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Cloud Point Extraction and Spectrophotometric Determination of Codeine in Pharmaceutical and Biological Samples

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Cloud point extraction process using non-ionic surfactant, Triton X-114, to extract codeine from aqueous solution was investigated. The method was based on the extraction of codeine and bromothymol blue from acetate buffer media to surfactant-rich phase and formed a charge transfer-ion pair complex. The extracted surfactant-rich phase was diluted with ethanol and its absorbance was measured at 430 nm. The effect of different variables such as pH, Triton X-114 concentration, cloud point temperature and time was established. The calibration graph was linear in a wide range of 100.700 ng ml^{-1} of codeine with $r = 0.998$ ($n = 7$). The detection limit based on three times standard deviation of the blank (3s) was 4.6 ng ml^{-1} and relative standard deviation (R.S.D) is 2.15% for 500 ng ml^{-1} codeine ($n = 5$). The proposed method was applied to the determination of codeine in acetaminophen codeine tablets and blood samples.

Keywords: Codeine, Bromothymol blue, Cloud point extraction, Charge transfer complex

INTRODUCTION

Codeine is one of the main alkaloids in poppy seed having pharmacological and toxicological activity (Fig. 1). As a drug, codeine is widely used as a good analgesic and cough suppressant agent [1,2]. It is a derivative of morphine, one of the best known drug abuses. Excessive or habitual use of these drugs causes toxic symptoms. Additionally, it has been reported that addiction to poppy drug abuse leads to a lot of sever social problems [3]. Thus, the rapid separation and determination of small level of codeine in biological fluid is of vital interest in clinical toxicology, control of drug abuse and forensic cases.

A wide rang of analytical methods have been reported for the determination of codeine such as gas chromatography [4,5], high performance liquid chromatography [6,7] and ion selective electrode [8]. Sample preparation techniques prior to instrumental analysis were based on liquid-liquid extraction (LLE) [9] and solid phase extraction (SPE) [10-13]. Unfortunately, these methods require a large sample volume and are time consuming. In particular, the traditional liquid-liquid extraction method used large amounts of hazardous, volatile organic solvents. So, in recent years, the green liquid-liquid extraction and cloud point extraction (CPE) have been employed in analytical chemistry. The use of preconcentration

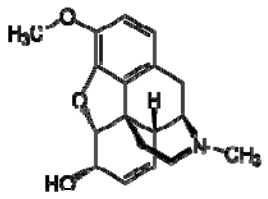

Fig. 1. Molecular structure of codeine.

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step based on phase separation by cloud point technique offers a convenient alternative to more conventional extraction system. Compared with the traditional organic liquid-liquid extraction, cloud point extraction requires a very small amount of relatively nonflammable and nonvolatile surfactants that are friendly to the environment. The first applications of phase separation based on cloud point phenomenon refer to the extraction of metal ions forming complexes sparingly soluble in water. Another application focuses on the isolation and purification of species of biological interest, mainly proteins [14]. This methodology was used first by Bordier [15] for the separation of hydrophilic proteins in biological membranes, using solution of Triton X-114 as an extractant [16,17]. Furthermore, this surfactant has been used for the preconcentration of vitamins A and E without their decomposition. The efficiency extraction with Genapol X-080 for vitamins A and E in blood and serum samples has been shown to be comparable to that obtained using an official method, which validates this method for quantification of the said vitamins in blood samples [18]. Furthermore, surfactant aggregates have been widely used as drug delivery vehicles [19,20] because they have low viscosity, small aggregate size, simple preparation and long shelf-life. In fact, micellar solubilization is one of the most important properties of surfactant solution, widely used in pharmaceutical, food, detergency and cosmetic industries, enhanced oil recovery and so forth [21].

For organic species, the parameters susceptible to optimization stem from the properties of the surfactant medium that is applied. However, for inorganic species, where the quantitative formation of a hydrophobic complex is an essential prerequisite for efficient CPE, the properties of the surfactant system have to be optimized more carefully, taking into account the variables of complex formation. Apart from the selection of the appropriate chelating agent, common parameters for both organic and inorganic species, which have to be examined to make CPE successful, are: pH, ionic strength, surfactant type and concentration, temperature, and, equilibrium and centrifugation time [22].

This method is still in its initial stages which means that only few reports on the extraction of environmental pollutants such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCBs), vitamins, pesticides and other organic compounds are available in literature [23-26].

All these indicate that CPE has great analytical potential as an effective enrichment method. To the best of our knowledge, no research ahs been reported on how to extract codeine through cloud point technique. In this study, we suggest a spectrophotometric method for the determination of codeine in a simple CPE process. The method is based on the cloud point extraction of charge transfer (CT)-ion pair of codeine-bromothymol blue (BTB). Triton X-114 was used as an extraction solvent.

EXPERIMENTAL

Apparatus

A Perkin Elmer UV-Vis spectrophotometer model Lambda 25 was used for recording absorption spectra and absorbance measurements using 1cc quartz cell. A CRISON digital pH meter model GLP22 with combined glass electrode was used for measuring the pH. A Hettach centrifuge model EBA20 and a Julabo ED thermostatic bath model F12, maintained at the desired temperature, were used for cloud point temperature experiments.

Reagents

All reagents were analytical grade and used without further purification. A stock solution of 10^{-3} M of codeine phosphate (Merck) was prepared by dissolving an appropriate amount of the reagent in distilled water and diluting to 50 ml in a volumetric flask.

BTB solution 10^{-3} M was prepared by dissolving an appropriate amount of it (Merck) in alkaline solution and diluting in a volumetric flask with distilled water to the mark. The acetate buffer pH 4.5 was prepared by adding NaOH solution to a 0.1 M acetic acid solution and adjusting pH to 4.5 using a pH meter. Triton X-114 (1% v/v) was prepared by dissolving 1 ml of concentrated solution (Fluka) in 99 ml distilled water.

Procedure

An aliquot of codeine solution (whose final concentration would be in the range of 100-700 ng ml^{-1}), 2 ml of acetate buffer (0.1 M, pH 4.5), 2 ml of triton X-114 1% (v/v) and 50 µl of 10^{-3} M BTB solution were added to 10 ml volumetric

flask and diluted to the mark with distilled water. The resulting solution was transferred to a centrifuge tube and equilibrated at 40° for 15 min. Then the phase separation was accelerated by centrifugation for 7 min at 3500 rpm. The mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily removed using a syringe pipette. The surfactant-rich phase was diluted with 0.3 ml of ethanol. A minimum volume was used for diluting the surfactant-rich phase to measure the absorbance and maximize the preconcentration factor. Then the ethanolic surfactant-rich phase was transferred into a 1 cc quartz cell to measure its absorbance at 430 nm.

Preparation of Acetaminophen Codeine Tablets and the Biological Sample

Ten tablets were properly ground and 0.300 g (equal to one tablet) of the resulting powder was dissolved in doubly distilled water, and filtered with filter paper. The transparent solution was transferred to a 50.0 ml volumetric flask and diluted to the mark with doubly distilled water. Working solutions were prepared by taking a suitable aliquot of the sample and its codeine content was determined by the above-mentioned procedure.

Blood serum samples were supplied by the Damavand Clinical Laboratory, Tehran, Iran, by a single centrifugation of the whole blood. The proteins were precipitated by adding saturated ammonium, shaking for 15 min and centrifuging to separate the precipitate. The samples did not undergo any pre-treatment before the analysis.

RESULTS AND DISCUSSION

The experiments were carried out to develop a simple and sensitive cloud point extraction for the spectrophotometric determination of codeine. Bromothymol blue is the most widely used dye for the determination of amines of pharmaceutical interest by photometric method (27, 29 and references therein). The BTB forms a CT-ion pair complex with codeine. The CT complex was formed by the interaction of the codeine base as n-electron donor and BTB as π-acceptor. The absorption spectra of BTB and the CT-ion pair complex after CPE process with triton X-114 are shown in Fig. 2. As can be seen, the absorbance at λ_{max} (430 nm) increased after

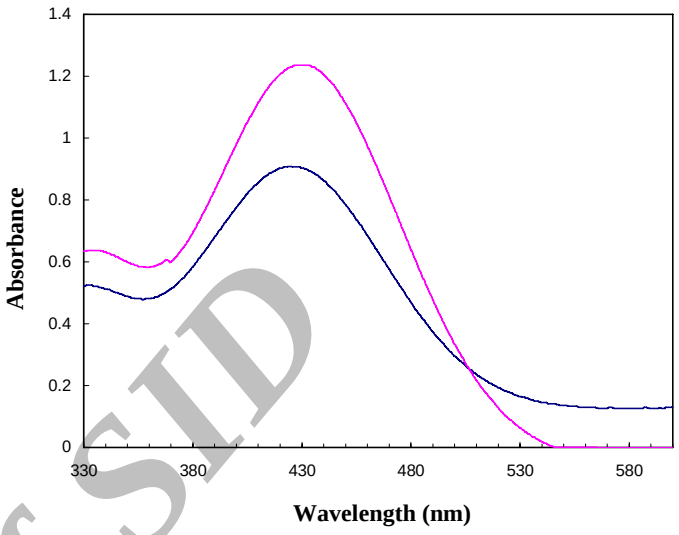

Fig. 2. Absorption spectra of: (a) BTB, (b) BTB-codeine CT-ion pair complex after CPE process. Conditions: 0.2% (v/v) Triton X-114, 5.0×10^{-6} M BTB, pH = 4.5 (acetate buffer, 0.1 M), and 500 ng ml^{-1} of codeine.

the formation of this charge transfer complex. Therefore, all measurements were carried out at this wavelength.

The extraction process can be altered by different factors, such as equilibration temperature and time, pH, concentration and nature of the surfactant, and addition of salt. Hence, the effect of these factors on the percentage extraction of the codeine was studied.

Effect of pH

For organic molecules, pH is perhaps the most critical factor regulating the partitioning of the target analyte in the micellar Phase. Especially, for ionizable species such as phenols and amines, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte prevails.

The effect of pH on the extraction of CT-ion pair complex in CPE process was studied for 400 ng ml^{-1} of codeine in the range of 2.5-7.0 by the addition of appropriate buffer. As can be seen in Fig. 3, maximum extraction was obtained at pH 4.5.

The extraction equilibria can be represented as follows:

$$COD_{(aq)} \leftrightarrow COD_{(org)}$$

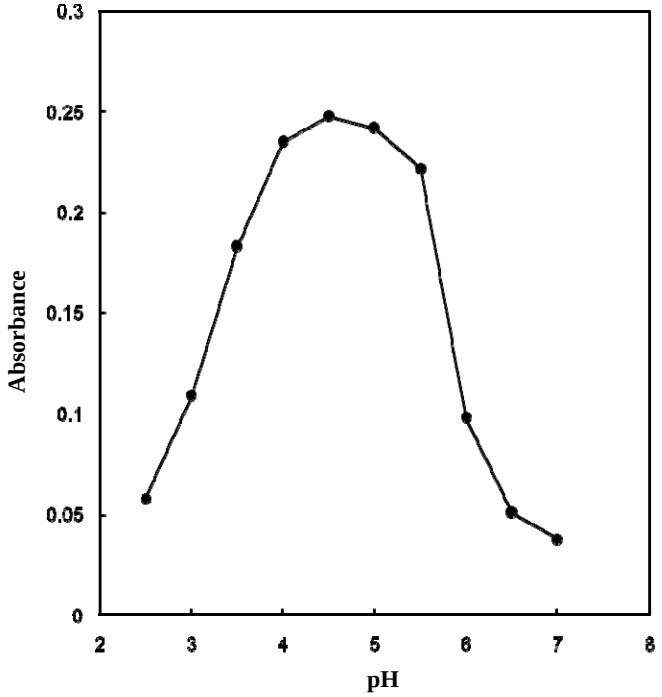

Fig. 3. Effect of pH on absorption of the complex. Conditions: 0.2% (v/v) Triton X-114, 5.0×10^{-6} M BTB and 400 ng ml^{-1} of codeine.

$BTBH_{(aq)} \leftrightarrow BTBH_{(org)}$

$COD_{(org)} + BTBH_{(org)} \leftrightarrow CODHBTB_{(org)} \leftrightarrow CODH^{+}\text{-}BTB^{-}_{(org)}$ (charg transfer complex)

where COD and HBTB represent the codeine and the bromothymol blue, respectively, and the subscript (aq) and (org) refer to the aqueous and surfactant rich phases, respectively. At pH higher than 4.5, the extraction of bromothymol blue and codeine to the organic phase was decreased, hence the formation of CT complex and absorbance was diminished. In addition, at lower pH value the extraction of protonated codeine molecule to the surfactant phase was decreased.

Effect of Buffer Concentration

From among the several buffer agents such as format, phosphate, and acetate that we studied, the best results regarding stability, preconcentration factor and kinetics of phase separation, belonged to acetate buffer. The influence of buffer concentration after CPE process was investigated. The results are shown in Fig. 4. As can be seen, the maximum extraction efficiency was obtained with 2.5×10^{-3} M acetate buffer solution. Hence, in all the experiments, the pH of aqueous solutions was adjusted to 4.5 with 2.0 ml of 0.1 M acetate buffer solution.

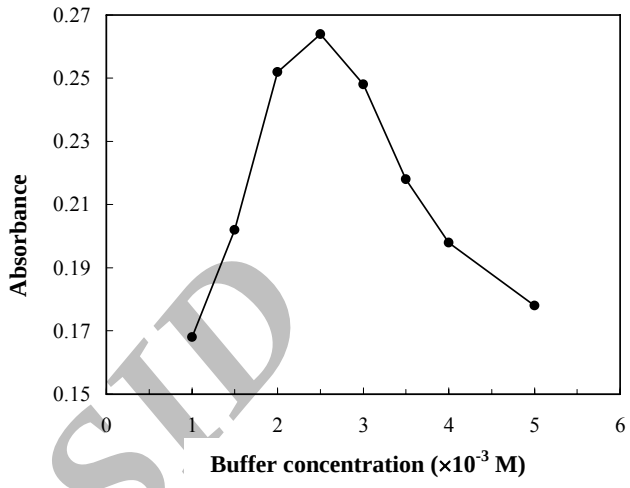

Fig. 4. Effect of buffer concentration on the absorption of the complex. Conditions: 0.2% (v/v) Triton X-114, 5.0×10^{-6} M BTB, 400 ng ml^{-1} of codeine and pH 4.5.

Effect of BTB Concentration

The extraction was studied at different concentrations of BTB and the results are shown in Fig. 5. As it is seen, the signal increases up to a known concentration of BTB, reaching a plateau, which is considered as the complete extraction. Therefore, a concentration of 5.0×10^{-6} M was chosen as the optimum amount.

Effect of Surfactant Nature and Concentration

The nature of the surfactant used in CPE is a critical factor for its low cloud point temperature (CPT) and high density. Between Triton X-100 and Triton X-114, non-ionic surfactants, as are usually used in CPE, Triton X-114 was chosen because the main experimental difficulty to overcome when working with Triton X-100 as the extracting agent is its high critical point (above 65 °C) with the consequent loss of

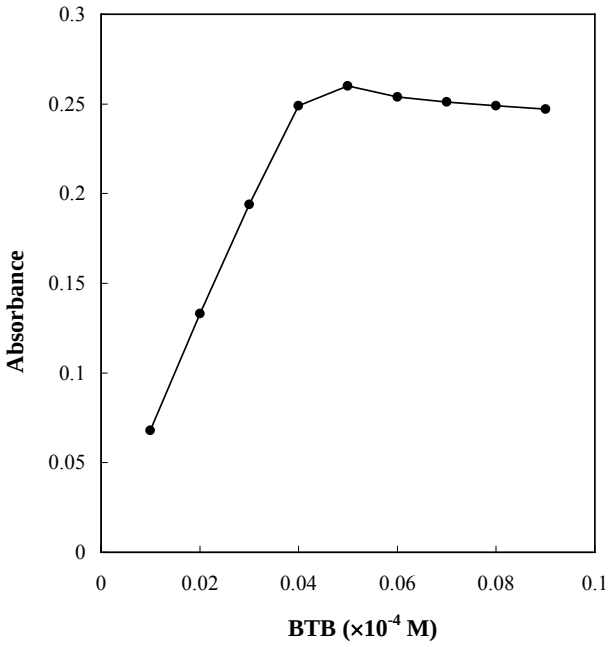

Fig. 5. Effect of BTB concentration on the absorption of the complex. Conditions: 0.2% (v/v) Triton X-114, pH 4.5 and 400 ng ml^{-1} of Codeine.

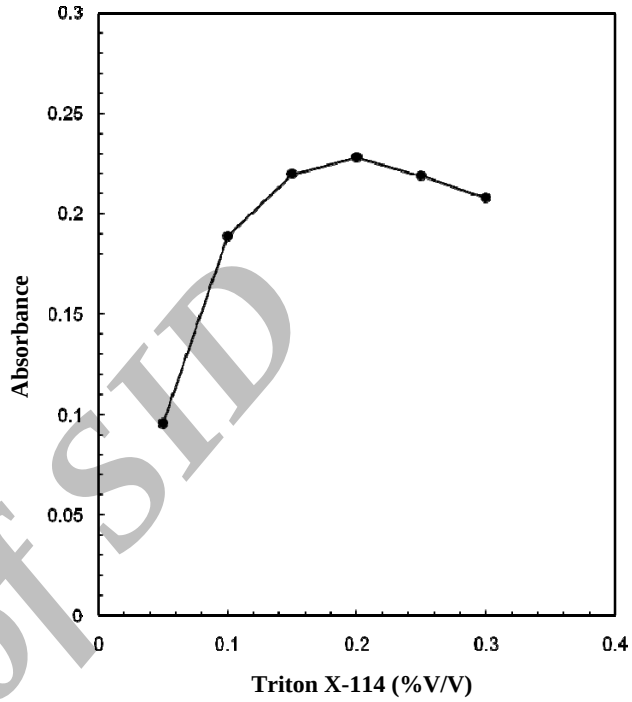

Fig. 6. Effect of Triton X-114 concentration on the absorption of the complex. Conditions: 5.0×10^{-6} M BTB, pH 4.5 and 400 ng ml^{-1} of codeine.

extraction efficiency during the centrifugation/phase separation step.

It is important to investigate the effect of surfactant concentration on CPE. There is a narrow range within which easy phase separation, maximum extraction efficiency and analytical signal are accomplished. Increasingly, outside this optimal range, the analytical signal is observed to deteriorate due to the increase in the final volume of the surfactant that causes the preconcentration factor to decrease. Hence, the variation on extraction efficiency was studied within the surfactant concentration range: 0.05.0.3% (v/v). As is shown in Fig. 6, the best result was found to be at 0.2% v/v of triton X-114 which was chosen in all stages.

Effect of Added Electrolyte

The cloud point of micellar solutions can be controlled by the addition of salts, alcohols, non-ionic surfactants and some organic compounds (salting-out effect). To date, most of studies conducted have revealed that ionic strength has no appreciable effect on the extraction efficiency. An increase in the ionic strength in the CPE does not seriously alter the efficiency of extraction of the chemical forms. Moreover, the addition of a salt can enhance phase separation process [29]. We observed that the addition of NaCl and $NaNO_3$ within the interval of 0.01-0.03 Mhad no significant effect on the efficiency. It is not necessary to use additional NaCl or $NaNO_3$ for salting effect because the buffer would have two roles in such a case, one as part of buffering solution for pH control, and the other as salting-out effect.

Effect of the Equilibration Temperature and Time

The recovery percentage depends on the time that the analytes have to interact with the micelles and get into their core. It has also been demonstrated that the analyte preconcentration factor and percent recovery in the CPE process increase as the equilibration temperature for phase separation is progressively increased to above that of the cloud-point temperature of the system. Similarly, an increase in the cloud-point temperature leads to a slight decrease in the

volume of the surfactant-rich phase. As the temperature, or the equilibration time, increases, the amount of water in a surfactant-rich phase decreases, and hence, the volume of that phase decreases [30]. Therefore, the effect of equilibration temperature and time were investigated. The results showed that a temperature of 40 °C is adequate for the proposed CPE method. The dependence of extraction efficiency upon incubation time was studied in the range of 5-20 min. An incubation time of 15 min was optimal for the quantitative extraction.

Analytical Performance

Table 1 shows the analytical characteristics of the method. Calibration graph was obtained by preconcentrating 10 ml of a sample containing given amounts of codeine under the optimized experimental conditions. Under the specified experimental conditions, the calibration curve was linear from 100 to 700 ng ml^{-1}. The detection limit obtained from 3S of blank was 4.6 ng ml^{-1}. The relative standard deviation (R.S.D) for five replicate experiments was 2.15% for 500 ng ml^{-1} codeine.

The preconcentration factor was calculated from the equation:

$$F_C = V_{in}/V_{fin}$$

Where F_C, V_{in}, and V_{fin} are preconcentration factor, initial volume (10 ml sample), and final volume (0.3 ml surfactant volume + 0.3 ml ethanol). Hence, the preconcentration factor was found to be 16.66.

Table 1. Analytical Characteristics of the Proposed Method

Linear range (ng ml^{-1})	100-700
Calibration equation (µg ml^{-1})	A = 0.613C + 0.016
Correlation coefficient (*r*)	0.998 (n = 7)
%RSD (500 ng ml^{-1})	2.1
LOD (ng ml^{-1})	4.6 (±0.1)
Preconcentration factor	16.7 (±0.3)

Analytical Applications

In order to validate the accuracy and precision of the proposed CPE method under the optimum conditions, acetaminophen codeine tablet, human blood serum and spiked samples were tested. The results are shown in Table 2. The amount of codeine in a tablet was found to be 19.6 (±0.5) mg/tablet by the proposed method. The results were evaluated by standard addition method and were in good agreement with those reported by Alborz Daroo Company (20 ± 1 mg /tablet) that was obtained by HPLC method. As shown in Table 2, in all cases the spiked recoveries were satisfactory, showing no obvious matrix interferences.

Table 2. Determination of Codeine in Acetaminophen Codeine Tablet and Human Serum Samples

Sample	Added (µg)	Found (µg)	Recovery of added amount (%)
Acetaminophen codeine tablet[a]	-	2.2 (±0.2)[b]	-
	0.5	2.7 (±0.1)	100.0
	1.0	3.1 (±0.2)	90.0
	2.0	4.0 (±0.3)	90.0
Blood sample	-	ND[c]	-
	3.0[d]	2.9 (±0.2)	96.7
	5.0	5.1 (±0.2)	102.0
	7.0	6.9 (±0.2)	98.6

[a]The first solution contains 6 µl of the tablet solution which was prepared as discussed in section 2-4. [b]Values in parenthesis are standard deviation for 3 replicated results. [c]Not detect. [d]3 µg codeine phosphate was added to 1ml blood serum.

CONCLUSIONS

In this paper, we introduced a novel and sensitive cloud point extraction procedure as a rapid, safe and inexpensive method for the extraction, preconcentration, and determination of codeine spectrophotometrically. The method validation yielded good results and included linearity, repeatability/ reproducibility, sensitivity, recovery and accuracy. Triton X-114 was chosen for the formation of surfactant rich phase due to its low cloud point temperature, almost at room temperature. The proposed method can be applied to the determination of codeine in acetaminophen codeine tablet and human blood serum.

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