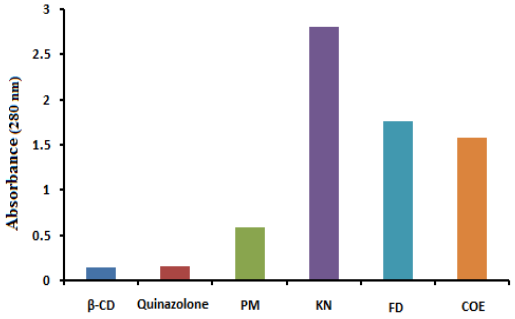


Fig. 2: Efficiency of different methods for complex formation

Vibrational spectroscopic studies with theoretical calculation of the 2-phenyl-4H-3,1- benzoxazine-4-one is reported earlier. This helped us to conclusively report the formation of the complex from its IR studies.

The most intense IR-band of the compound is the C=O stretching band which occurs at 1760-1763 cm^{-1} in IR KBr and 1760.9 cm^{-1}in Nujol. The slight shift in lower wave number in nujol is the indication that in non-polar environment the carbonyl stretching frequency can shift slightly to lower frequency. In the complex, the band appeared at 1762.8 cm^{-1} which can be easily characterized. This indicates that in spite of the hydrophobic environment inside the β- cyclodextrin, the stretching of the band is highly restricted due to the encagement into the cyclodextrins cavity. In PM, this band appeared at 1772.5 cm^{-1} indicating more polar environment which has dampened the frequency to about 10cm^{-1}. Hence, it indicates the fact that the compound remained outside the β-cyclodextrin.

The asymmetric stretching of C-O generally appears in the range of 1255±10cm^{-1}. This band was identified in IR at 1258cm-1(KBr), at 1257.5 cm^{-1} (Nujol) in PM and in the complex, and remained unchanged in all the cases.

Another strong band of the titled compound is the C=N stretching occurs at 1692cm^{-1} as a sharp band in nujol. This band for the complex appeared at 1607 cm^{-1} which shows that the band is very sensitive to environmental change; in fact, we have identified the band as weak in the PM and very weak in the complex. The related band, phenyl carbon-nitrogen band, identified at 1319 cm^{-1} in KBr, 1313 cm^{-1} in nujol is also sensitive to the change in polarity of the environment that appears at 1319 cm^{-1} in the complex and 1309.6 cm^{-1} in the PM.

The out of plane deformation band of phenyl ring at around 755±15 cm^{-1} is also a characteristic band. In the compound, the band is identified in KBr at 765cm-1, in nujol at 763 cm^{-1}. This band appeared for the complex at 763.8 cm^{-1} and for PM at 769.5 cm^{-1}. This band is characteristics of mono substituted benzene ring derivatives. The observed changes in their occurrence indicate that this ring is also encaged inside the β- cyclodextrins ring (Fig. 3). Thus, in a nutshell the observed changes in trivial bands clearly designates that the full molecule is encaged inside the cavity of β-cyclodextrins ring in the complex and residing outside in PM.

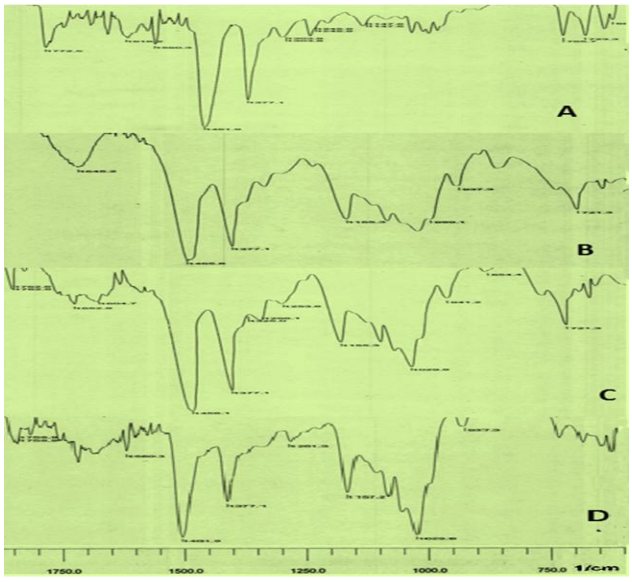


Fig. 3: IR spectra of (A) 2-phenyl-4H-3,1- benzoxazine-4-one (B) β-cyclodextrin (C) PM and (D) KN.

In order to design the best formulation of inclusion complexes, β -cyclodextrin was mixed with a small amount of warm water to make a slurry and then kept at the 50 °C for 12 h. The slurry was maintained for 12 h with occasional mauling. After 12 h, equimolar amount of the 2-phenyl-4H-benzo[d][1,3]oxazin-4-one and the β -cyclodextrin slurry was mixed by triturating in a mortar with a small volume of water-ethanol solution. The thick slurry was kneaded for 45 min and air dried at 50 °C. The crushing time for the complex preparation was varied as follows: 0min, 20 min, 30min, 40 min and 60min. The 1:1 mixture of compounds and β-cyclodextrin was used in every preparation. Dried mass was sieved through a 100 micron mesh. It is found that after 40 min crushing, the product yield was optimized. It is observed that crushing time has also played an important role in the complexation process. With the increase in the time of crushing during making a PM, the absorbance increased. This serves to be a very basic and simple novel experiment that shows how the complex formation depends upon the crushing time. It showed a plateau after 40 minutes of crushing of the drug, which revealed that 40 minute crushing time is the optimum (Fig. 4).

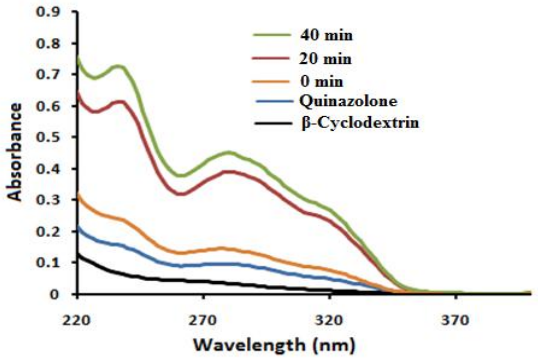


Fig. 4: Effect of crushing time on complex formation

Molecular modeling was carried out in the gas phase, and in cosmosolvation in water as a solvent. The energies calculated for the stoichiometry systems 1:1 and 1:2 (drug: cyclodextrin) are described. In all cases, the more stable conformations were attained in the presence of water. The 1:1 stoichiometry was the one which presented the higher stability. The possible structure of the complex produced is shown in the Fig 5**.** It was found that water played a pivotal role in the formation of the complex. In the absence of any water in the system, the complex did not form i.e. compound 2-phenyl-4H-benzo[d][1,3]oxazin-4-one did not enter into the cavity of cyclodextrin, but with the addition of water, it readily entered into the cavity and formed an inclusion complex. This observation is supported by computational study also.

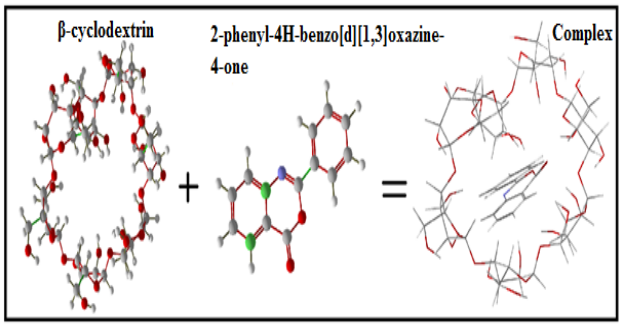


Fig. 5: The probable structure of inclusion complex

CONCLUSION

The solubility of the stated compound was successfully enhanced with water by the formation of their inclusion complexes with β-cyclodextrin. The results obtained by different mode of preparation clearly indicated that the kneaded method is the best method for preparing the complex. These results have supported our approach to enhance the solubility of quinazoline compounds by β-Cyclodextrin which is an easy and economical method. The method may consequently increase the bioavailability of the drug molecule to improve its pharmaceutical potential.

ACKNOWLEDGEMENT

Authors are thankful to Amit Kumar Mandal, SRF, Department of Biotechnology , University of North Bengal for his assistance. BKT was provided with a fellowship funded by DBT Sanction No. BT/BIO-CARe/06/141/2010-2011.

REFERENCES

1. Waisser K, Petrlikova E, Perina M, Klimesova V, Kunes J, Palat K, Kaustova J, Dahse H, Mollmann U. A note to the biological activity of benzoxazine derivatives containing the thioxo group. Eur J Med Chem 2010 ; 45: 2719-2725.
2. Li X, Liu N, Zhang H, Knudson SE, Slayden RA, Tonge PJ. Synthesis and SAR studies of 1,4-benzoxazine MenB inhibitors: Novel antibacterial agents against *Mycobacterium tuberculosis.* Bioorg Med Chem Lett 2010 ; 20: 6306-6309.
3. Nanjwade BK, Derkar GK, Bechra HM, Nanjwade VK, Manvi FV. Design and Characterization of Nanocrystals of Lovastatin for Solubility and Dissolution Enhancement. Nanomedic Nanotechnol 2011 ; 2: 107.
4. Singh M, Sharma R, and Banerjee UC. Biotechnological applications of cyclodextrins. Biotechnol Adv 2002; 20:341-359.
5. Ogoshi T, Chujo Y. Synthesis of Organic–Inorganic Polymer Hybrids by Means of Host–Guest Interaction Utilizing Cyclodextrin. Macromolecules 2003; 636:654.
6. Wulff M, Alden M, Tegenfeldt J. Solid-State NMR Investigation of Indomethacin–Cyclodextrin Complexes in PEG 6000 Carrier. Bioconjugate Chem 2002; 13: 240-248.
7. Asanuma HA, Hishiya T, Komiyama M. Tailor-Made Receptors by Molecular Imprinting. Adv Mater 2000; 12: 1019-1030.
8. Bassani, VL, Krieger D, Duchene D, Woue D. Enhanced water-solubility of albendazole by hydroxypropyl-b-cyclodextrin complexation. J Incl Phenom Macrocycl Chem 1996; 25: 149–152.
9. Buvari-Barcza A, Barcza L. Changes in the solubility of bcyclodextrin on complex formation: guest enforced solubility of b-cyclodextrin inclusion complexes. J Incl Phenom Macrocycl Chem 2000; 36: 355–370.
10. Nasongkla N, Wiedmann AF, Bruening A, Beman M, Ray D, Bornmann WG, Boothman DA, Gao J. Enhancement of solubility and bioavailability of b-lapachone using cyclodextrin inclusion complexes. Pharm Res 2003; 20: 1626–1633.
11. Ammar HO, Salama HA, Gharab M, Mahmoud AA. Implication of inclusion complexation of glimepiride in cyclodextrin–polymer systems on its dissolution, stability and therapeutic efficacy. Int J Pharm 2006; 320: 53-57.
12. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci 1996; 85(10): 1017-1025.
13. Ghodkea D S, Nakhatb PD, Yeole PG, Naikwade N S, Magduma CS, Shaha RR. Preparation and Characterization of domperidone Inclusion Complexes with Cyclodextrin: Influence of Preparation method. Iran J Pharm Res 2009; 8 (3): 145-151.
14. Patil DR, Dalal DS. One-Pot, Solvent free synthesis of Hantzsch 1, 4-Dihydropyridines using β-cyclodextrin as a Supramolecular Catalyst. Lett Org Chem 2011; 8(7): 477-486.
15. Rajamohan R, Nayaki SK, Swaminathan M. Host–guest complexation between 5-aminoisoquinoline and b-cyclodextrin and its effect on spectral and prototropic characteristics. J Incl Phenom Macrocycl Chem 2012; 73: 99–108.
16. Sharma MC, Sharma S. Formulation and Spectroscopic Studies and Dissolution Behavior of Levofloxacin-β Cyclodextrins Inclusion Complex. Int J Pharm Tech Res 2011; 3(3): 1883-1888.
17. Nanda AK, Ganguli S, Chakraborty R. Antibacterial activity of some 3-(Arylideneamino)-2-phenylquinazoline-4(3H)-ones: synthesis and preliminary QSAR studies. Molecules 2007;12: 2413-2426.
18. Ambujakshan KR, Tresavarghese H, Mathew S, Ganguli S, Nanda AK, Panicker CY. Vibrational spectroscopic studies and theoretical calculations of 2-phenyl-4H-3,1-benzoxazin-4-one. Orien J Chem 2008; 24: 865-874.