Chapter 6

Preparation and Characterization of β-Cyclodextrin inclusion complex of Quinazoline-4(3H)-ones

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6.1 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 (Waisser *et al.,* 2010; Li *et al.,* 2010). Despite their great medicinal value to emerge as successful drugs, aqueous solubility is one of the key limiting determinants (Nanjwade *et al.,* 2011). 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 (Figure 6.1) (Asanuma *et al.,* 2000; Singh *et al.,* 2002; Wulff *et al.,* 2002; Ogoshi *et al.,* 2003). The hydrophobic nature inside its cavity with an outside hydrophilic part enables β-CD (Figure 6.2) 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 (Figure 6.3) has been frequently used to increase oral bioavailability (Basson *et al.,* 1996; Buvari & Barcza, 2000; Nasonglela *et al.,* 2003). In this approach some drugs gain shelf life (Amma *et al.,* 2006) to a certain extent, and additionally it contributes to controlled drug release rate, improved organoleptic properties and maximized gastrointestinal tolerance (Loftsson & Brenster, 1996). Thus, increased solubility of a drug plays a very important role in absorption, which ultimately affects its bioavailability (Ghodkea *et al.,* 2009).

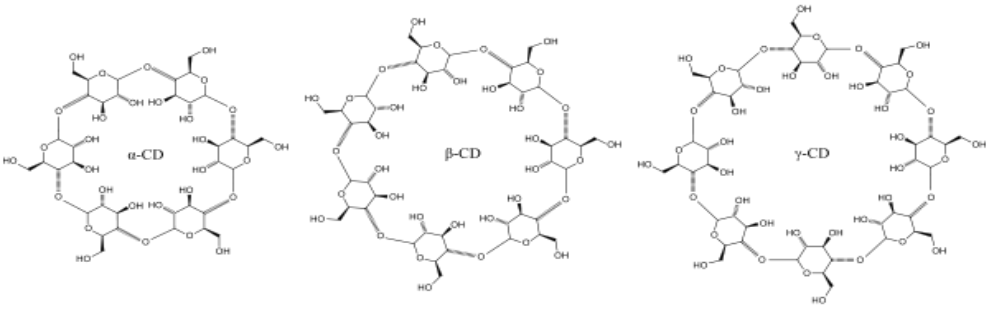

Figure 6.1: Representing the α-CD, β-CD and γ-CD.

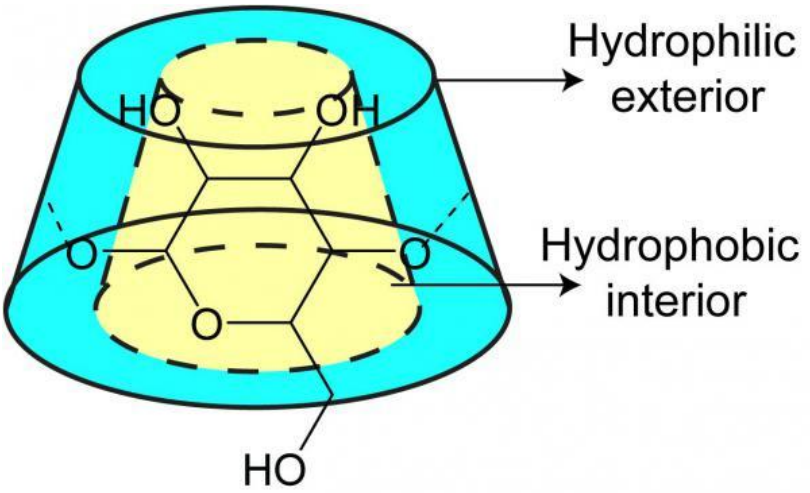

Figure 6.2: Interior Hydrophobic region and Exterior Hydrophilic region of β-cyclodextrin

A number of quinazoline products based upon cyclodextrins complex were reported. Most of the studies however remained confined to established drugs while very few emphasized to increase the solubility of potentially biologically active quinazoline-4(3H)-ones 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 (Patel & Dalal, 2011). The host–guest complexation between 5-aminoisoquinoline and β-CD was also studied (Rajamohan *et al.*, 2012). It is therefore important to develop methods which can be applied to enhance the solubility.

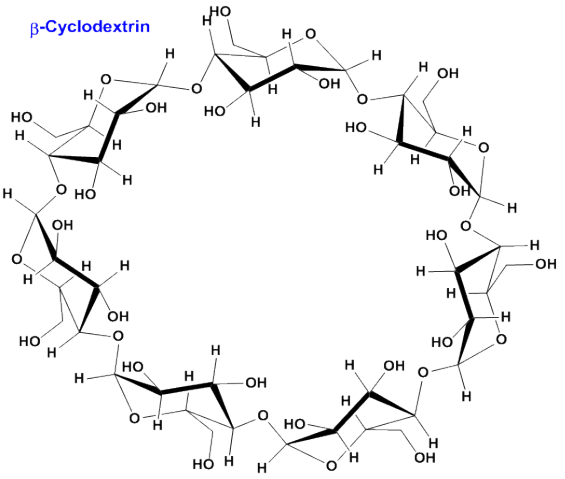

Figure 6.3: Structure of β-cyclodextrin

6.1.2 Techniques for solubility enhancement

There are various techniques available to improve the solubility of hydrophobic drugs. Some traditional and novel approaches to improve the solubility are:

a. *Particle Size Reduction*

The solubility of drug is often intrinsically related to drug particle size. As particle becomes smaller, the surface area to volume ratio increases. The larger surface area allows a greater interaction with the solvent which cause increase in solubility.

b. *Solid Dispersion*

Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption and therapeutic efficacy of drugs in dosage forms. The concept of solid dispersions was originally proposed by Sekiguchi and Obi, who investigated the generation and dissolution performance of eutectic melts of a sulphonamide drug and a water-soluble carrier in the early 1960s **(**Obi & Sekiguchi, 1961**)**. The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug.

c. *Solvent Evaporation Method*

Solvent Evaporation Method is also useful for improvement and stability of solid dispersions of poor water soluble drugs. Tachibana and Nakumara **(**Tachibana & Nakamura, 1965**)** were the first to dissolve both the drug and the carrier in a common solvent and then evaporate the solvent under vacuum to produce a solid solution. This enabled them to produce a solid solution of the highly lipophilic β-carotene in the highly water soluble carrier polyvinylpyrrolidone. Many investigators studied solid dispersion of meloxicam naproxen and nimesulide using solvent evaporation technique (Chaumeli, 1998; Blagden *et al.,* 2007; Vogt *et al.,* 2008)

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d. *Nanosuspension*

Nanosuspension technology was developed as a promising candidate for efficient delivery of hydrophobic drugs. This technology is applied to poorly soluble drugs that are insoluble in both water and oils. A pharmaceutical nanosuspension may be called as biphasic system consisting of nano sized (average particle size ranging between 200 and 600 nm.) drug particles stabilized by surfactants for either oral and topical use or parenteral and pulmonary administration (Muller *et al.,* 2000).

e. *Precipitation Techniques*

In the precipitation technique, drug is dissolved in a solvent, which is then added to nonsolvent to precipitate the crystals. The basic advantage of precipitation technique is the use of simple and low cost equipment. The basic challenge of this technique is that during the precipitation procedure the growing of the drug crystals needs to be controlled by addition of surfactant to avoid formation of microparticles. The limitation of this precipitation technique is that the drug needs to be soluble in at least one solvent and this solvent needs to be miscible with the non-solvent. Moreover precipitation technique is not applicable to drugs, which are simultaneously poorly soluble in aqueous and non-aqueous media (Riegelman, 1971).

f. *Inclusion Complex Formation Based Techniques*

Lipophilic drug-cyclodextrin complexes, commonly known as inclusion complexes, can be formed simply by adding the drug and excipients together, resulting in enhanced drug solubilization. Among all the solubility enhancement

techniques, inclusion complex formation technique was employed more precisely to improve the aqueous solubility, dissolution rate, and bioavailability of poorly-water soluble drugs.

In this chapter, the inclusion complexes were prepared and characterized by different spectroscopy and PXRD. It was observed that the effects of complexation by β-CD showing enhancement of the aqueous solubility of the compound.

6.2 Materials and method

6.2.1 Chemicals*:* β- cyclodextrin (HiMedia) was used in this study. All other reagents were of analytical grade

6.2.2 Preparation of inclusion complex

***Preparation 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 (KND): 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 ^{o}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 were stirred for 2 days. The suspension was freeze-dried and the freeze-dried complex thus produced was pulverized and sieved through a mesh.

6.2.3 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 280 nm from this standard curve.

6.2.4 Molecular modelling

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).

6.2.5 Characterization of inclusion 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: 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}.

Raman Spectroscopy: Raman spectra of the stated compounds, β-CD, physical mixture of compounds and the inclusion complex were recorded on Varian FT-Raman and Varian 600 UMA. All the samples were scanned in the region 4000-400 cm^{-1}.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was carried out with a Shimadzu DSC-60 instrument (Shimadzu, Kyoto, Japan). Samples weighing 3–5 mg were heated in opened aluminum pans at a rate of 10 K/min under nitrogen gas flow of 35 mL/min.

Powder X-Ray Diffraction

X-ray Powder Diffraction (XRPD) patterns were recorded on a X'Pert Philips PW3020 diffractometer (Philips, The Netherlands) over the 2y range of 58–408, using graphite monochromatized Cu Ka radiation (1.54184 A °), in aluminum sample holders, at room temperature.

6.3 Results and Discussion

6.3.1. Standardization of methods and mulling time

Before switching on to the formation of inclusion complex, we have performed simple computational study to get scheme/suggestion for best way to form inclusion complex. In this context, molecular modelling was carried out in the gas phase, and in cosmosolvation in water as a solvent. The energies calculated for the stoichiometric systems 1:1 and 1:2 (compound: 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 inclusion complex produced is shown in the Figure 6.4**.** 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 inclusion 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.

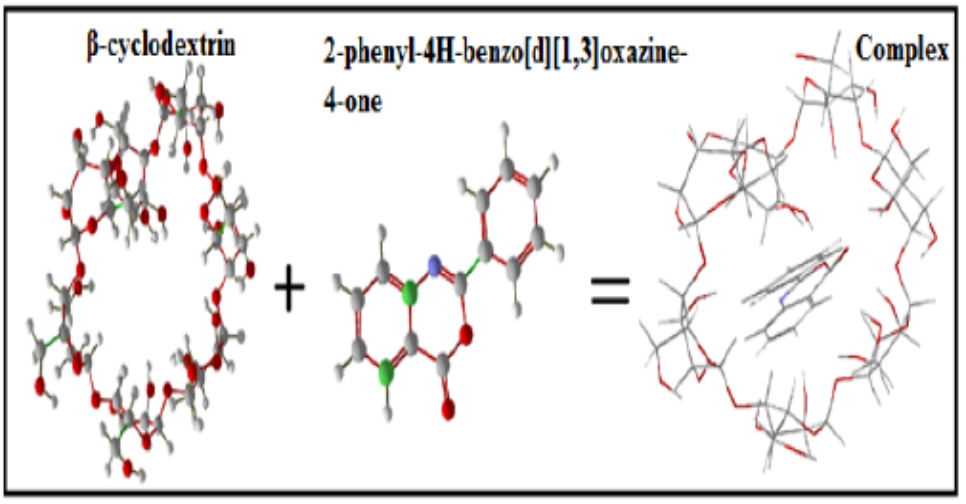

Figure 6.4: The probable structure of inclusion complex

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 Figure 6.5A, 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.

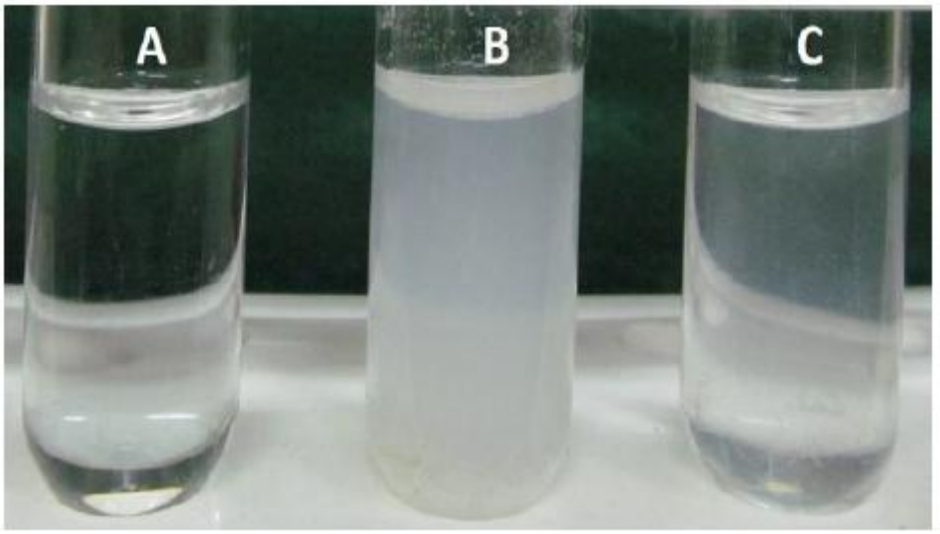

Figure 6.5: Solution in water (A) β-CD (B) Physical mixture (C) Inclusion Complex

In order to design the best formulation of inclusion complexes, we have used the information obtained by molecular modelling. The β -cyclodextrin was mixed with a small amount of warm water to make slurry and then kept at the 50 ^{o}C for 12 h. We allowed extra time so that the dry β- CD swells to full. 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 ^{o}C. The crushing time for the complex preparation was varied as follows: 0 min, 20 min, 30min, 40 min and 60 min. 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 complex, the absorbance of aqueous solution increased. It was found that upto 40 minutes the UV absorption in water solution increases. This serves to be a very basic and simple novel experiment that shows how the complex formation depends upon the crushing time. It showed no further enhancements of the peaks beyond that time *i.e.* a plateau after 40 minutes of crushing of the compound, which revealed that 40 minute crushing time is the optimum. The time for optimization of inclusion complex is shown in Figure 6.6.

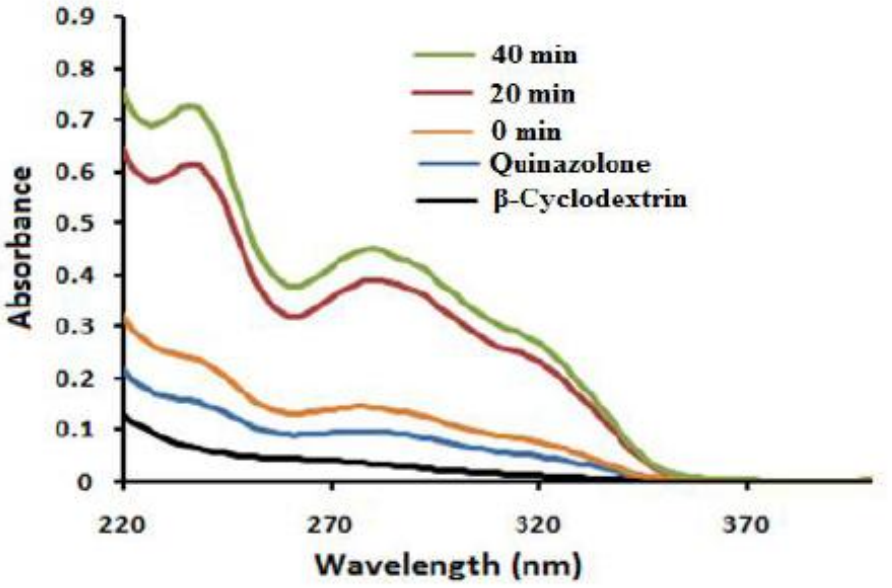

Figure 6.6: Effect of crushing time on inclusion complex formation

We used UV-absorption as an indicator for the formation of inclusion complex; since the compounds under study possessed chromophores which display appreciable UV-absorption; but they are insoluble in water. After formation of inclusion complex, expectedly the water solution displayed versus absorption and the amount of absorption was used as a good indicator of the complex formation. In the UV spectra, the relative absorbance of the compound was changed due to the complex

formation as shown in Figure. 6.7. It was found that the complex produced in the physical mixture (PM) without the addition of the water was very low in comparison to the complex produced by the kneaded (KND) 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.

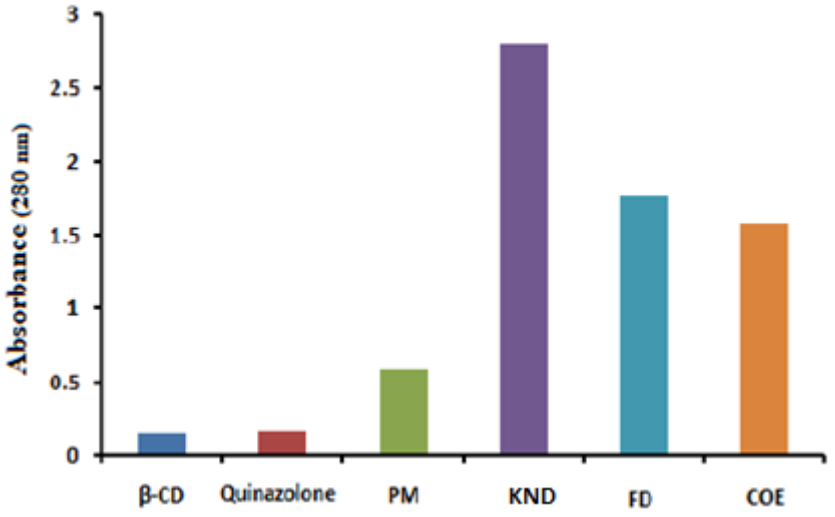

Figure 6.7: Efficiency of different methods for formation of inclusion complex.

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 10 cm^{-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±10 cm^{-1}. This band was identified in IR at 1258 cm^{-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 which occurred at 1692 cm^{-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 765 cm^{-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 characteristic of mono substituted benzene ring derivatives. The observed changes in their occurrence indicate that this ring also encaged inside the β- cyclodextrins ring (Figure 6.8).

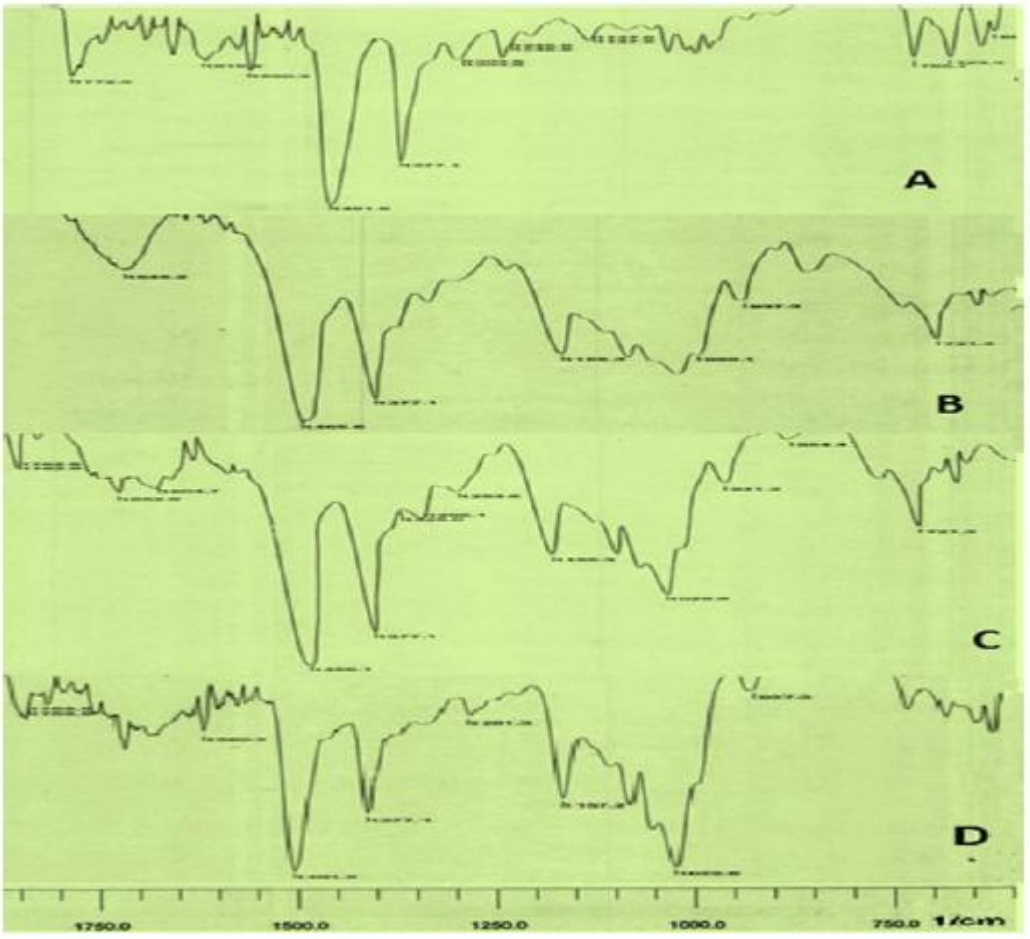

Figure 6.8: IR spectra of (A) 2-phenyl-4H-3,1- benzoxazine-4-one (B) β-cyclodextrin (C) Physical mixture (PM) and (D) Inclusion complex (KND).

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.

After getting a clear indication of the complex formation of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one with CD, we have tried for more biologically active synthesized compounds (3a, 3b and 3c) for inclusion complex study in more detail. In our previous studies, we have reported the vibrational spectroscopy and respective DFT calculation of these three compounds. So, we have chosen them for inclusion complex study. The inclusion complexes were prepared with same methods used for 2-phenyl-4H-3,1- benzoxazine-4-one.

6.3.2 Vibrational spectroscopy study of inclusion complex

Infrared and Raman spectra of the compounds (3a, 3b and 3c) and inclusion complex and physical mixture of compunds with β- CD are shown in Figure 6.9a, b and c and 6.10a, b and c respectively. An infrared spectrum was used to evaluate the functional groups of compounds involved in the complexation. Infrared and raman spectra of compounds are characterized by identification of the carbonyl (C=O), methyl. In the spectra of the inclusion complex, these bands were shifted towards higher frequencies and the asymmetrically vibration peak of C=O band was obtained as three intensity peaks increases, suggesting formation of the inclusion complex. The IR spectrum of β-CD is characterized by intense bands at 3000–3600 cm^{-1} that are associated with the absorption of the hydrogen bonded –OH groups of β-CD. The vibrations of the CH-CH groups appear in the 2897– 3250 cm^{-1} region. Thus, spectral changes were always concerned with COOH, -CH_3 and CH groups of the β- CD.

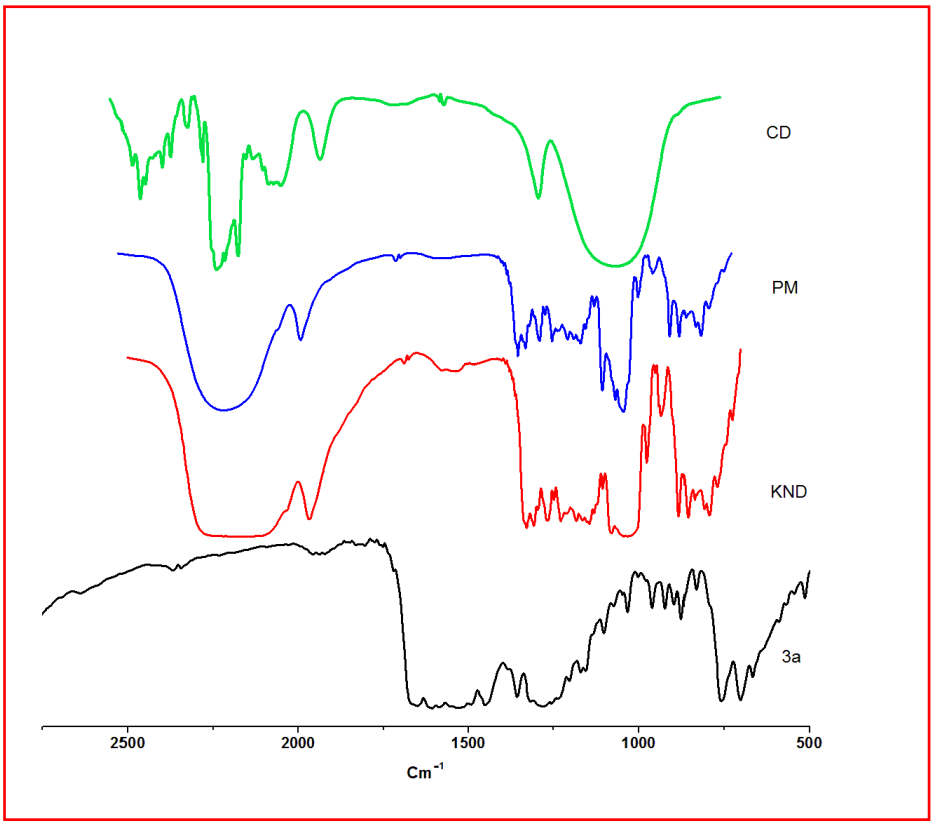

Figure 6.9(a) : FTIR spectra (KBr pellets): Compound 3a; Physical mixture (PM) of 3a and β-CD; 3a/β-CD inclusion complex by kneaded method (KND) and β-Cyclodextrin (CD).

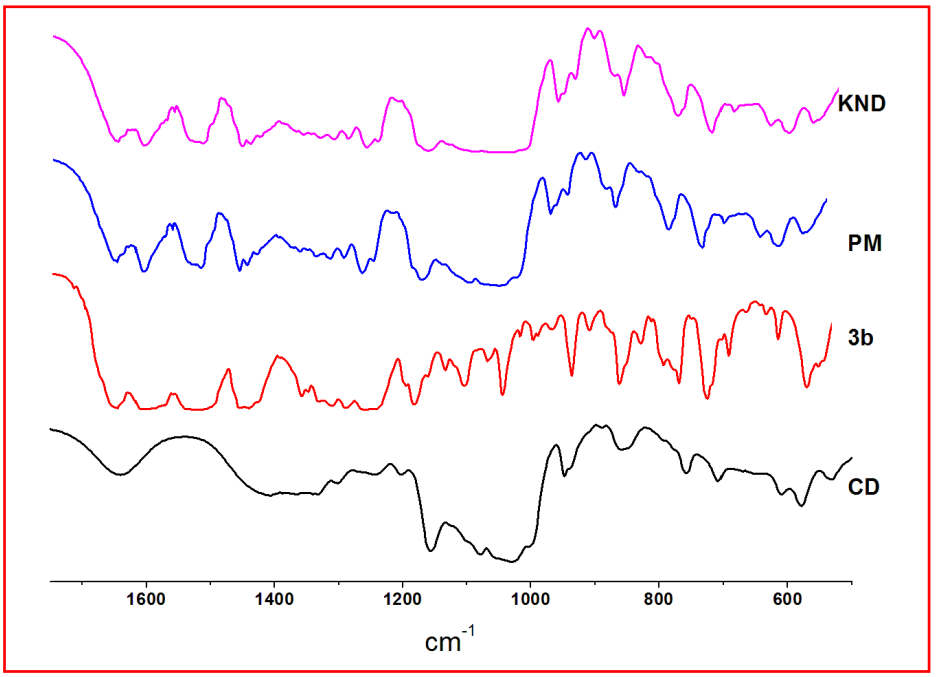

Figure 6.9(b): FTIR spectra (KBr pellets): Compound 3b; Physical mixture (PM) of 3b and β-CD; 3b/ β-CD inclusion complex by kneaded method (KND) and β-Cyclodextrin (CD).

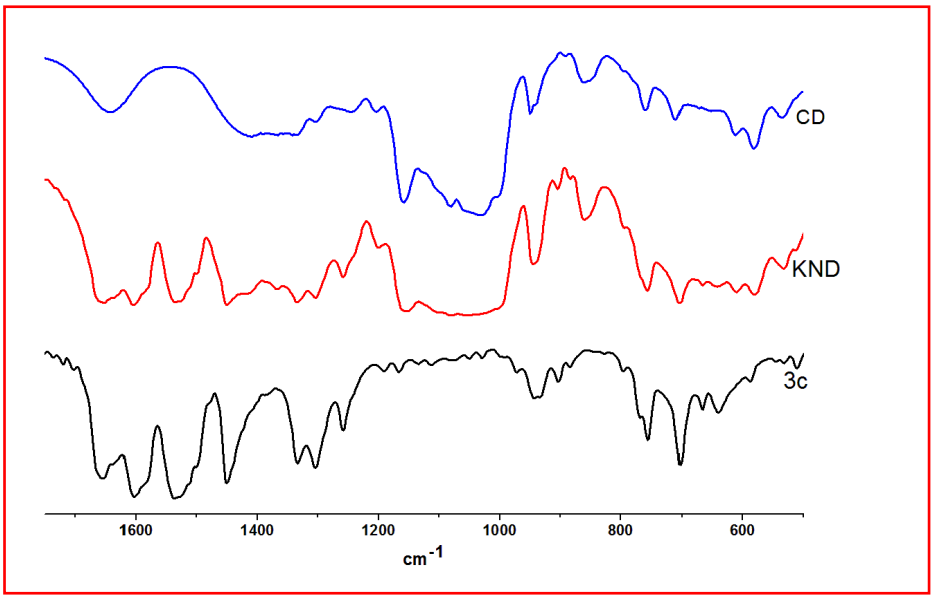

Figure 6.9(c): FTIR spectra (KBr pellets): Compound 3c; Physical mixture (PM) of 3c and β-CD; 3c/ β-CD inclusion complex by kneaded method (KND) and β-Cyclodextrin (CD).

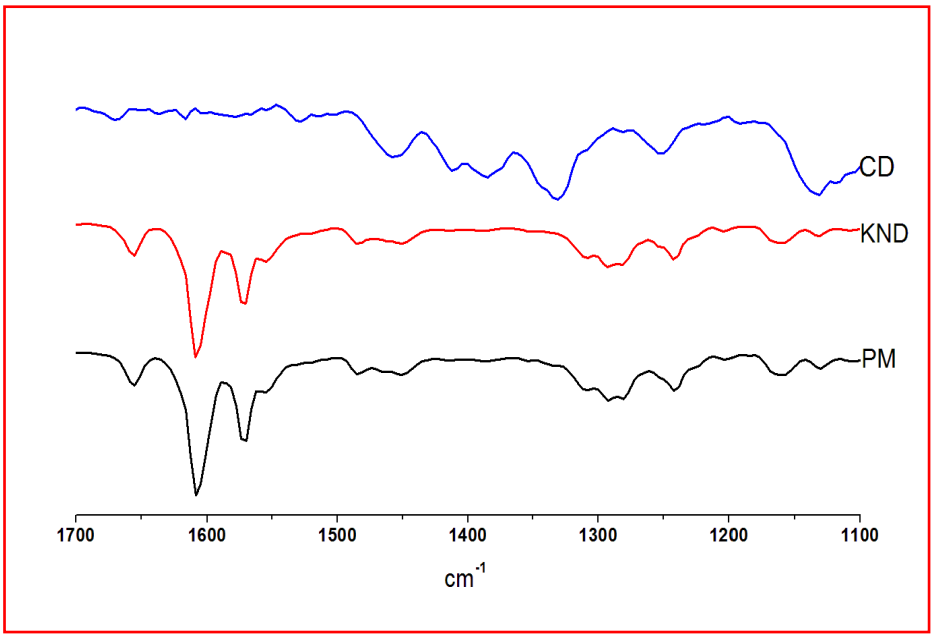

Figure 6.10(a): Raman spectra: Compound 3a; 3a/ β-CD inclusion complex (KND) and β-Cyclodextrin (CD).

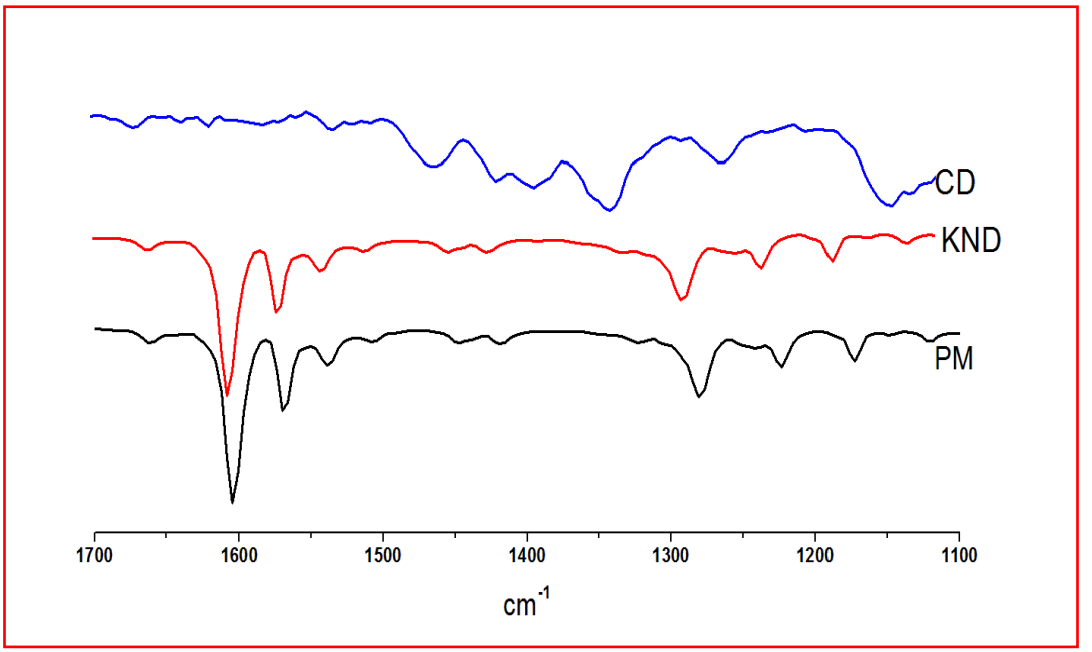

Figure 6. 10 (b): Raman spectra: Compound 3b; 3b/ β-CD inclusion complex (KND) and β-Cyclodextrin (CD).

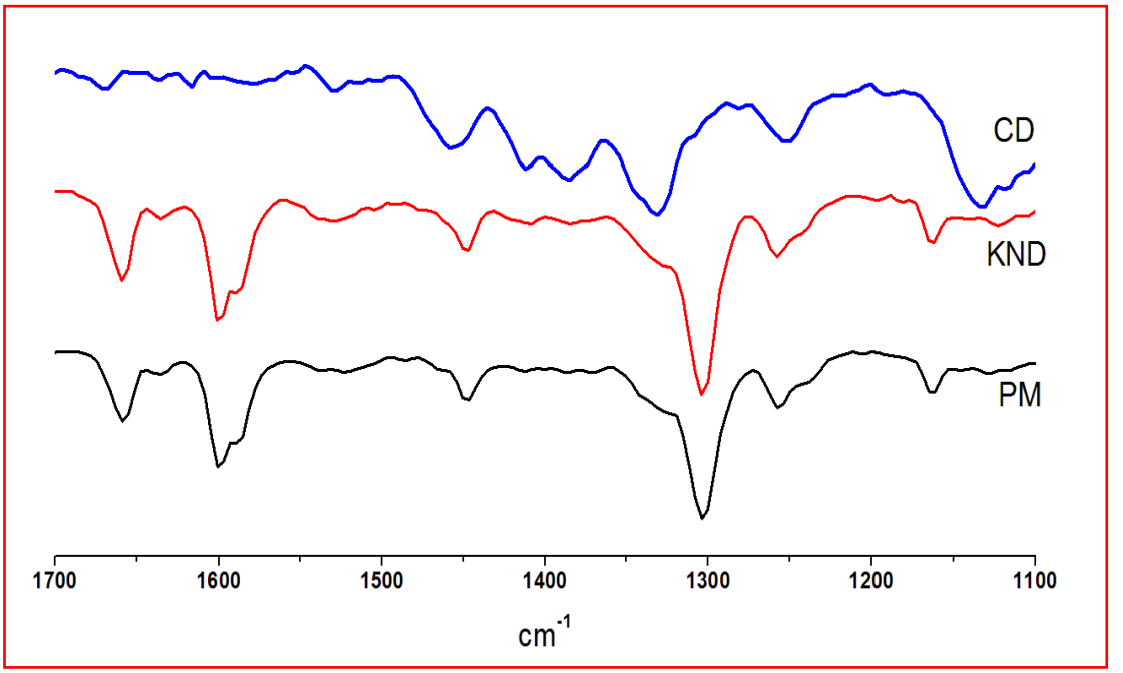

Figure 6. 10(c) Raman spectra: Compound 3c; 3c/ β-CD inclusion complex (KND) and β-Cyclodextrin (CD).

6.3.3 Powder X-ray diffraction study of inclusion complex

True inclusion complexes have its diffraction pattern altered from those of pure components. The powder X-ray pattern for individual components, complex and physical mixture is shown in Figure 6.11a, b and c. The diffraction pattern of complex was found to be different than diffraction pattern of pure β-CD and compounds. Comparing the pattern for β- CD-compounds complex with that of physical mixture reveals mark differences. In complex, new peaks were found and shift in the peak positions were also found where as the physical mixture has peaks which are superimposition of two individuals. The intensity of certain peaks in the complex are also enhanced thereby confirming complex formation.

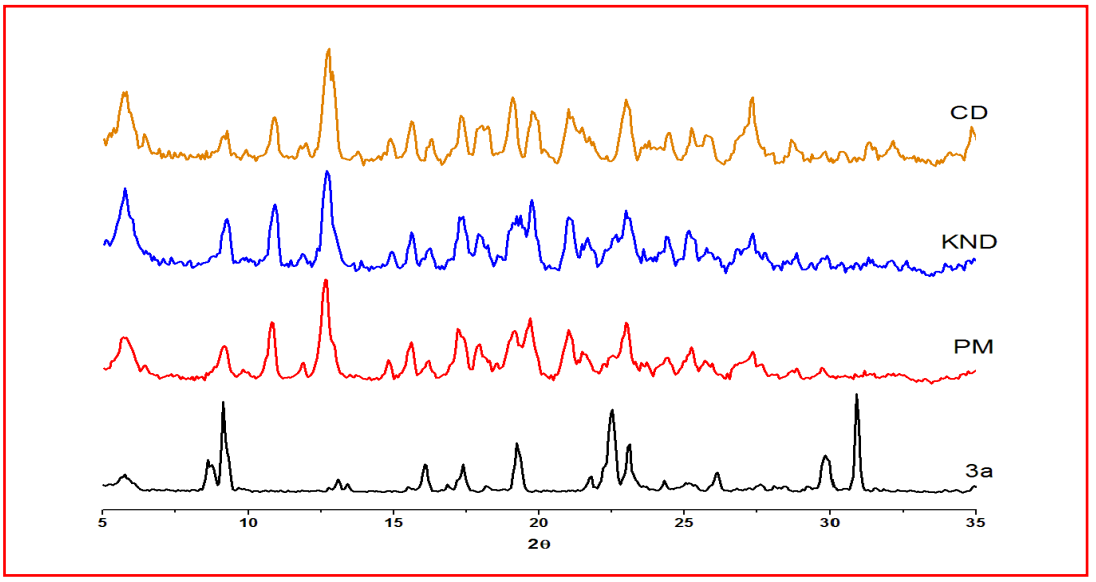

Figure 6.11(a): PXRD patterns: 3a; Physical mixture (PM) ; 3a/ β-CD inclusion complex (KND) and β- CD

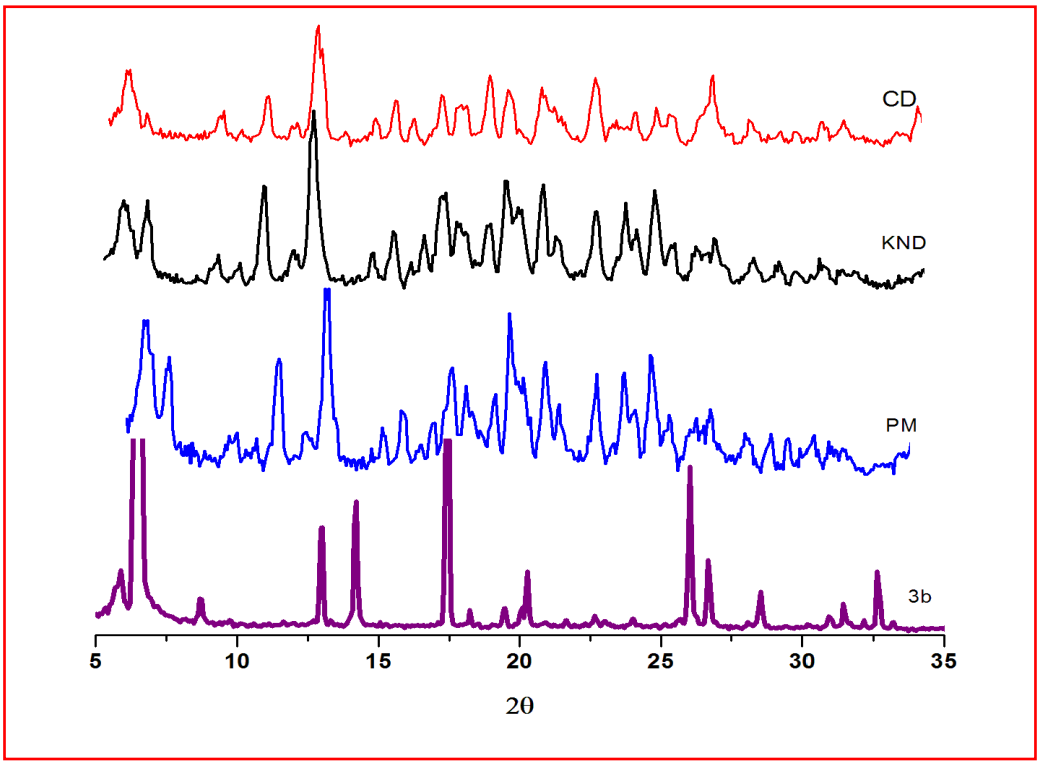

Figure 6. 11 (b): PXRD patterns: 3b; Physical mixture (PM) ; 3b/ β-CD inclusion complex (KND) and β-CD

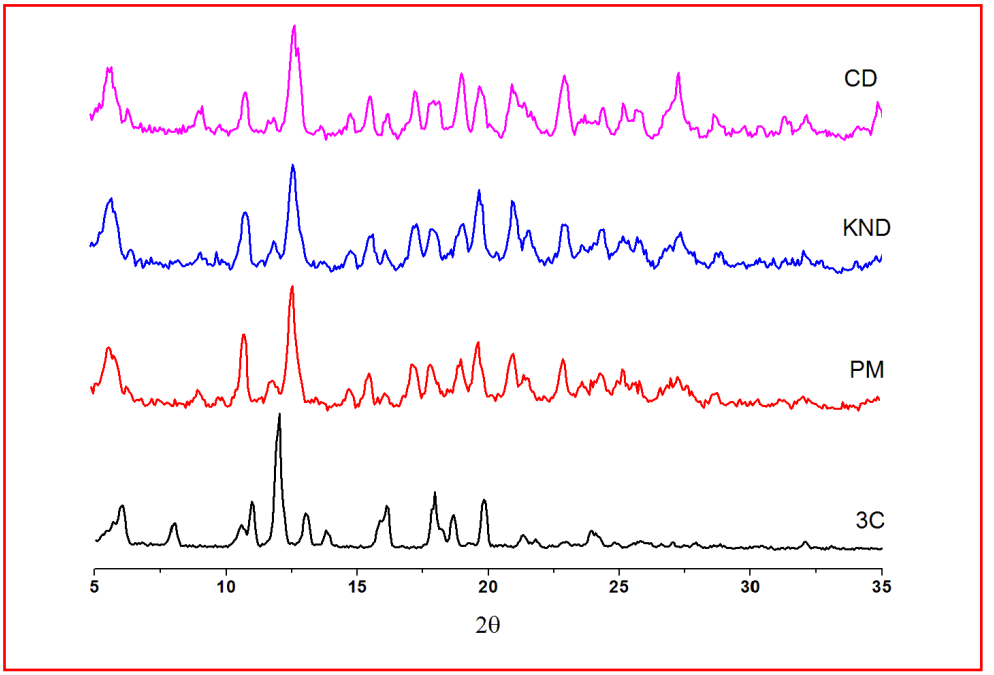

Figure 6. 11 (c): PXRD patterns: 3c; Physical mixture (PM) ; 3c/ β-CD inclusion complex (KND) and β- CD

6.3.4 Differential scanning calorimetry study of inclusion complex

DSC is a fast technique to examine and verify the drugs that form inclusion complex with β-CD and to confirm the absence of the drug melting endotherm. The thermal analysis has been reported as an important method for recognition and characterization of CDs complexes. When guest molecules were embedded in CDs cavities or in the crystal lattice, their melting point is generally shifted to a different temperature and the intensity decrease or disappears. The results of DSC thermograms for given samples are shown in Figure 6.12a, b and c. The DSC curve of compounds showed an endothermic reaction and its melting peak was at the respective onset temperature. The thermal behaviour of β-CD exhibited a sharp endothermic peak due to its melting. Physical mixture showed a sharp endothermic peak. Therefore it was concluded that some part of the compound is complexes with β-CD but some part remained outside of the complex.

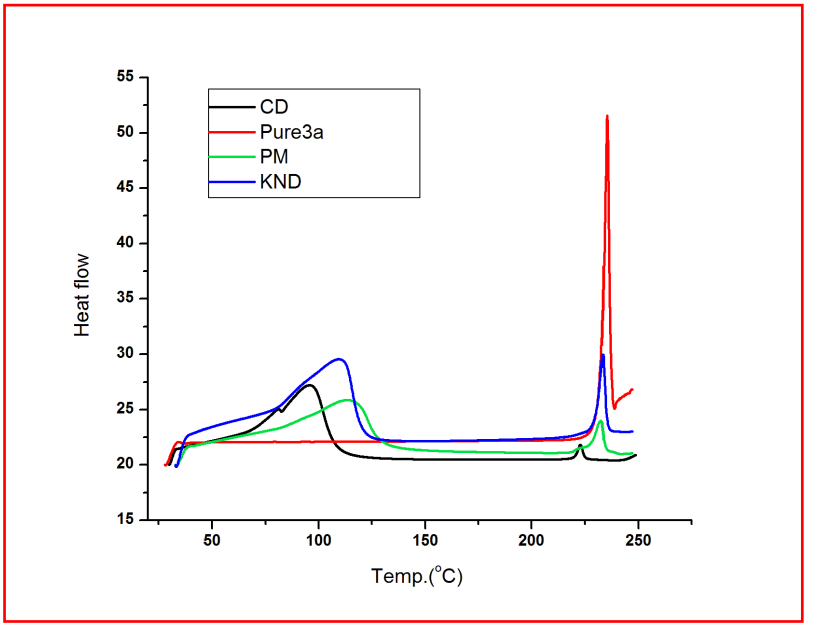

Figure 6.12(a): DSC curves for: β-CD; 3a; physical mixture (PM) and inclusin complex (KND)

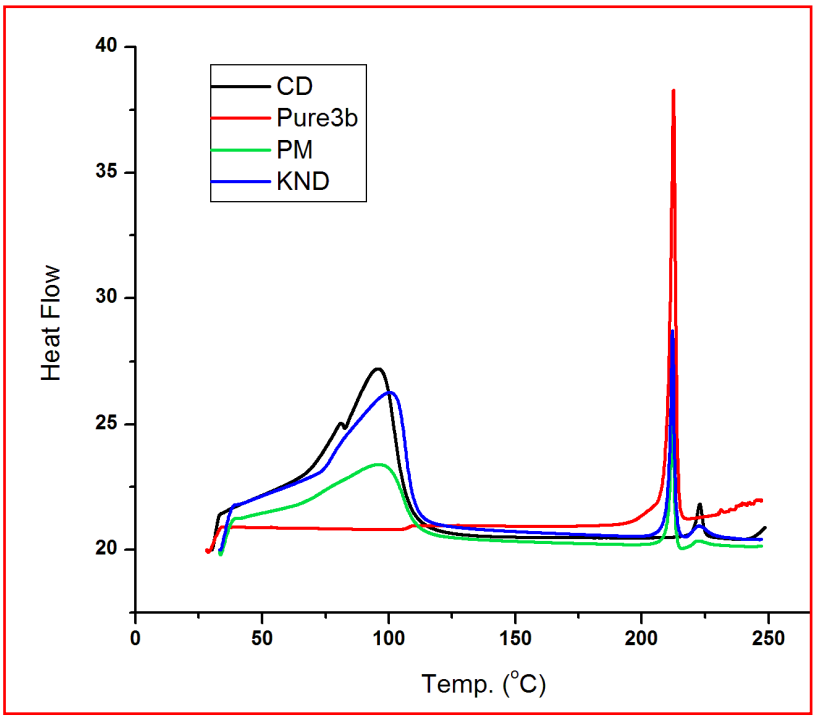

Figure 6.12(b): DSC curves for: β-CD; 3b; physical mixture; and inclusion complex.

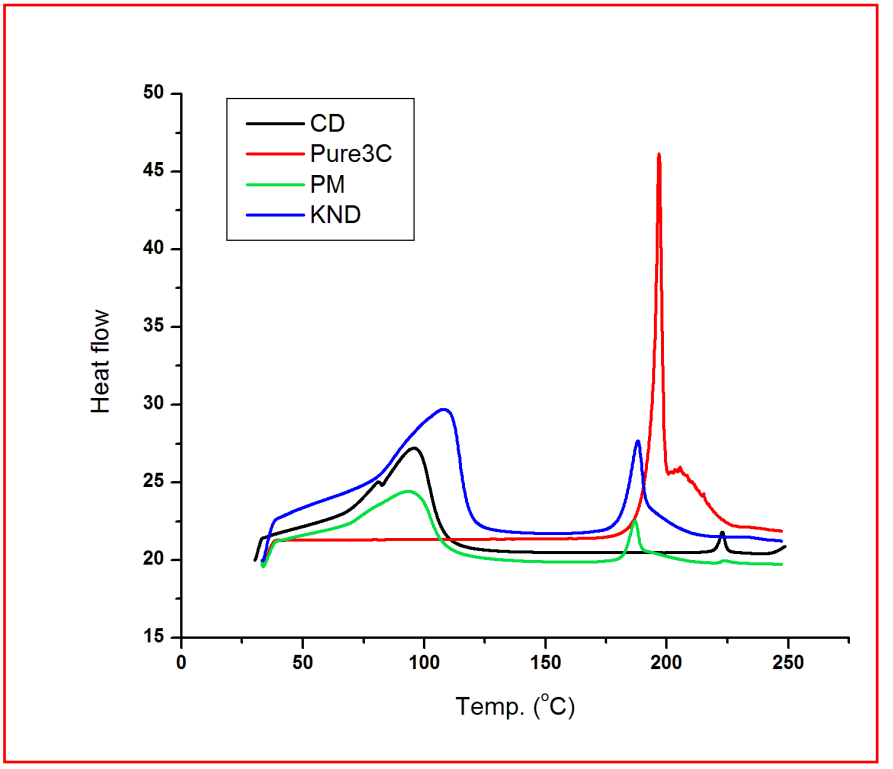

Figure 6.12(c): DSC curves for: CD; 3c; physical mixture (PM); and inclusion complex (KND).

The FT-IR, FT-Raman, DSC and PXRD results produced important evidences in support of compounds-CD inclusion complex formation. The solubility of the stated compounds was successfully enhanced with water by the formation of their inclusion complexes with β-cyclodextrin. 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.