Complexation of ibuprofen with water soluble *p*-sulfonatocalix [4]arene: A potential candidate for drug delivery application

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Abstract: Complexation of ibuprofen with water soluble *p*-sulfonatocalix[4]arene (3) was evaluated. Both molecules exhibit a host and guest type complexation. pH, complex stoichiometry and binding constant were determined by UV-Vis and FT-IR spectroscopy. The maximum complexation of 3 with ibuprofen occurs at pH 2. Stability constant values (9.897) show that there is favorable complex formed due to vital role of *p*-sulfonatocalix[4]arene, while the thermodynamic parameters, i.e. ΔG, ΔH and ΔS have been found as -24.09 KJ/mol, 0.012 KJ/mol and 0.12 KJ/mol. K, respectively. The results show that 3 has efficiency to carry the drug at particular conditions and can be used for drug delivery as a carrier.

Keywords: Synthesis, inclusion complex, *p*-sulfonatocalix[4]arene**,** thermodynamic**,** drug delivery.

INTRODUCTION

Most important characteristic of any drug is its effectiveness, but due to incapability to deliver the drug at a particular site, *i.e.* tissues or cells; effectiveness of the drug may often be reduced. After administration, actual quantity of the drug reduces, when it reaches to the site while passing through various physiological barriers. However, a model drug must contain the characteristics such as stability, solubility and tissue specificity; but such properties have rarely or not been found before therefore; it is very important to develop a drug carrier system with such characteristics (Kolhe *et al*., 2003). In this regard, Yan Xue *et al*. showed the pH triggered delivery of ciprofloxacin from amphoteric calix[8]arene having negative upper rim and positive lower rim (Xue *et al*., 2013). On the other hand, Kui Wang *et al*. worked on the recognition ability of *p*-sulfonatocalix[4]arene and its controllable release for doxorubicin (Wang *et al*., 2011).

The calixarenes are third generation macromolecules in supramolecular chemistry after cyclodextrins and crown ethers, which contain an upper rim with *para* substituents at phenolic rings, a lower rim with phenolic hydroxyl groups and a central annulus (Gutsche, 1998). Both rims can be readily and selectively functionalized to function as hosts due to their specific skeleton and ‘‘basket’’ shaped structure that plays an essential role in host-guest chemistry, (Memon *et al*., 2012). They are widely utilized as synthetic materials, which allow the organic and inorganic "guests" to coordinate/sorb onto their cavity. This prompts their application in the fields of polymer, separation science, sensing, complexation, molecular recognition, pharmaceutical, water treatment, and food industries (Meenakshi *et al*., 2013; Memon *et al*., 2013). Besides this, in biomedical science, calixarenes have been used in antiviral, antibacterial and antifungal activities (de Fatima *et al*., 2009) as well as in protein complexation (Perret *et al*., 2006). Consequently, water soluble calixarenes with high aqueous solubility, extra binding sites and low-toxicity have played vital role as carrier for drugs (Saluja and Sekhon, 2013).

2-(4-Isobutylphenyl) propionic acid is the first member of propionic acid derivatives commonly known as ibuprofen (a non-steroidal drug) (Oladiran and Batchelor, 2007). It is generally used in the treatment of inflammation, pain, and rheumatism (Sakhiyani *et al*., 2012). A variety of pharmaceutical formulations of ibuprofen are available such as oral tablets, transdermal gels as well as creams (Zheng *et al*., 2007). Due to the appropriate size of molecule (*i.e.* 1.0-0.6 nm), excellent pharmacological activity and short biological half life (about 2 hrs) (Nayak and Jain, 2011), a large number of scientists have worked on ibuprofen and employed it as a model drug for controlled/sustained delivery (Öner *et al*., 2011). Thus, the objective of present study has been focused on the synthesis of *p*-sulfonatocalix[4]arene (3), which is non-cytotoxic, non-immunogenic and biocompatible compound (Shah and Agrawal, 2012); and it has also been aimed to examine its physical interaction with ibuprofen (a small molecular drug) in order to prepare *p*-sulfonatocalix[4]arene-ibuprofen complex. The stoichiometry, stability constants at various temperatures and other thermodynamic parameters of the inclusion complex has also been evaluated.

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MATERIALS AND METHODS

Chemicals and reagents

All the regents used in synthesis and solution preparation were of analytical grade. Ibuprofen used in this study was procured from commercially available source. Formaldehyde was purchased from VWR international Ltd. (England). *p-tert*-butylphenol was obtained from Alfa Aesar, a Johnson Matthey Company (Kalsruhe, Germany). Phenol was purchased from Sigma-Aldrich (Taufkirchen, Germany). All compounds, i.e. *p-tert*-butylcalix[4]arene (1) (Gutsche *et al*., 1986), calix[4]arene (2) (Gutsche and Lin, 1986) and *p*-sulfonatocalix[4]arene (3) (Shinkai *et al*., 1987) were synthesized according to previously reported methods as shown in (Scheme 1). Deionized water was used for the preparation of various aqueous solutions. KOH (0.1 M) and HCl (0.1 M) were used to adjust the pH from 2 to 12.

Instrumentation

FT-IR spectra were recorded on a Thermo Nicolet AVATAR 5700 FT-IR spectrometer using KBr pellets (at the spectral range from 4000 to 400 cm^{-1}).

Elemental analysis was performed using a CHNS instrument model Flash EA 1112 elemental analyzer (20090; Rodano, Milan, Italy).

UV-Vis. spectra were recorded on a Perkin Elmer Lambda-35 UV-Vis. spectrophotometer using standard (1.00 cm) quartz cells. Analytical TLC was performed on pre-coated silica gel plates (SiO_2, Merck PF254).

pH study was performed using pH meter (781-pH/Ion meter, Metrohm, Herisau, Switzerland) with glass electrode and internal reference electrode.

Synthesis of p-sulfonatocalix[4]arene

The synthetic route of *p*-sulfonatocalix[4]arene is described in the Scheme 1. The starting material (1) was synthesized by base catalyzed phenol-formaldehyde condensation followed by Friedal Crafts dealkylation of *p-tert*-butylcalix[4]arene (2) as described in literature (Gutsche *et al*., 1986, Gutsche and Lin, 1986). Finally, *p*-sulfonatocalix[4]arene (3) was prepared according to Shinkai's report (Shinkai *et al*., 1987). The different techniques such as TLC, melting point, FT-IR, UV-Vis. and elemental analyses were used to confirm the synthesis of compounds (1-3).

Job's method of continuous variation

The stoichiometric composition of the complex was determined using job's method of continuous variation. The solutions were prepared by mixing the equimolar concentrations (2.5×10^{-4} M) of host (3) and guest (ibuprofen) molecules in different ratios varying from 1:9 to 9:1. Then by plotting the graph between absorbance and concentration, the actual stoichiometric ratio could be determined conveniently.

Preparation of inclusion complex

The solid complex was prepared by co-evaporation of 1:1 host-guest solution that was obtained by mixing their stoichiometric amounts in 5% acetic acid. The mixture solution was stirred at room temperature for 24 hrs, poured in petri dish and evaporated the entire solvent system and resultant complex powder was obtained and vacuum dried.

RESULTS

Solvatochromic study

In order to evaluate the behavior of compound (3), different solvents such as deionized water, tetrahydrofuran (THF), ethanol and acetic acid were used at the concentration of $1x10^{-3}$ M. The UV-Vis. spectra of compound (3) in different solvents are shown in fig. 1.

Complexation study

The complexation efficiency of compound (3) with ibuprofen was explored by UV-Vis. spectroscopy. Solvatochromic study suggests 5% acetic acid as a good solvent for complexation due to the best response of compound (3) in it. Generally, the changes in the absorption spectra of the complex such as shift in band towards shorter or longer wavelengths, enhancement in the absorbance compared to free host are the common indications of complexation. However, to check out the complexation behavior of compound (3) ($1x10^{-3}$ M) with the ibuprofen in 5% acetic acid, UV-Vis. study was carried out.

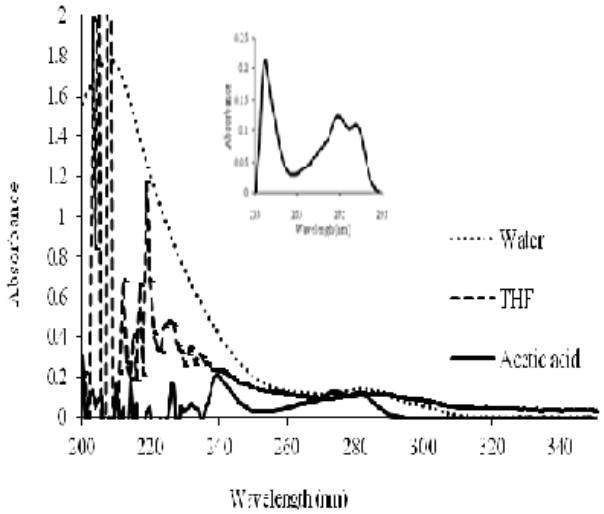

Fig. 1: UV-Vis. spectra of *p*-sulfonatocalix[4]arene in different solvent ($1x10^{-3}$ M) systems.

Job's plot

Usually Job's plot is employed for the spectroscopic determination of complex stoichiometry between host and guest molecules. The stoichiometric ratio is determined by plotting mole fraction (*X*) versus absorbance (Harris, 1995). For 3-ibuprofen complex, the maximum

absorbance was found at mole fraction value=0.5 at fixed wavelength of 236 nm, which indicates 1:1 ratio of host and guest in the complex as shown in fig. 4.

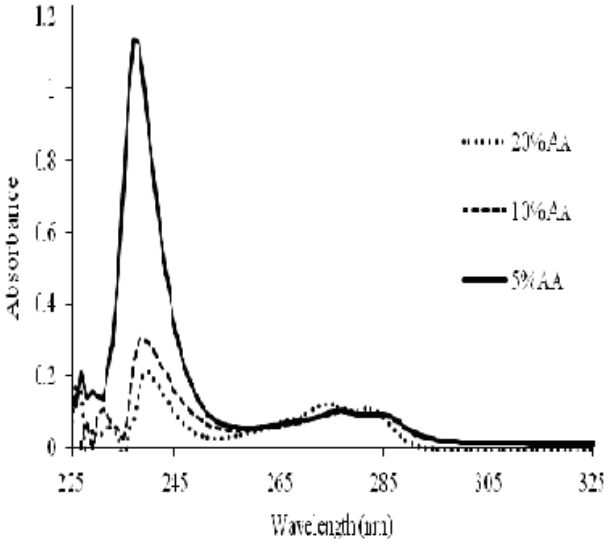

Fig. 2: UV-Vis. spectra of *p*-sulfonatocalix[4]arene ($1x10^{-3}$ M) in different concentrations of acetic acid.

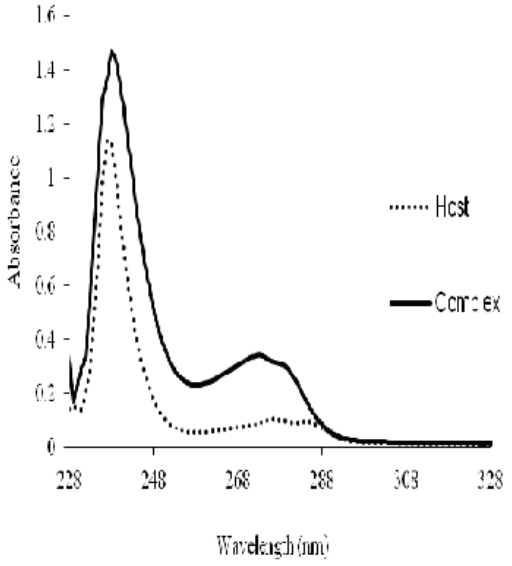

Fig. 3: Comparative UV-Vis. spectra of *p*-sulfonatocalix [4]arene and complex.

FT-IR study

Complex compound was further characterized by FT-IR spectroscopy by comparing the spectra of host and guest molecules. The complex was formed by various interactions including π–π (π-stacking) interactions and hydrogen bonds. These non-covalent interactions play a dominant role in many forefront areas of modern chemistry, especially in drug design in molecular biology.

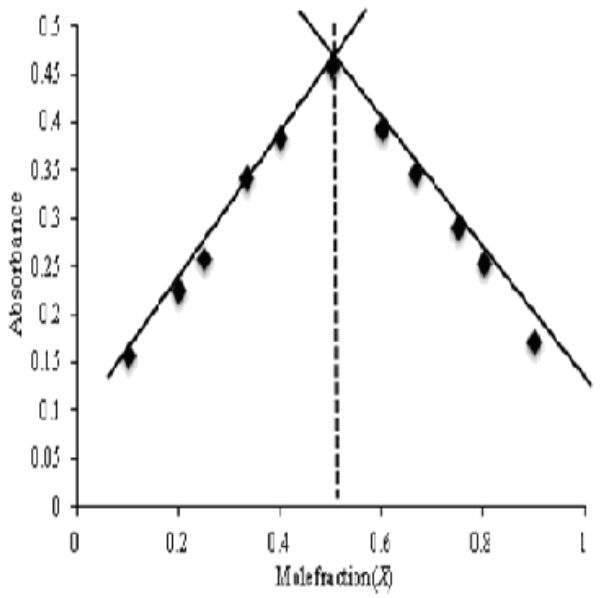

Fig. 4: Job's plot of *p*-sulfonatocalix[4]arene ($2.5x10^{-4}$ M) and ibuprofen ($2.5x10^{-4}$ M).

pH study

For further study, pH of the complex formation was examined and monitored through UV-Vis. study. It is evident from fig. 7 that by varying pH from 2–12, the maximum absorbance was observed at acidic pH value of 2.

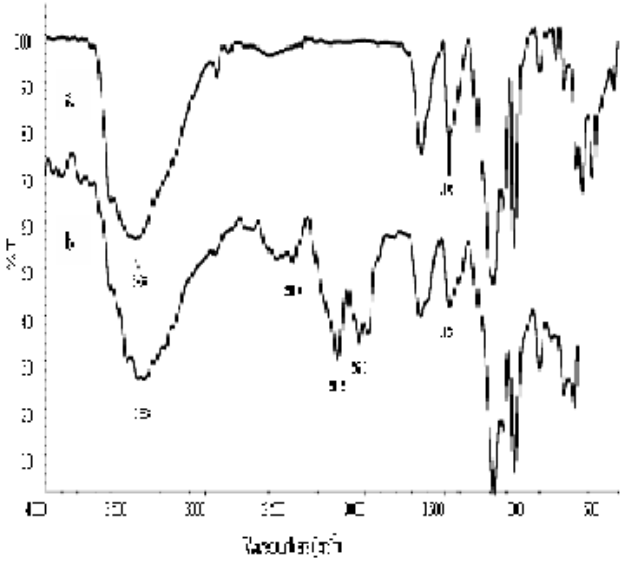

Fig. 5: FT-IR spectra of (a) host *p*-sulfonatocalix[4]arene and (b) complex.

Stability constant

Spectrophotometric method was used to calculate the stability constants (table 1) and thermodynamic parameters (table 2.) of 3-ibuprofen complex at different temperatures (*i.e.* 25, 29, 33, 37 and 41°C).

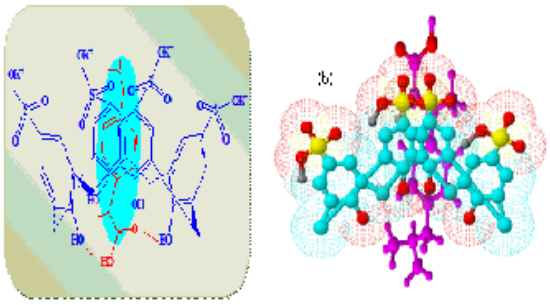

Fig. 6: The proposed structure of complex (a) structural model (b) ball and stick model.

Table 1: Stability constants of *p*sulfonatocalix[4]arene-ibuprofen complex at various temperatures.

$1/T\ 10^{-3}$	ln*K*
3.33	9.659
3.31	9.683
3.26	9.761
3.22	9.814
3.18	9.897

From stability constants, the values of ΔG, ΔH, ΔS were evaluated, which are given in table 2.

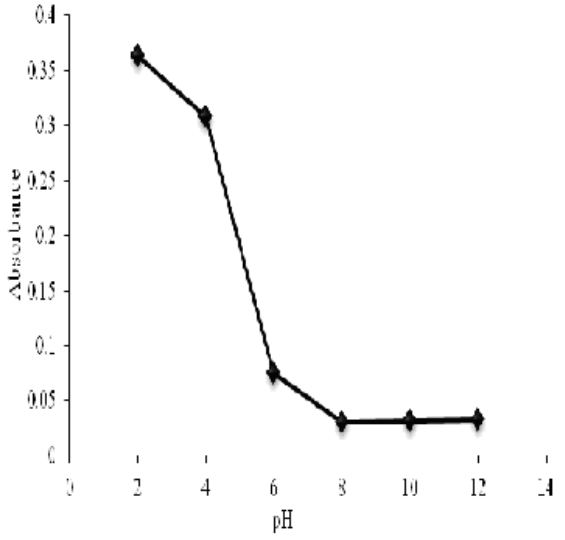

Fig. 7: Effect of pH on complexation behavior of *p*-sulfonatocalix[4]arene.

Initially, molar absorption coefficients (□) were determined using Beer's law (A = □bc) at various temperatures followed by calculation of stability constants. The graph was plotted between ln*K* and 1/T as depicted in fig. 7 ΔG was calculated for 3-ibuprofen complex. In addition ΔS and ΔH were calculated from the straight line equation obtained from graph using equations 1-3:

$$\Delta G = -RT \ln K \quad \rightarrow \quad (1)$$

$$\ln K = -\frac{\Delta G}{RT} = \frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \rightarrow \quad (2)$$

$$\ln K = -\frac{\Delta H}{R} \times \frac{1}{T} + \frac{\Delta S}{R} \quad \rightarrow \quad (3)$$

Besides this, the slope was found from -ΔH/R, while the intercept was obtained from ΔS/R using graph (fig. 8).

As shown in table 1, the values of stability constants of the given complex increase with increase in temperature, which shows that the complex is stable at higher temperature. There is spontaneous formation of complex as shown by negative value of ΔG. The low positive ΔH value indicates that interaction between ibuprofen and (3) occurs at room temperature. These results suggested that the entropic driving force also favor the formation of 3-ibuprofen complex. The relevant free energy change for this system indicates that this inclusion process is an energetically favored process. The positive value of ΔS confirms that the complexation is entropic ally allowed. The smaller value of ΔS for the inclusion complex may be related to steric factor (Panda and Tripathy, 2013, Panhwar *et al*., 2010, Zhou *et al*., 2011).

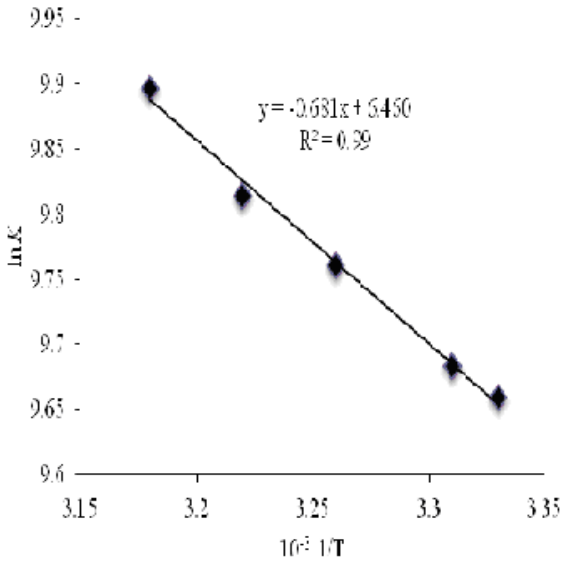

Fig. 8: Plot of ln*K* vs. 1/T for the determination of thermodynamic parameters of complex.

DISCUSSION

It is generally observed that in solvatochromic study, the ground state is less Polar than the excited state for almost all the molecules. Therefore, excited state will be stabilized more than the ground state by the polar solvents. Consequently, by increasing the solvent polarity,

a red shift (bathochromic effect) is observed in the spectrum, which is known as positive solvatochromism. For less polar solvents, a blue shift occurs in the spectrum, which indicates the negative solvatochromism. This study of solvatochromic effect helps to choose the solvent for a particular molecule/compound that could not show similar spectra in different solvents. From various studies, it is investigated that solvent has greater impact on complexation concerning neutral/ionic species, macrocyclic molecules and calixarenes in particular (Hadjmohammadi *et al*., 2008, Harikrishnan and Menon, 2008, Kunsági-Máté *et al*., 2002). It is clearly shown that spectral changes of compound (3) in solvents of different polarity indicate its sensitivity in various solvents environment. In fig. 1, the compound (3) exhibits red shift in its absorption maxima as the solvent polarity increases. In water, it is 209 nm and in acetic acid it is 240 nm, which indicates the positive solvatochromism. In THF, compound (3) shows too much noise. This study suggested that acetic acid is the most suitable solvent for current study. Further attempts were made to observe the behavior of host in different concentrations of acetic acid. For this purpose 20%, 10% and 5% solutions of acetic acid were tried to select appropriate one. It may be concluded from fig. 2 that compound (3) shows good behavior in 5% acetic acid. Thus, 5% acetic acid was selected as a suitable solvent for complexation.

UV-Vis. spectra of free host show a strong band at 237 nm, which is attributed to the $\pi \rightarrow \pi^*$ and another band at 277-284 nm appears due to $n \rightarrow \pi^*$ transition. It can be observed from fig. 3 that addition of guest to the host molecule causes shift in the wavelength and enhance the intensity of the band, which confirms the complexation. Since, *p*-sulfonatocalix[4]arene possesses three types of functionalities, i.e. sulphonate at upper rim, hydroxyl groups at lower rim and central annulus with π electronic cloud. On the other hand, guest molecule has carboxylic and methyl groups. Thus, here weak electrostatic forces of attraction among these groups might control the overall mechanism of complexation. Aromatic hydroxyl protons may engage in hydrogen bonding with acid part of the guest molecule. In this regard, oxygen atoms at lower rim drift their electrons toward protonic part of the acid group in guest and similarly oxygen atoms of carboxyl groups share electrons with phenolic protons of the host molecule. On the other hand π electrons of aromatic rings of calixarene moiety share π- π interaction, which help the host molecules to accommodate guest molecule in its cavity; Likewise, other members of supramolecular family, i.e. cyclodextrins possess hydroxyl groups similar to the calixarenes and utilize weak electrostatic forces for complexation with foreign guest molecules. This is the reason for extensive applications of calixarenes in different fields.

In fig. 5 the most important characteristic peak that undergoes significant shift is present at 3438 cm^{-1} in the host molecule that is found at 3383 cm^{-1} in the complex evidencing that complex has been formed. This shift is indicative of hydrogen bonding between host and guest molecules because at lower frequency value -O---H is hydrogen bonded while at higher value free –OH group shows absorption frequency. In addition, there is also peak broadening in the spectrum of complex molecule that may also be the distinct and recognizable argument regarding the complex formation. Several new peaks are also appeared in the spectrum of complex molecule at around 2027, 2162 and 2440 cm^{-1} demonstrating that the significant interaction has occurred. Among the important interactions of non-covalent type (π-π interactions) between host and guest molecules (*i.e.* in complex), the aromatic C=C gives frequency value at 1469 cm^{-1} in the host molecule, which reduces to 1458 cm^{-1} in complex spectrum indicating such interactions. Absence of C=O (1700-1750 cm^{-1}) stretching vibration for carboxylic group indicates that the drug was solely intercalated in the cavity of host molecule forming endo complex. Thus, FT-IR study fully supports the formation of complex. The proposed structure of the complex is presented in fig. 6.

By varying pH from 2–12, the maximum absorbance was observed at acidic pH value of 2, which indicates the maximum interaction between host and guest molecules. Because, at this pH phenolic protons of calixarene molecule remain in contact and take part in complexation with guest. As we go from acidic to neutral value the absorbance was decreased continuously that means the interaction becomes weak at neutral side and finally at pH 8 the absorbance becomes minimum. It means that at this point, the interaction is negligible due to the removal of phenolic protons, which were the basis for complexation and results in release of the drug.

CONCLUSION

The *p*-sulfonatocalix[4]arene (3) was synthesized and its interaction was examined with ibuprofen. Results suggest that the complex formation between the compound (3) and ibuprofen is due to non-covalent interactions, *i.e*. π–π stacking and H-bonding that might change with the change in pH. The complex was characterized by UV-Vis. as well as FT-IR spectroscopy. In addition, the Job's plot was carried out to know the stoichiometry that was found as 1:1 host guest ratio for the complex. The ΔG, ΔS and ΔH values support the formation of a inclusion complex. Thus, from this study, it has been revealed that the formation of complex is thermodynamically favorable and suggesting that *p*- sulfonatocalix[4]arene (3) can be used as a tool for drug delivery system.

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Table 2: Thermodynamic parameters of 3-ibuprofen complex at various temperatures

Host	Guest	Temp (K)	ln*K*	-ΔG (KJ/mol)	ΔH (KJ/mol)	ΔS (KJ/mol.K)
p-sulphonatocalix[4]arene	Ibuprofen	300	9.659	24.091	0.0129	0.123
		302	9.683	24.312		
		306	9.761	24.832		
		310	9.814	25.294		
		314	9.897	25.837		

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