

## Separation/Analysis of Chrysophanol by Ionic Liquid Loaded $\beta$ -Cyclodextrin Polymer Coupled with Ultraviolet Spectrophotometry

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### Abstract

A novel method for separation/analysis of chrysophanol (Chry) in drugs is described. The work is based on the utilization of ionic liquid ([C4min] PF6) (IL) loaded  $\beta$ -cyclodextrin cross-linked polymer (IL- $\beta$ -CDCP) for effective adsorption of Chry coupled with ultraviolet spectrophotometry for the analysis of Chry. The inclusion interaction of IL- $\beta$ -CDCP with Chry is studied by FTIR and  $^{13}\text{C}$ -NMR. Under the optimum conditions, linear range, Detection Limit (DL) and the relative standard deviation are 0.10-20.0  $\mu\text{g mL}^{-1}$ , 0.02  $\mu\text{g mL}^{-1}$ , 0.45% ( $n=3$ ,  $c=4.0 \mu\text{g mL}^{-1}$ ), respectively. This technique is successfully applied for determination of Chry in drug samples.

**Keywords:** Chrysophanol; Ionic Liquid Loaded  $\beta$ -Cyclodextrin Polymer; Solid-Phase Extraction; Inclusion Interactions; Ultraviolet Spectrophotometry

### Introduction

Chrysophanol (Chry, Figure 1), 1,8-dihydroxy-3-methylanthraquinone, is a free anthraquinone compound and a secondary metabolite of medicinal plant rhubarb. Chry has been reported to have many pharmacological effects such as anti-inflammatory activity [1], anti-microbial activity [2] and anti-cancer action [3]. Therefore, the detection of Chry is quite important and interesting. Until now, various techniques have been developed for the determination of Chry to date, including high-performance liquid chromatography [4] and fluorescence spectrophotometry [5]. However, the direct determination with spectrophotometry is difficult owing to matrix effects and low concentration of Chry in samples. Therefore, the necessity of separation/preconcentration procedure of Chry is significant (Figure 1).

Solid-phase extraction (SPE) is of interest because of its simplicity, higher preconcentration factor, and rapid phase separation. Some novel functional materials such as activated carbon [6], zeolite [7], clay [8], different polymeric adsorbents [9,10] and cyclodextrin polymers (CDs) [11] have been used for SPE. CDs have attracted particular attention, due to their unique physico-chemical characteristics, low cost, availability, and presence of various reactive groups on its backbone chain [12]. The functionalization of CDs can, in particular, alter their physical properties to make them suitable for a wide range of applications [13]. Common methods for the functionalization of resin include load technology, bonding technology and sol-gel technology. The load technology is proposed as an alternative green and economic process [14]. Recent

research on application of functionalized cyclodextrin for separation/analysis includes: biological-based chitosan grafted  $\beta$ -cyclodextrin-benzoic acid [15];  $\text{Fe}_3\text{O}_4$ /cyclodextrin polymer nanocomposites-heavy metals [16];  $\beta$ -cyclodextrin-ionic liquid polyurethanes-organic pollutants/ heavy metals [17]. Ionic liquid (IL) load  $\beta$ -cyclodextrin cross-linked polymer as SPE material seems to be lacking.

In this work, IL was synthesized and loaded onto the  $\beta$ -cyclodextrin cross-linked polymer (IL- $\beta$ -CDCP). FTIR and  $^{13}\text{C}$ -NMR were used to study the inclusion interactions of IL- $\beta$ -CDCP and Chry. The IL- $\beta$ -CDCP-solid phase extraction technique followed by ultraviolet spectrophotometry was applied to separation/analysis Chry in real samples.

### Experimental

#### Materials and Reagents

Centrifuge (Anke Scientific Instrument Factory, Shanghai), timing multifunctional oscillator (Guohua Limited Company, China), digital water-bath (Guohua Limited Company, China), and pH meter (Shanghai Jinjke Limited Company, China) were purchased from their respective companies.

FTIR spectra were measured with a Bruker Tensor 27 spectrometer (Bruker Company, Germany). Samples were pressed in KBr pellets and recorded at frequencies from 4000 to 400  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ . Solid-State NMR (Bruker Company, Germany) spectrum was operated at 400 MHz at room temperature. A SEM Hitachi S-4800 II instrument was used to obtain micrographs of the polymers. Chromatographic separation of analytes was achieved with a LC-10A HPLC (Shimadzu Corporation, Japan), the mobile phase were methanol:water (80:20).

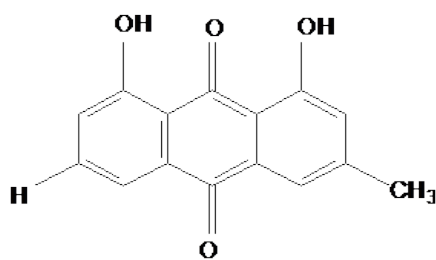


Figure 1: Chemical structure of Chry.

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The flow rate of the mobile phase was maintained at  $1 \text{ mL min}^{-1}$  and the injection volume of sample solution was  $10 \mu\text{L}$ .  $\beta$ -CD, NaOH, HCl, carbinol, ethanol, epoxy chloropropane, Chry were purchased from Shanghai Chemical Reagent Corporation, (China). Stock solution of  $100 \mu\text{g mL}^{-1}$  Chry was prepared by directly dissolving it into ethanol and kept in the dark. Maren pills (Taifu Pharmaceutical Corp. Ltd, China), sanhuang tablets (Jinchen Pharmaceutical Corp. Ltd, China).

### Synthesis of the IL-B-CDCP

Hydrophobic ionic liquids  $[\text{C}_4\text{min}] \text{PF}_6$  was synthesised according to the literature [18].  $\beta$ -cyclodextrin cross-linked polymer ( $\beta$ -CDCP) was prepared following a published procedure [19]. 5 grams of  $\beta$ -CDCP were stirred for 3 days in 20 mL of  $[\text{C}_4\text{min}] [\text{PF}_6]$  medium (ionic liquid: ethanol=1:3) for effective impregnation. The adsorbent was dried at  $90^\circ\text{C}$  for 24h. Then the product was sonicated for 1 day and dried at  $90^\circ\text{C}$  for 24 h again, and the resulting polymer was IL- $\beta$ -CDCP.

### Real Sample Pretreatment

A certain amount of sanhuang tablets/maren pills were grinded into powder. Then 3.0 g of sanhuang powders/4.5 g of maren powders were accurately weighed into the beaker, dissolved in 15.0 mL of ethanol. The above clear liquid was fixed with ethanol into a 25 mL of flask and stored for determination. The solution was diluted with water before determination. pH of the solution was always kept the same (pH=7.0).

### Static Adsorption of Chry

At  $15^\circ\text{C}$ , the amount of Chry ( $10 \text{ mL}$ ,  $4.0 \mu\text{g mL}^{-1}$ ) and  $0.1 \text{ g}$  IL- $\beta$ -CDCP was mixed, controlling the appropriate acidity. The mixture ( $10 \text{ mL}$ ) was shaken on the multifunctional oscillator for 30.0 min and then centrifugal. Supernatant was determined with ultraviolet spectrophotometry at  $317 \text{ nm}$  (the reference solution was distilled water). And retention efficiency of Chry on IL- $\beta$ -CDCP was defined as: Adsorption (%) =  $[(C_0 - C_e)/C_0] \times 100\%$  ( $C_0$  is the initial solution concentration,  $C_e$  is the supernatant solution concentration) [15].

## Results and Discussion

### Characteristics of the Synthesized Polymer (IL-B-CDCP)

**FTIR analysis:** Figure 2 shows the FTIR spectra of IL,  $\beta$ -CDCP and IL- $\beta$ -CDCP, it could be seen that [1] the peaks at  $3170 \text{ cm}^{-1}$  and  $3125 \text{ cm}^{-1}$  in IL- $\beta$ -CDCP are the aromatic part of the  $\sigma_{\text{C-H}}$  vibrations of IL and the characteristic absorption band of imidazole ring in IL from  $1404$  to

$1620 \text{ cm}^{-1}$  present in IL- $\beta$ -CDCP (curve a, b); [2] the characteristic peak at  $3430 \text{ cm}^{-1}$  in IL- $\beta$ -CDCP is the spectrum of  $\sigma_{\text{C-O-H}}$  in  $\beta$ -CDCP and the peaks from  $1000$  to  $1110 \text{ cm}^{-1}$  in IL- $\beta$ -CDCP corresponds to  $\sigma_{\text{C-O-H}}$  and  $\sigma_{\text{C-O-C}}$  vibrations in  $\beta$ -CDCP (curve a c). Therefore, all necessary peaks of  $\beta$ -CDCP and IL are present in the spectrum of IL- $\beta$ -CDCP, which indicates that IL have impregnated onto  $\beta$ -CDCP successfully (Figure 2).

### Analytical Application

#### Optimization of Adsorption Step

**Effect of pH:** Chry and emodin are all anthraquinone compounds, and always coexist in drugs. In this work, the adsorption behavior of Chry and emodin on IL- $\beta$ -CDCP and Chry on  $\beta$ -CDCP are studied, respectively (Figure 3a). As shown in Figure 3b that [1] emodin cannot be adsorbed quantitatively on IL- $\beta$ -CDCP (curve b); [2] the retention efficiency of Chry on IL- $\beta$ -CDCP (curve a) is always higher than  $\beta$ -CDCP (curve c) (Figure 3c), this results indicated that the adsorption ability of  $\beta$ -CDCP for this analyte is greatly improved due to the existence of IL improving the hydrophobicity of polymer; [3] the retention efficiency of Chry on IL- $\beta$ -CDCP is above 90.0% in the range of pH 6.0–10.0 and lower at pH (6.0 or) 10.0, it reaches the highest value 97.8% when pH=7.0. As the dissociation constant ( $\text{pK}_a$ ) of Chry is 9.64 [20], the major existence form of Chry was molecule form in neutral solution, the hydrophobicity of molecule form was stronger than its ionic form, so, it was favorable for the Chry enter into IL- $\beta$ -CDCP. Thereafter, all experiments were conducted at pH 7.0 (Figure 3).

**Effect of the Sample Volume:** The amount of Chry was fixed at  $4.0 \mu\text{g mL}^{-1}$ , and the volume of the sample solution increased from  $5.0 \text{ mL}$  to  $50.0 \text{ mL}$ . It could be seen from Figure 4 that the retention efficiency was greater than 85% in the volume of sample  $5.0$ – $10.0 \text{ mL}$ . In this work, the sample volume of  $10.0 \text{ mL}$  was adopted. The adsorption temperature and time of Chry on IL- $\beta$ -CDCP were also studied; the experiment was carried out at  $15.0^\circ\text{C}$ , 30.0 min.

#### Optimization of Desorption

**Selection of Eluants:** The IL- $\beta$ -CDCP could be reproduced by eluants. Chry could be adopted on IL- $\beta$ -CDCP due to the hydrophobic grouping of Chry enter into IL- $\beta$ -CDCP in neutral solution, so some hydrophobic organic solvent and NaOH was selected as eluants. Different eluants as ethanol, SDS, CTAB, NaOH were investigated

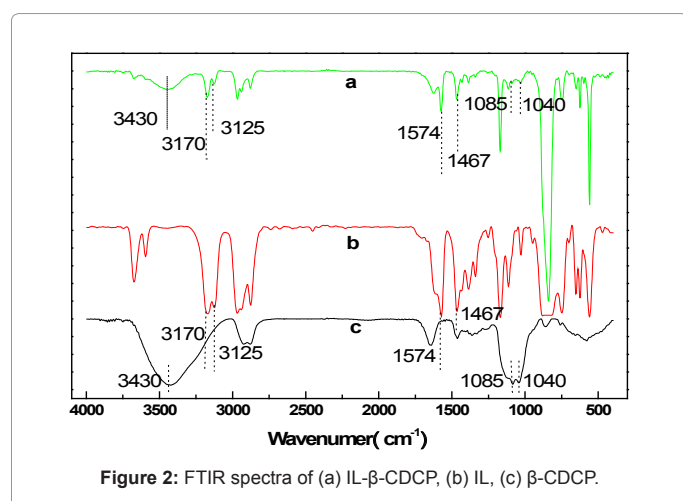


Figure 2: FTIR spectra of (a) IL- $\beta$ -CDCP, (b) IL, (c)  $\beta$ -CDCP.

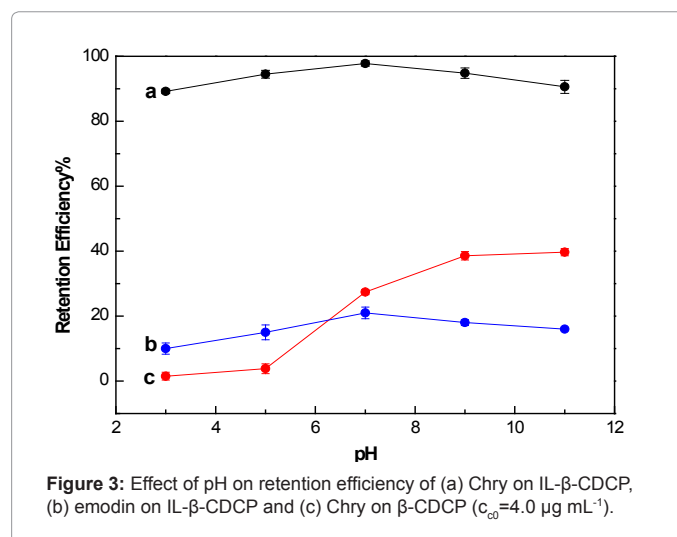
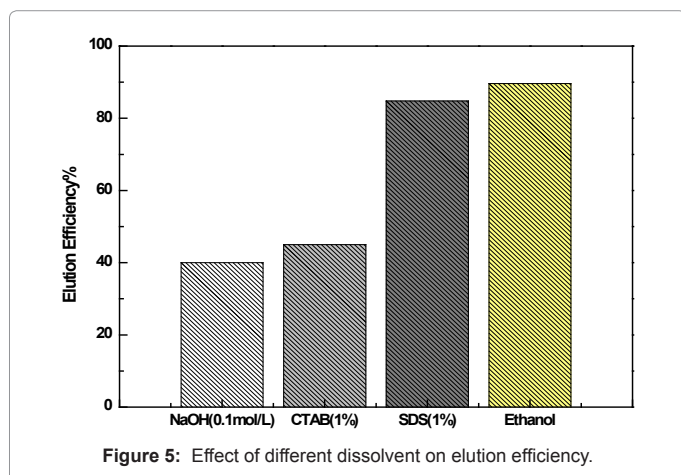
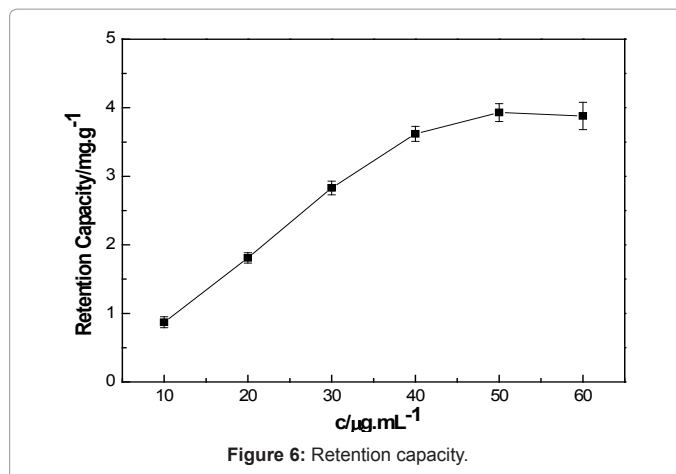
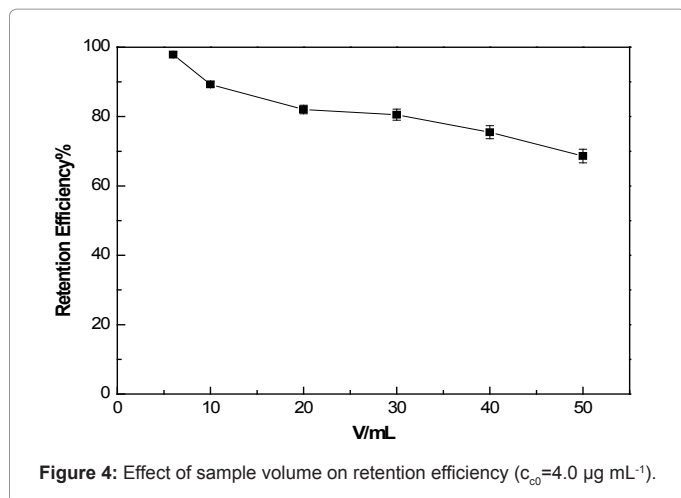


Figure 3: Effect of pH on retention efficiency of (a) Chry on IL- $\beta$ -CDCP, (b) emodin on IL- $\beta$ -CDCP and (c) Chry on  $\beta$ -CDCP ( $c_0=4.0 \mu\text{g mL}^{-1}$ ).



Interferent	Tolerance ratio
phenol	500
$\text{H}_2\text{PO}_4^-$	400
tartaric acid, glucose, Vc	250
citric acid	125
$\text{SO}_4^{2-}$	80
$\text{Mg}^{2+}$	30
geniposide, ursolic acid	5
emodin	1.2

Table 1: Tolerance of interference ions.

(Figure 5). The order of elution efficiency was ethanol (89.6%) SDS (84.8%) CTAB (45.0%) NaOH (40.0%). It indicates that Chry cannot be quantitatively eluted by NaOH and CTAB, but can be completely eluted by ethanol and SDS, since lots of air bubble was formed in the process of elution in SDS solution and the hydrophobicity of ethanol was stronger than other eluants. So, ethanol was chosen as eluent.

Elution efficiency of Chry with 3.0-8.0 mL of ethanol was studied. The elution efficiency of Chry is above 85% from 4.0 mL to 8.0 mL. The preconcentration factor is 2.5 (the quotient of volume before absorption and after elution). Therefore, the optimum volume of ethanol solution chosen for this work is 4.0 mL. In this experiment, the elution temperature and time of Chry on IL- $\beta$ -CDCP were studied; the experiment was conducted at 50.0°C, 30.0 min.

**Sorption Capacity:** The sorption capacity, defined as the maximum amount of Chry sorbed per gram of the polymer IL- $\beta$ -CDCP, is an important factor for evaluation of the synthesized IL- $\beta$ -CDCP. As shown in Figure 6, when the concentration of Chry is 50  $\mu\text{g mL}^{-1}$ , the retention reaches a maximum value. The retention capacity of the polymer is therefore calculated as 3.94  $\text{mg g}^{-1}$ .

**Effect of Foreign Ions:** Determination of Chry (4.0  $\mu\text{g mL}^{-1}$ ) in the presence of foreign substances was investigated. With a relative error of less than  $\pm 5\%$ , the tolerance limit for various foreign substances is in Table 1.

**Analytical Parameters:** Under optimum conditions described above, IL- $\beta$ -CDCP shows a linear calibration curve within the concentration ranging of 0.10 to 20.0  $\mu\text{g mL}^{-1}$ . The least square equation is:  $A=0.051 C (\mu\text{g mL}^{-1})+0.047$  ( $R^2=0.9930$ ). The limits of detection (DL), which is defined as  $C_{DL}=3S_b/m$ , where  $S_b$  is the standard deviation of eleven replicate blank signals and  $m$  is the slope of the linear section of the calibration curve after preconcentration, is found to be 0.02  $\mu\text{g mL}^{-1}$  for a sample volume of 10 mL. The relative standard deviation for three separate batch experiments with 0.1 g of sorbent for determination of 4.0  $\mu\text{g mL}^{-1}$  Chry in 10 mL of water is 0.45%. The preconcentration factor was calculated to be 2.5.

The result of the present method for extraction and determination of Chry are compared with other reported methods [21-24]. Table 2 lists the linear range for the analysis of Chry in real samples with those reported in literatures. It is obvious that the present work has wide linear range, and easy operation (Table 2).

**Sample Analysis:** As shown in Table 1, emodin was the main interference substance in determination of Chry. The proportion of emodin and Chry was lower than 1.2 in sanhuang tablets/maren pills [22,25], which illustrate the existence of emodin would not influence the determination. The sanhuang tablets/maren pills were analyzed with standard curve method (listed in Table 3). The results of this method and HPLC method were checked by t test method ( $P=0.95$ ,  $f=3$ ) and had no significant difference (Table 3).

### The Adsorption Mechanism of IL- $\beta$ -CDCP and Chry

The inclusion interactions of IL- $\beta$ -CDCP and Chry are the primary mechanism for the adsorption of Chry on IL- $\beta$ -CDCP [26]. In this paper, it is evidenced by FTIR and  $^{13}\text{C}$ -NMR.

**FTIR Analysis:** Figure 7 shows the FTIR spectra of the inclusion complex of Chry, IL- $\beta$ -CDCP and IL- $\beta$ -CDCP-Chry. The intensity of

Method	Linear range ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Limit of detection ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Reference
HPLC	6.41-102.60	-	21
RP - HPLC	3.181-101.8	-	22
HPLC - FLD	0.2560~25.60	-	23
CPE - HPLC	0.126-16.2	-	24
ILS- $\beta$ -CDSP SPE/UV	0.1-20.00	0.02	The proposed method

Table 2: Comparison with previously reported methods.

Sample	Found	HPLC value	Recovery (%)
sanhuang tablets	3.14 mg/g	3.20 mg/g	98.13
maren pills	35.61 $\mu\text{g}/\text{g}$	36.22 $\mu\text{g}/\text{g}$	98.32

The sanhuang and maren solution was diluted 100 and 10 times before detection, respectively.

Table 3: The results for the determination of Chry in drug samples.

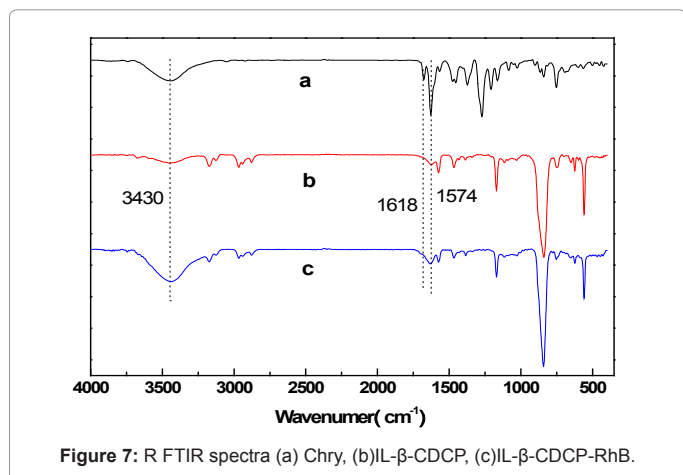


Figure 7: R FTIR spectra (a) Chry, (b) IL- $\beta$ -CDSP, (c) IL- $\beta$ -CDSP-RhB.

$\sigma_{\text{C=C}}$  peaks from 1404 to 1620  $\text{cm}^{-1}$  in imidazole ring of IL- $\beta$ -CDSP-Chry (curve. c) are much higher than that of IL- $\beta$ -CDSP-Chry (curve. b). It is probably that the IL bond interactions play a crucial role in the inclusion of IL- $\beta$ -CDSP-Chry.  $\sigma_{\text{O-H}}$  Peak (3430  $\text{cm}^{-1}$ ) in IL- $\beta$ -CDSP-Chry (curve. c) increases, which demonstrated that the hydroxy part in Chry (curve. a) is enclosed in the hydrophobic cavity of IL- $\beta$ -CDSP-Chry.

**Solid-State NMR Analysis:**  $^{13}\text{C}$ -NMR spectra of the IL- $\beta$ -CDSP and IL- $\beta$ -CDSP-Chry are presented in Figure 8. The splitting of  $\text{C}_1$  peak in IL- $\beta$ -CDSP (curve a) disappear completely after adsorbing Chry, which illustrates the formation of the inclusion IL- $\beta$ -CDSP-Chry (curve b). This result indicated that the existence of Chry makes the conformation of CD ring in IL- $\beta$ -CDSP more symmetrical and  $\text{C}_1$  is almost in the same chemical environment [27] (Figure 8).

**Inclusion constants:** The inclusion constant  $K$  is a significant parameter which illustrates inclusion properties of host-guest molecules. The inclusion complex can be easily formed at a higher  $K$ . The inclusion constants of the monomers of two kind of polymers ( $\beta$ -CD and IL+ $\beta$ -CD) and Chry were measured. The form of inclusion and the inclusion constant can be calculated by UV-visible absorption spectroscopy and Hildebrand-Benesi equation [28,29].

The double reciprocal plots of the  $\beta$ -CD-Chry inclusion complex (curve. a) and (IL+ $\beta$ -CD)-Chry inclusion complex (curve. b) are shown in Figure 9. It can be concluded that  $\beta$ -CD and IL+ $\beta$ -CD form the

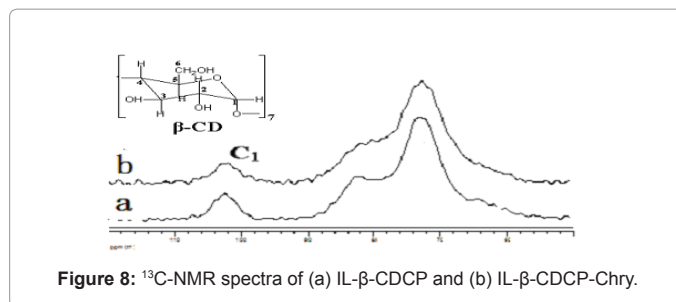


Figure 8:  $^{13}\text{C}$ -NMR spectra of (a) IL- $\beta$ -CDSP and (b) IL- $\beta$ -CDSP-Chry.

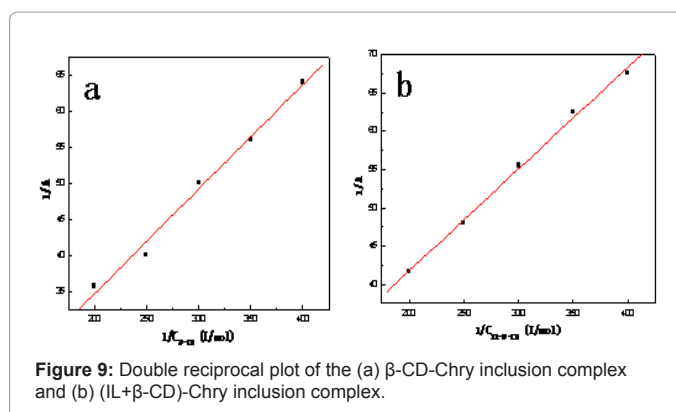


Figure 9: Double reciprocal plot of the (a)  $\beta$ -CD-Chry inclusion complex and (b) (IL+ $\beta$ -CD)-Chry inclusion complex.

inclusion complexes with Chry at ratio of 1:1. The two double reciprocal plots show good linearity with correlation coefficients of 0.9946 for  $\beta$ -CD and 0.9983 for IL+ $\beta$ -CD. The inclusion constant of  $\beta$ -CD-Chry inclusion complex  $K$  is 38.54  $\text{L mol}^{-1}$  and 113.64  $\text{L mol}^{-1}$  for (IL+ $\beta$ -CD)-Chry inclusion complex, which are acquired by the slope and intercept of the double reciprocal plots. Inclusion constant reflects inclusion ability of host molecule to guest molecule. All above illustrates that the inclusion ability of IL+ $\beta$ -CD towards Chry is stronger than of  $\beta$ -CD. That is one reason why IL- $\beta$ -CDSP has higher adsorption efficiency than  $\beta$ -CDSP (Figure 9).

## Conclusion

IL- $\beta$ -CDSP used as solid phase extraction material (SPE) to pre-concentrate/separate Chry coupled with ultraviolet spectrophotometry for the analysis of Chry was established. The proposed method has some advantages, such as increase of contact area between IL and water, reduction of amount of IL, little loss of IL and was an easy, safe and inexpensive methodology for the separation and determination of Chry in drugs.

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