International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 4, 2022

Short Communication

DETERMINATION OF AURINTRICARBOXYLIC ACID (A VIRAL INHIBITOR) USING MIXED MICELLAR CLOUD POINT EXTRACTION PROCEDURES

RADHA KRISHNA DIMMITI, AMEER KHAN SHAIK, SHYAMALA PULIPAKA*

Department of Physical and Nuclear Chemistry and Chemical Oceanography, School of Chemistry, Andhra University, Visakhapatnam 530003, Andhra Pradesh, India

***Email: dr.pshyamala@andhrauniversity.edu.in**

Received: 22 Jan 2022, Revised and Accepted: 11 Apr 2022

ABSTRACT

Objective: Development of micelle mediated cloud point extraction (CPE) and preconcentration of aurintricarboxylic acid (ATCA) (a viral inhibitor).

Methods: The method is based on the ion-pair formation between ATCA and a cationic surfactant tetrabutylammonium bromide (TBAB), which is extracted into the Triton X-114 (TX-114, a non-ionic surfactant) at a pH 4.4. The effect of different important parameters like pH, the concentration of surfactants (TX-114 and TBAB), salt, temperature, and time for the extraction of ATCA was optimized.

Results: Extraction efficiency of 90.28 % for CPE of ATCA was obtained using mixed micelles of TX-114 and TBAB. The linear range and limits of detection for ATCA was found to be 8.44-84.47 µg ml^{-1} and 8.14 ng $ml^{-1,}$ respectively.

Conclusion: The suggested CPE method has been applied to the determination of ATCA in aqueous solutions.

Keywords: Cloud point extraction (CPE), Aurintricarboxylic acid (ATCA), Ion-pair, Triton X-114 (TX-114)

DOI: https://dx.doi.org/10.22159/ijap.2022v14i4.44206. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

Aurintricarboxylic acid (ATCA) is an anionic aromatic compound and potent inhibitor of protein-nucleic acid interactions [1]. According to molecular docking-based virtual screening [2] shows that ATCA inhibits the replications of viruses such as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), causing severe acute respiratory syndrome [3, 4]. ATCA has wide applications in pharmaceutical industries and determination of ATCA in trace levels present in various samples like natural and wastewater, and biological samples is important. The direct determination of ATCA using various instrumental methods is difficult due to matrix effects and low concentrations. Out of various instrumental techniques, the spectrophotometry method is featured by its speed, simplicity, accuracy and inexpensiveness [5]. In this context, the following green chemistry technique, such as cloud point extraction (CPE) is used with spectrophotometry. CPE is an excellent pre-concentration technique based on the separation of two phases was obtained by heating of non-ionic surfactants to a particular temperature called cloud point temperature (CPT). The main advantages of CPE are; utilizing of low cost, non-toxic and non-flammable solvents and compliance with green chemistry principles [6]. Percentage recovery of dye increases by using mixed micelles (non-ionic surfactant with cationic or anionic) when compared to single micelles [7]. In this work, a simple CPE method has been developed for the pre-concentration of ATCA prior to spectrophotometric determination using Triton X-114(TX-114) and tetrabutylammonium bromide (TBAB) mixed micelles.

Stock solution of ATCA, Triton X-114, TBAB and sodium chloride (NaCl) was prepared in double-distilled water.

In a 15 ml centrifuge tube, pH 4.4 corresponds to an acetate buffer, different concentrations of ATCA (8.44-84.47 µg ml^{-1}), 2.2 % (w/v) of TX–114, 0.4 % (w/v) of TBAB and 1.8 % (w/v) of NaCl were added. The solution was heated at 70 °C for 20 min so that the CPT was obtained and separation of two phases (aqueous phase and surfactant phase) was obtained. The two phases were separated by centrifugation for 5 min at 3000 rpm followed by cooling for fifteen minutes. 20 % methanol was used to dissolve the surfactant phase, which contains ATCA. The amount of ATCA was then determined by UV-VIS 1800 (Shimadzu) spectrophotometer at a wavelength of 522 nm, which is the wavelength of maximum absorbance for ATCA.

Various analytical parameters affecting the CPE of ATCA are pH; TX-114, TBAB, NaCl, temperature and time were optimized (shown in fig. 1(a), (b), (c) and (d)).

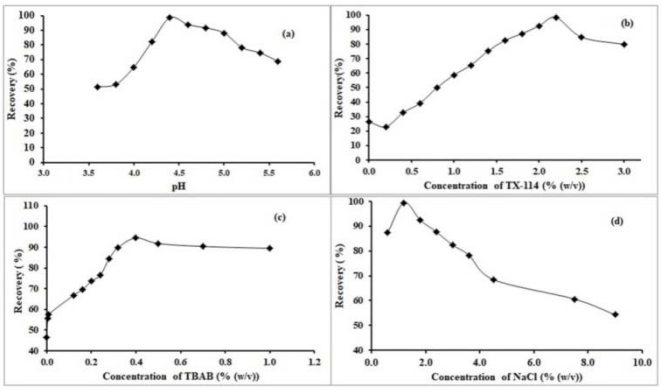

Fig. 1: Optimized parameters affecting CPE of ATCA (a) pH (b) Concentration of TX-114 (c) Concentration of TBAB (d) Concentration of NaCl (Experimental conditions: Concentration of TX-114 is 2.2 % (w/v), concentration of TBAB is 0.4 % (w/v), concentration of NaCl is 1.8 % (w/v), temperature is 70 °C and time is 20 min)

The pH is evaluated as critical parameter for regulating the partition of the analyte in the surfactant phase, which controls the extraction efficiency. Generally non-ionic surfactants were used in CPE because they have a high interfacial activity and are composed of both polar and nonpolar regions [8]. Here TX-114 (non-ionic surfactant) was used for the development of the surfactant phase due to its low CPT (23 °C-26 °C) near to room temperature, which makes easy phase separation. To enhance the percentage of recovery of dye, TBAB was used along with TX-114 so that ATCA forms an ion pair complex with TBAB and the obtained hydrophobic species, which can be easily extracted into TX-114. Since the addition of TBAB will increases the CPT to 90-100 °C. Generally, a salting-out agent(NaCl) was added to the mixture of TX–114 and TBAB decreases the CPT to 50 °C. The optimum conditions obtained are shown in table 1.

Table 1: Optimized parameters affecting CPE of ATCA*

Parameter	Optimized range	Optimum value
pH	3.6-5.2	4.4 (Acetate buffer)
TX-114 % (w/v)	0.2-4.0	2.2
TBAB % (w/v)	0.0-1.2	0.4
NaCl % (w/v)	0.0–6.0	1.8
Temperature (° C)	30-100	70
Time (min)	5-60	20

*n=3

Under optimum conditions, linear calibration curves were in the range of 8.44-84.47 µg ml^{-1} and the limits of detection was found to be 8.14 ng ml^{-1} (n=3). The equation for the calibration curve is A=0.0117 [ATCA]+0.0383, with a correlation coefficient (R^2) is 0.995 (fig. 2). Comparison of the present method with other methods reported for the cloud point extraction of dyes/drugs has been given in table 2.

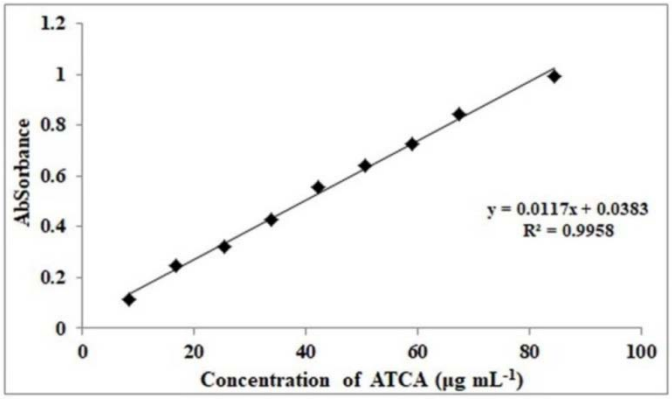

Fig. 2: Calibration graph for ATCA under optimized conditions

Table 2: Comparison of the present method with other methods for the determination of dyes/drugs after mixed micellar CPE

Micellar system	Dyes/Drugs	Matrix	Reference
CPC and TX-114	Ponceau 4R	Beverage samples	[9]
SDS and PONPE 7.5	Ofloxacin and gatifloxacin	spiked plasma, spiked urine, and urine samples	[10]
CTAB and TX-114	CFD-2,5-DMP	Pharmaceutical samples	[11]
CTAB and TX-100	Erythrosine, Quinoline yellow and indigo carmine	Pharmaceutical samples	[12]
TBAB and TX-114	Aurintricarboxylic acid	Tap water and seawater sample	Present work

The proposed CPE technique was effectively used to determine the ATCA in different water samples yielding 83.6-91.8 % recoveries. The results are summarized and shown in table 3.

Table 3: Recoveries of ATCA in different water samples and their spike recoveries using CPE method*

Samples	Taken (µg ml^{-1})	Found (µg ml^{-1})	Recovery (%)[b]
Tap water	–	ND[a]	–
	25.34	23.19	91.5±0.22
	59.12	49.43	83.6±0.14
Seawater	–	ND[a]	–
	25.34	23.27	91.8±0.66
	59.12	52.89	89.4±1.42

*n=3, [a]Not detected, [b]mean±standard deviation

In conclusion, the developed cloud extraction method combined with spectrophotometry shows that it is a sensitive, selective, economical and accurate method for the determination of ATCA in trace levels. Extraction efficiency of 90.28 % for CPE of ATCA was

obtained using mixed micelles of TX-114 and TBAB. The results show that the suggested CPE method has been successfully applied to the determination of ATCA in aqueous solutions.

ACKNOWLEDGMENT

SAK is thankful to the University Grants Commission (UGC), India, for financial support under the senior research fellowship (SRF) scheme (Ref. No. 30-09-2015-118875).

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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