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Application of Mixed Micelle-Mediated Extraction for Selective Separation and Spectrophotometric Determination of *P*-Aminophenol Impurity in Pharmaceutical Formulations

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A procedure for separation/preconcentration of *para*-aminophenol (PAP) in pharmaceutical formulation, based on cloud point extraction (CPE) as a prior step to determination by UV-Vis spectrophotometry, has been developed. The analyte reacted with 4-dimethylamino benzaldehyde (DAB) in acidic media to form a shift base, which were separated and preconcentrated in a mixed micelle of the anionic surfactant sodium dodecylsulfate (SDS) and the nonionic surfactant Triton X-114. The parameters affecting the extraction efficiency of the proposed method, such as acid concentration, reagent concentration, amount of surfactants, and incubation time were optimized. Under the optimum conditions, the linear range was 50-1000 ng ml^{-1} of PAP with a correlation coefficient (*r*) of 0.998. The limit of detection was 30 ng ml^{-1}. Relative standard deviation (*RSD*) for the five replicate determinations of 200 ng ml^{-1} PAP was 1.4%. In this work, the concentration factor of 10 was reached. Also the improvement factor of the proposed method was 3.3. The proposed method was successfully applied for determination of *p*-aminophenol in pharmaceutical formulations. The advantages of this method are simplicity of operation, rapidity and low cost.

Keywords: *Para*-aminophenol, Cloud point extraction, Preconcentration, Separation, Pharmaceutical formulation, Spectrophotometric determination

INTRODUCTION

Acetaminophen (N-acetyl-*p*-aminophenol or paracetamol) is a widely used analgesic drug formulated in a variety of dosage forms *e.g.* tablets, syrups and suspensions, both in the single-ingredient and multi-component ones. It is accepted as a very effective treatment for the relief of pain and fever in a variety of patients including children, pregnant women, and the elderly [1]. Paracetamol is a *p*-aminophenol derivative that is synthesized by acetylation of *p*-aminophenol and acetic anhydride. *p*-Aminophenol is the main impurity in paracetamol preparations that may be formed during the storage in some conditions such as high temperature, acidic or basic media, or may be originated during the synthesis of paracetamol [2]. It was reported that *p*-aminophenol may cause nephrotoxicity and teratogenicity; therefore, its amount should be strictly controlled [3]. The United States and British pharmacopeia (BP) have limited the amount of *p*-aminophenol in paracetamol substance at 0.005% (w/w). The limits of *p*-aminophenol may vary in different products depending on the dosage forms and formulations. The monograph of paracetamol tablets in BP, *p*-aminophenol, is limited to 0.1% (w/w), while no impurity testing method is presented in USP31 [4,5]. The BP uses a spectrophotometric assay method for the determination of paracetamol tablets. The USP31 recommends methods for paracetamol determination using HPLC method. All of these

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methods determine only the amount of paracetamol, none of them includes degradation product determination.

Generally, separation/preconcentration procedures based on cloud point extraction (CPE) have attracted considerable attention mainly because it is in agreement with the principles of the "green chemistry". CPE is a green method for the following reasons: (i) it uses the extractor media diluted solutions of the surfactants that are inexpensive, resulting in the economy of reagents and generations of few laboratory residues, and (ii) surfactants are not toxic, not volatile, and not easily flammable [6]. Micelle-mediated extraction procedures have found wide applications in different areas of analytical chemistry, and their advantages over the conventional liquid-liquid extraction technique have been well documented in the literature [7-9]. The formation of micelles consists of the aggregation of a certain number of surfactant monomers [10]. Aqueous solutions containing a non-ionic or zwitterionic surfactant above its critical micellar concentration (CMC) become turbid, because the surfactant molecules associate spontaneously, forming aggregates of colloidal dimensions [11]. Any species that originally present associates and binds to these micellar aggregates can be extracted from the initial solution and preconcentrated in a small volume of the surfactant-rich phase. The clouding phenomenon can be induced by changing the temperature, additive content, or pressure by which results in the separation of a single isotropic micellar phase into two isotropic phases: (i) a surfactant-rich phase of small volume composed mainly of surfactant, and (ii) an aqueous phase containing surfactant with the concentration level near to CMC [9].

This work is mainly focused on the suitability of CPE combined with UV-Vis spectrophotometry for determination of PAP. The influence of the different experimental parameters on the reaction and extraction-steps are discussed. To evaluate the applicability of the proposed method, it was then applied for the determination of PAP in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

A Lambda 25 model UV-Vis spectrophotometer with 1-cm quartz cells (1.0 ml) was used for recording absorbance spectra. A centrifuge with 10 ml calibrated centrifuge tubes (Hettich, Germany) was used to accelerate the phase separation process.

Chemicals and Reagents

All the solutions were prepared using reagent grade substances and triply distilled water. The surfactants, Triton X-114 and sodium dodecyl sulfate (SDS) (Merck) were used without further purification. A standard solution of *p*-aminophenol (100 μg ml^{-1}) was prepared by dissolving 0.100 g of *p*-aminophenol (Merck) in water and diluting to the mark with water in a 100 ml volumetric flask. A 2.0% (w/v) SDS was prepared by dissolving 2.0 g SDS (Merck) in water and diluting to the mark in a 100 ml volumetric flask. A 0.02 M *p*-(dimethylamino) benzaldehyde (DAB) solution was prepared by dissolving 0.298 g DAB in the 2.0% (w/v) SDS and diluting with SDS solution to the mark in a 100 ml volumetric flask. A 0.2 M HCl solution was prepared with diluting concentrated hydrochloric acid.

Cloud Point Extraction Procedure

For the cloud point extraction under optimum conditions, an aliquot of the *p*-aminophenol solution was transferred into a 10 ml Centrifuge tube with screw cap, so that its final concentration would be in the range of 50-1000 ng ml^{-1}, then 2.5 ml of 0.02 M DAB solution and 0.8 ml of 0.2 M HCl solution were added. Then, the solution was left for 5 min under the room temperature until its color turned into yellow. Then, 1.0 ml of 2.0% (w/v) of Triton X-114 solution and 4.0 ml of 20% (w/v) NaCl solution were added and the final volume was brought to the mark with distilled water. A homogeneous solution with yellow color was obtained. The solution separation of the aqueous and surfactant-rich phase were accomplished by centrifugation for 5 min at 3500 rpm. The mixture was cooled in an ice-salt bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily decanted. The surfactant rich phase of this procedure was dissolved and diluted to 1.0 ml with ethanol and transferred to a 1.0-ml quartz cell for absorbance measurement at 450 nm against a blank solution.

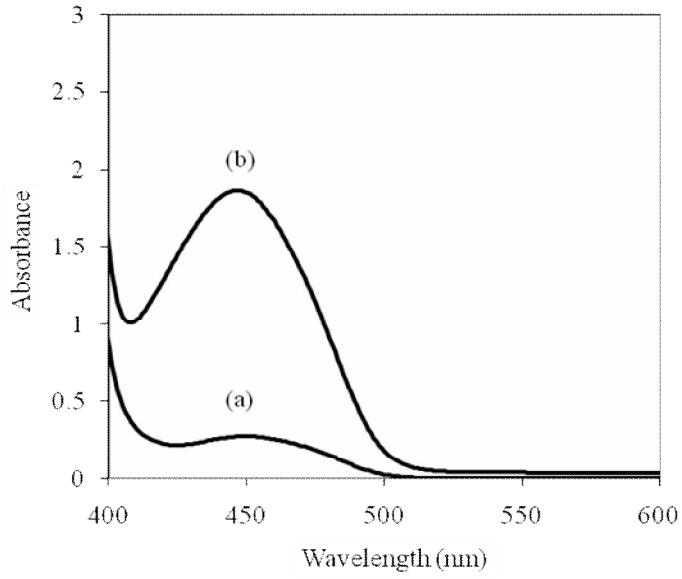


Fig. 1. Absorption spectra of *p*-aminophenol derivative (a) 2000 ng ml^{-1} PAP before CPE (b) 800 ng ml^{-1} *p*-aminophenol after CPE, Conditions: DAB, 5 mM; HCl, 0.16 mM; SDS , 0.2% (w/v); Triton X-114, 0.2% (w/v); NaCl, 8% (w/v).

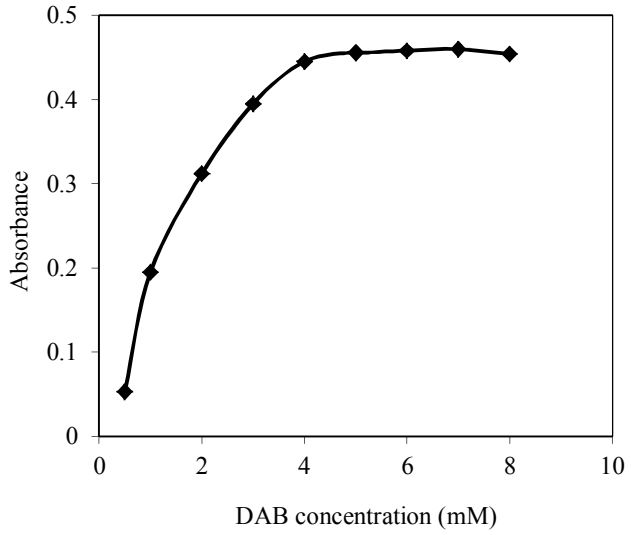
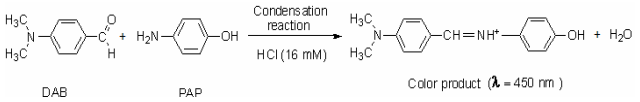


Fig. 2. Effect of DAB concentration on the CPE pre concentration efficiency of the system. Conditions: DAB, 5 mM; HCl, 16 mM; SDS, 0.2% (w/v); Triton X-114, 0.2% (w/v); NaCl, 8% (w/v).

RESULTS AND DISCUSSION

In the SDS micellar media, the condensation reaction of *p*-aminophenol with *p*-(dimethylamino) benzaldehyde (DAB) produces a color product according to stoichiometric equation given below [12]:



The color product shows an absorption spectrum with a maximum absorbance at 450 nm. It was observed that addition of the neutral surfactant Triton X-114 and sodium chloride makes the solution turbid. Therefore, the color product can be extracted by CPE method. After separation of surfactant-rich phase, the absorbance was measured in 450 nm against a reagent blank as the reference (Fig. 1).

Parameters Affecting the CPE

In order to obtain a high sensitive determination, the preliminary experiments were investigated and suitable ranges for the influencing parameters were found.

Effect of the DAB Concentration

The effect of DAB concentration on the absorbance of the system was investigated within the range of 0.50-8.0 mM. The results revealed that the absorbance increases by increasing the reagent concentration up to 5 mM, and does not change at higher concentrations (Fig. 2). Therefore, a concentration of 5 mM DAB was applied in the proposed method.

Effect of the HCl Concentration

The effect of hydrochloric acid concentration on the reaction of *p*-aminophenol with DAB was studied in the range of 2.0-30 mM. As Fig. 3 shows, for the reaction of *p*-aminophenol with DAB the absorbance increases by increasing HCl concentration up to 16 mM and remained nearly constant at higher concentrations. Therefore, 16 mM HCl was used as the optimum concentration.

Effect of the SDS concentration. It was found that cloud point extraction of color product is more efficient in the presence of anionic surfactant, sodium docecyl sulfate (SDS). The color product forms an ion pair with SDS and is extracted into non-ionic surfactants, Triton X-114. Therefore, the effect of SDS concentration on the extraction and determination of p-aminophenol were investigated in the range of 0.03-0.4% (w/v). As Fig. 4 shows, sensitivity of method increases by increasing the SDS concentration up to 0.2% (w/v) and decreases at higher concentrations. Therefore, a concentration of 0.2% (w/v) SDS was selected as optimum.

Effect of Triton X-114 Concentration

The performance of cloud point was observed in the presence of Triton X-114 as a non-ionic surfactant which can affect the extraction of *p*-aminophenol and sensitivity of the method. Therefore, the influence of Triton X-114 concentration in the range of 0-0.32% (w/v) on the CPE was investigated. The results showed that the absorbance of the surfactant-rich phase increases by increasing Triton X-114 concentration up to 0.202% (w/v) and remains nearly constant at higher concentrations. Therefore, 0.2% (w/v) Triton X-114 was used as optimum concentration.

Effect of Electrolyte Concentration

The addition of an electrolyte to aqueous solutions of non-ionic surfactant usually alters the cloud point due to the salting-out effect. Some electrolytes reduce the cloud point temperature while others present a contrary effect. When small amounts of inorganic salts are added to the system, a decrease in the cloud point temperature occurs [13,14]. As pointed out by Gu and Galera-Gomez [15], if the concentration of the added electrolyte is high enough, the cloud points of some mixed systems could be even lower than those of the pure nonionic surfactant solution. In this work, it was observed that the addition of NaCl electrolyte to the Triton X-114/SDS system reduces drastically the cloud point, thus allowing phase separation at room temperature. The effect of NaCl concentration on the absorbance of the system was investigated within the range of 0-12 (%w/v). The results revealed that the absorbance increases by increasing the reagent concentration up to 8 (%w/v), and decreases at higher concentrations (Fig. 6). Therefore, a concentration of 8 (%w/v) NaCl was applied in the proposed method.

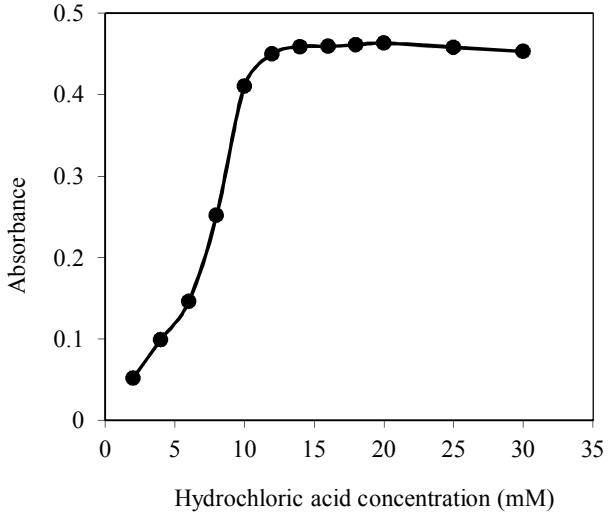


Fig. 3. Effect of hydrochloric acid concentration on the CPE preconcentration efficiency of the system. Conditions: DAB, 5 mM; SDS, 0.2% (w/v); Triton X-114, 0.2% (w/v); NaCl, 8% (w/v).

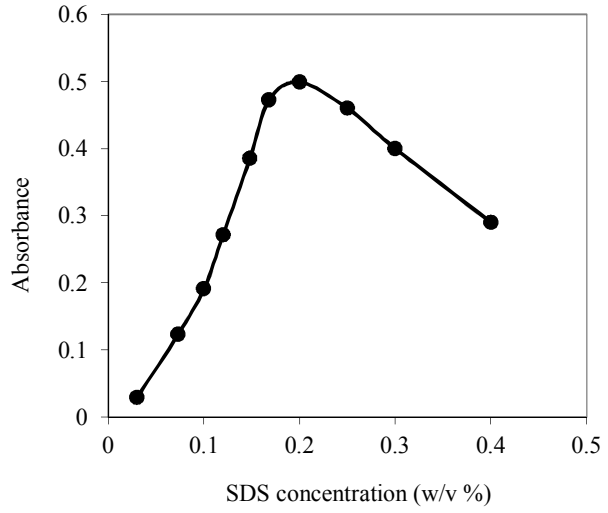


Fig. 4. Effect of SDS concentration on the CPE pre concentration efficiency of the system. Conditions: DAB, 5 mM; HCl, 16 mM; Triton X-114, 0.2% (w/v); NaCl, 8% (w/v).

Effects of equilibration temperature and incubation time on the extraction performance. Optimal incubation time and equilibration temperature are necessary to complete the reaction, and to achieve easy phase separation and preconcentration as efficient as possible. The greatest analyte preconcentration factors are thus expected under conditions where the CPE is conducted using equilibration temperatures that are well above the cloud point temperature of the surfactant. It was desirable to employ the shortest equilibration time and the lowest possible

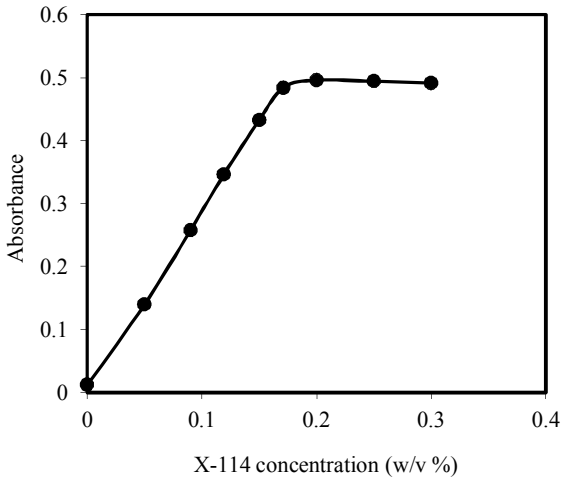


Fig. 5. Effect of the Triton X-114 concentration on the CPE pre concentration performance of the system. Conditions: DAB, 5 mM; HCl, 16 mM; SDS, 0.2% (w/v); NaCl, 8% (w/v).

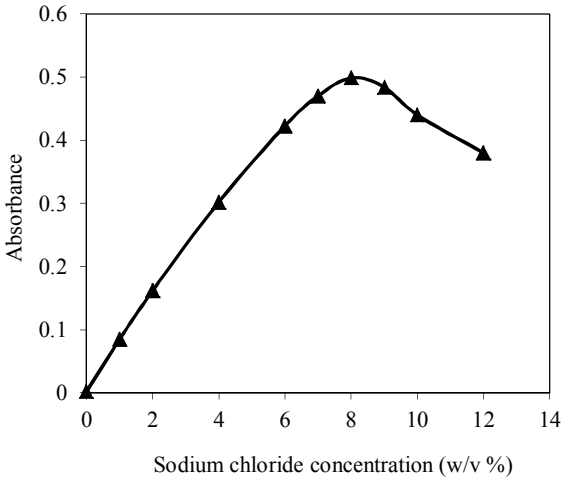


Fig. 6. Effect of NaCl concentration on the CPE pre-concentration performance of the system. Conditions: DAB, 5 mM; HCl, 16 mM; SDS, 0.2% (w/v); Triton X-114, 0.2% (w/v).

equilibration temperature, which compromise completion of the reaction and efficient separation of phases. Therefore, the effect of equilibration temperature in the range of 20-70 °C was studied. It was found that 25 °C is adequate for the quantitative analysis. The dependence of absorbance upon equilibration time was also studied within the range of 30-60 min. Five min was chosen as optimal time for equilibration and incubation.

Analytical Properties of Merit

From the measurements made under the optimum conditions described above, the calibration graph was found

Table 1. Determination of *p*-aminophenol in Pharmaceutical Formulations by the Proposed Method[a]

Samples	*p*-aminophenol (ng ml^{-1})		
	Spiked	Found	Recovery (%)
Paracetamol	0.0	Not detected	-
	200	198 ± 1.14	99.0
	400	408 ± 3.21	102
Acetaminophen	0.0	Not detected	-
Codeine	200	205 ± 2.41	102.5
	400	391 ± 3.66	97.8
Novafen	0.0	Not detected	-
	200	196 ± 2.03	98
	400	389 ± 4.18	97.3
Coldox	0.0	Not detected	96
	200	207 ± 3.51	103.5
	400	388 ± 2.98	97.0
	0.0	Not detected	-
Acetaminophen	200	193 ± 2.78	103.5
Syrup	400	409 ± 1.98	103

[a]Average of three determinations.

to be linear in the range of 50-1000 ng ml^{-1}. The calibration equation is A = 0.00197C + 0.065 with a correlation coefficient of 0.998 (n = 10), where *A* is the absorbance and *C* is the concentration of *p*-Aminophenol in the initial sample solution in ng ml^{-1}. Detection limit based on three times of the standard deviation of the blank ($3s_B$) was 30 ng ml^{-1} and the relative standard deviation (R.S.D.) for 200 ng ml^{-1} of *p*-aminophenol was 1.4% (n = 5). The solution was concentrated by a factor of 10, because the amount of *p*-Aminophenol in 10 ml of sample solution is measured after preconcentration by CPE in a final volume of nearly 1 ml. The enhancement factor, defined as the ratio of the slope of the calibration graph for the CPE method to that of the slope of the calibration graph in micellar media without preconcentration, was 3.3.

Selectivity

In order to assess the possible analytical applications of the proposed CPE method, the effect of some common excipients frequently found along with PAP in drug samples such as paracetamol, 4-nitrophenol, caffeine, diphenhydramine hydrochloride, ibuprofen, phenylephrine,

Table 2. Comparison of the Performance of the Proposed Method with that of the other Reported Methods for Determination of *p*-aminophenol

Analytical method	Sample matrix	Linear range ($\mu g\ ml^{-1}$)	Detection limit ($\mu g\ ml^{-1}$)	Ref.
Colorimetric	Urinary	2-100	0.9	[16]
Sequential spectrophotometric	Pharmaceutical products	0.44-5.5	0.09	[17]
Automated spectrophotometric assay	Urine	20 -400	≤10	[18]
conventional spectrophotometry	Urine	1.5-12	40	[19]
High-performance liquid chromatographic (HPLC)	Multicomponent analgesic preparations	-	1	[20]
Carbon ionic liquid Electrode	Pharmaceuticals	0.076-109	0.01	[21]
Differential pulse Voltammetric	Urine Samples	0.02-10	0.037	[22]
Cloud point extraction-spectrophotometry	Pharmaceuticals	0.05-1.0	0.03	Proposed method

and some of cations and anions such as K^+, Ca^{2+} ,Na^+, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, SO_4^{2-}, NO_3^-, PO_4^{3-}, CO_3^{2-} were studied by analyzing synthetic sample solutions containing 200 ng ml^{-1} of PAP. A compound was considered as an interferent when it caused a variation greater than ±5% in the absorbance of the sample. The results indicated the investigated ions did not interfere even when present in 50-fold excess over the analyte. This shows the good selectivity of the proposed method.

Application of the Proposed Method

The extraction procedure was applied to the determination of *p*-aminophenol, using the selected experimental conditions and 10 ml of preconcentrated micellar solution. The accuracy of the proposed procedure was investigated through the recovery experiments in water samples, adding known masses of analyte to the analyzed matrices. The analyses were performed in triplicate, and as seen in Table 1, the proposed method was not susceptible to the matrix effects, providing recoveries between 97.0% and 107%.

CONCLUSIONS

This study proposes the use of CPE as a method for extraction and preconcentration of *p*-aminophenol impurity

in pharmaceutical formulation as a prior step to its determination by spectrophotometry. The extent of extraction is markedly influenced by using the mixture of anionic surfactant SDS and non-ionic surfactant, Triton X-114 as a mixed micelle. The CPE using mixed micelle is versatile, simple and provides good enrichment factors and efficient separation. Further, in comparison to solvent extraction methods, it is much safer, since only a small amount of the surfactant, which has a low toxicity, is used. The limit of detection of the method (LOD) is better or comparable to some of the previously reported techniques. A comparison of the results is given in Table 2. The results of this study clearly show the potential and versatility of this method, which could be applied to monitoring the *p*-aminophenol impurity in pharmaceutical formulation such as acetaminophen codeine, Novafen, Coldax tablet and acetaminophen syrup.

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