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Cloud point extraction and spectrophotometry in the determination of As(III) using amaranth in water samples of rivers located in industrial and non-industrial areas

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Cloud-point extraction (CPE) method was employed for the extraction of trace quantities of arsenic(III) (As(III)) from various water samples using spectrophotometry. For this mixed micelle-mediated extraction, amaranth, Triton X-114, cetyl trimethylammonium bromide (CTAB) and NaCl were applied as chelating, sensitizing agent, extraction and co-extraction agents respectively. The various effective parameters on CPE, including pH, type of surfactants and electrolytes, concentration of chelating agent, surfactant, sensitizing agent (CTAB), electrolyte, temperature and duration were investigated. Additionally, analytical performance was estimated (limit of detection, recovery, sensibility and relative standard deviation (RSD) and linear range). A linear calibration curve in the range

30–1500 $\mu\text{g l}^{-1}$ of amaranth was acquired. Under the optimized conditions, the limit of detection (LOD) was 2.8 $\mu\text{g l}^{-1}$ and RSD for 300 and 600 $\mu\text{g l}^{-1}$ was 2.23 and 1.73 respectively ($n = 10$). LOD was found to be 2.8 $\mu\text{g l}^{-1}$. The regression equation acquired by the least square method is $A = 2.05 \times 10^{-3} C_{\text{As}} + 1.21 \times 10^{-2}$ for 30–1500 $\mu\text{g l}^{-1}$ of As(III) with a correlation coefficient of 0.997 ($n = 12$), where A is the absorbance and C_{As} is the concentration of As(III) ($\mu\text{g l}^{-1}$). The results indicate that the As(III) quantity in water samples of the rivers located in industrial areas is considerably higher than that in the non-industrial areas.

Keywords: Amaranth, arsenic, cloud point extraction, spectrophotometry.

ARSENIC (As) is ranked first among the hazardous elements and has serious effects on plants, animals and human health¹. As contamination is a matter of concern world over, especially in India and Bangladesh². Millions of people worldwide are exposed to As in their drinking water and ingested As is an established cause of bladder, lung and skin cancer. In addition to cancer, As in water has also been associated with cardiovascular disease, skin lesions, diabetes, reproductive disorders, cognitive defects in children and other health effects³. Data indicate that As is cytotoxic and genotoxic to human lung primary cells. Reactive oxygen species (ROS) generation associated with As exposure is known to play a fundamental role in the induction of adverse health effects and diseases (cancer, diabetes, hypertension, cardiovascular and neurological diseases)⁴. Generally, the inorganic species of As are more toxic than the organic forms; the toxicity of As(III) is 60 times greater than As(V)⁵. In view of these, several researchers have devised various methods measuring of As^{6–13}.

In analytical chemistry, azo dyes play an important role as organic complexing reagents. These dyes are considered as tridentate ligands and form chelates with metal ions through oxygen atom of the ortho-hydroxyl group, nitrogen atom from pyridine, and one of nitrogen atoms of the azo group, giving two five-membered chelate rings. The complexes are stable with rather limited solubility in aqueous solution but much greater solubility in organic system. Because of their non-selectivity, azo dyes have been widely applied in different areas of analytical chemistry such as spectrophotometry, solid phase extraction (SPE), electrochemistry, liquid–liquid extraction and CPE¹⁴.

In the last decade, there has been increasing interest in the use of aqueous micellar solution in the field of separation science¹⁵. The cloud point extraction (CPE) technique has also been applied as a procedure for determination and removal of dyes and pigments as well as analysing metals^{16,17}. This simple technique enables us to refrain from hazardous organic solvents and allows us to reach a much higher concentration of analyte than in the case of

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liquid–liquid extraction, because the micellar phase volume is about 10–100-fold less than the volume of an aqueous phase¹⁸. The proposed method has advantages of being novel, with good selectivity, accuracy and limit of detection and also high recovery. It has been applied to the pre-concentration and determination of trace As(III) in tap water, river water (rivers located in industrial and non-industrial areas), well water and lake water with satisfactory results.

In the present study, absorption spectra and absorbance measurements were made with a Shimadzu UV-1800, UV–vis spectrophotometer using 1 cm quartz cells (1 ml). A Metrohm digital pH meter (model 691) with a combined glass electrode was applied to measure pH values. A Hettich universal 320 centrifuge was used to hasten the phase separation.

All reagents applied in the study were of analytical grade or better. The molecular structure of amaranth is shown in Figure 1. As standard working solutions were prepared from the 1000 mg l⁻¹ As(III) standard stock solution by dissolving appropriate amount of As₂O₃ (Merck, Darmstadt, Germany) in 3 M NaOH and pH 7.0 with 5 M HCl solution. The stock solution of 1000 mg l⁻¹ As(V) was prepared by dissolving 4.164 g of Na₂HAsO₄ (Merck) in water. Then 1 × 10⁻⁴ mol l⁻¹ of amaranth (Merck) solution was obtained by dissolving 0.006 g of amaranth in 100 ml water. Triton X-114 stock solution (3%, v/v) was prepared by dissolving 3 ml of concentrated solution (Sigma-Aldrich, Steinheim, Germany) in 100 ml double distilled water. Then 1 × 10⁻³ mol l⁻¹ of cetyl trimethylammonium bromide (CTAB) solution was prepared by dissolving 0.5 ml of this reagent (Merck) in bidistilled water and diluting to the mark in a 100 ml volumetric flask. The McIlvaine's buffer solution was provided by dissolving 7.71 ml of 0.2 M Na₂HPO₄ in 12.29 ml of 0.1 M citric acid. Stock solution of NaCl (1 × 10⁻¹) was prepared by dissolving 0.584 g NaCl in distilled water and diluting to 100 ml in a flask.

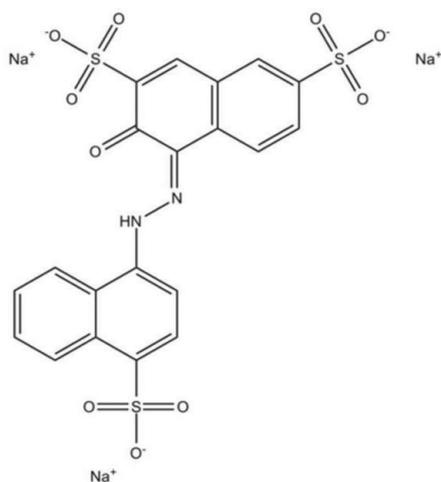


Figure 1. Molecular structure of Amaranth (E102).

In a typical CPE process, the standard solution containing As(III) (in the range 30–1500 µg l⁻¹), 2.25 ml of amaranth (1 × 10⁻⁴ mol l⁻¹), 1.5 ml of 3% (v/v) Triton X-114, 0.75 ml of 1 × 10⁻³ mol l⁻¹ CTAB, 1.5 ml of 0.1 mol l⁻¹ NaCl and 1.75 ml of McIlvaine's buffer (pH = 4) was transferred into a 15 ml tube and equilibrated at 60°C in a thermostat bath for 20 min. The separation into two phases was accelerated by centrifuging at 3500 rpm for 15 min. The contents of the tubes were cooled in an ice-bath, the surfactant-rich phase became viscous, and the upper aqueous phase was decanted. The surfactant-rich phase was then dissolved and diluted to 1 ml using methanol and transferred into a quartz cell. The absorbance spectrum of the solution was measured at 510 nm. A blank solution was prepared in the same way, except that distilled water was used instead of As(III). It was also submitted to the same course of action and its spectrum was measured.

Amaranth is an anionic dye that can form a complex with both As(III) and As(V) at pH 4, but As(III)–amaranth complex is more appropriate for spectrophotometric measurements due to formation of more stable ion-pair complex. The absorbance signal obtained from As(III)–amaranth complex is higher than that of As(V)–amaranth complex. Thus, As(III) was selected for further studies. Sodium thiosulphate (Na₂S₂O₃) is one of the most convenient and reliable reducing agents reported, which allows quick and complete reduction of As(V) to As(III) at room temperature without any interference of excess amount of thiosulphate in the As determination step¹⁰. After reduction of As(V) to As(III) with thiosulphate at fixed concentration of 15 mg l⁻¹, pH of the solution was adjusted to about 7 using 1 M NaOH and the proposed method was used for the determination of total As. The level of As(V) was calculated by the difference between total for As and As(III) concentrations. Appropriate amounts of water samples, including river water located in industrial (Karoon river, Ahvaz city) and non-industrial (Zohreh river, Gachsaran city) areas, well water, lake water and tap water were filtered through a 0.22 µm membrane to remove the suspended and floating particles.

In this study, As(III) ion interacted with amaranth, an anionic dye, which led to the formation of As–amaranth complex. This complex was extracted by mixed-micelle-mediated extraction through both cationic (CTAB) and non-ionic (Triton X-114) surfactants. The absorption spectra of As–amaranth complex showed a maximum absorption band at 510 nm. Therefore, all the measurements were carried out at this wavelength. To obtain the maximum absorbance and sensitivity, the important factors affecting CPE were optimized and a set of optimum conditions was established.

In the CPE method, pH plays a critical role on metal complex formation and subsequent extraction procedures. Therefore, in order to obtain favourable pre-concentration efficiencies, pH values were studied in the range 1.0–8.0.

For this, McIlvaine's buffer solution was used to adjust pH range¹⁹. The highest absorbance was obtained at pH 4 (Figure 2). At lower and higher pH values, the extraction yield is very low due to slow or incomplete complex formation. Thus, pH 4 was selected for further experiments by adding 1.75 ml of McIlvaine's buffer solution.

Amaranth is a dark red to purple azo dye that can be used as a chelating agent¹⁴. For studying the effect of amaranth on extraction of As, a solution containing As and various amounts of amaranth was used. The extraction yield was highest when $1.5 \times 10^{-5} \text{ mol l}^{-1}$ (2.25 ml) of amaranth was used. Concentrations greater than $1.5 \times 10^{-5} \text{ mol l}^{-1}$ had no effect on the extraction of As. At lower concentrations, the amount of ligand was insufficient to extract all the analyte in the solution. Absorbance increased with increasing the concentration of amaranth due to increase in As(III) and amaranth complex concentration. When all the As(III) ions were used, the process of complex formation was completed and after this it

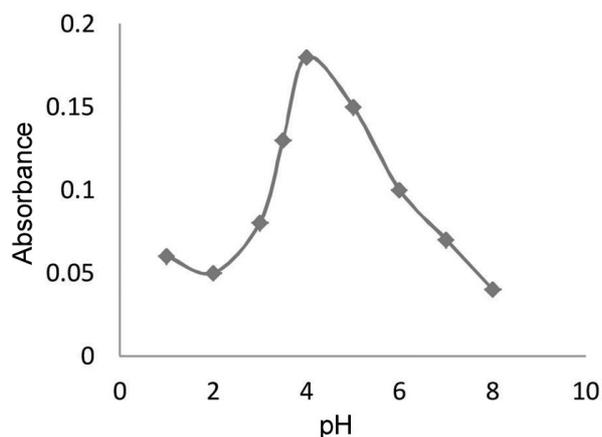


Figure 2. Effect of pH on cloud point extraction (CPE). (Experimental conditions: $1.5 \times 10^{-5} \text{ mol l}^{-1}$ of amaranth; $5 \times 10^{-5} \text{ mol l}^{-1}$ of CTAB; 0.3% (v/v) of Triton X-114; $1 \times 10^{-2} \text{ mol l}^{-1}$ of NaCl.)

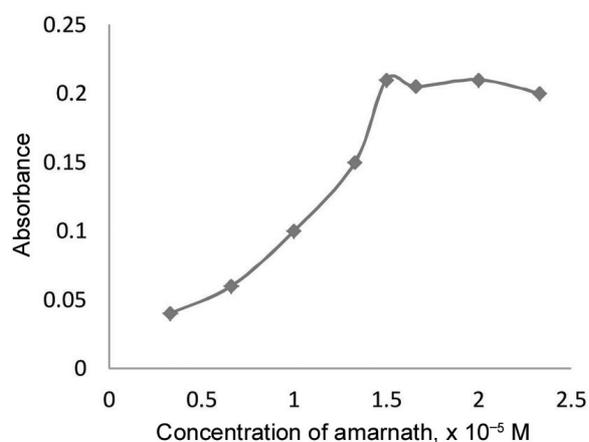


Figure 3. Effect of amaranth concentration on CPE. (Experimental conditions: pH 4; $5 \times 10^{-5} \text{ mol l}^{-1}$ of CTAB; 0.3% (v/v) of Triton X-114; $1 \times 10^{-2} \text{ mol l}^{-1}$ of NaCl.)

remained nearly constant at higher concentrations. In other words, the limiting factor is As concentration. Thus, $1.5 \times 10^{-5} \text{ mol l}^{-1}$ amaranth solution was chosen for subsequent experiments. The changes in absorbance as a function of concentration of amaranth are shown in Figure 3.

The variations of analytical signal as a function of concentration of ionic surfactants, cetyl pyridinium chloride (CPC) and CTAB which were chosen as sensitizing agents are shown in Figure 4. The sensitizing agents are useful to transfer metal–ligand complex from aqueous phase to surfactant-rich phase. The best yield of extraction was obtained in the presence of CTAB. The extraction of As(III) was found to increase up to $5 \times 10^{-5} \text{ mol l}^{-1}$

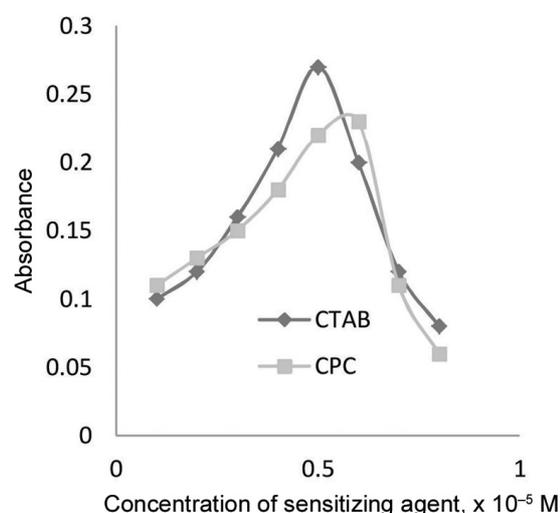


Figure 4. Effect of concentration of sensitizing agent on CPE. (Experimental conditions: pH 4; $1.5 \times 10^{-5} \text{ mol l}^{-1}$ of amaranth; 0.3% (v/v) of Triton X-114; $1 \times 10^{-2} \text{ mol l}^{-1}$ of NaCl.)

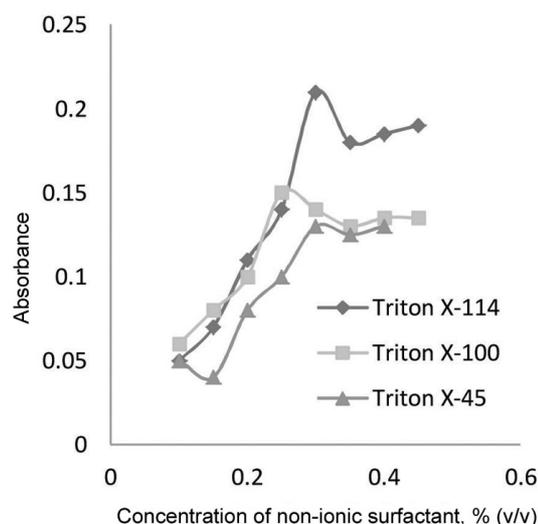


Figure 5. Effect of concentration of non-ionic surfactant on CPE. (Experimental conditions: pH 4; $1.5 \times 10^{-5} \text{ mol l}^{-1}$ of amaranth; $5 \times 10^{-5} \text{ mol l}^{-1}$ of CTAB; $1 \times 10^{-2} \text{ mol l}^{-1}$ of NaCl.)

Table 1. Determination of arsenic in different water samples and recovery tests

Sample	Added ($\mu\text{g l}^{-1}$)		Found ^a ($\mu\text{g l}^{-1}$)			Recovery (%)			Relative standard deviation (%)	
	As(III)	As(V)	As(III)	As(V)	Total As	As(III)	As(V)	Total As	As(III)	As(V)
River water ^b	–	–	55.25 \pm 0.10	29.38 \pm 0.11	84.63 \pm 0.14	–	–	–	1.9	2.4
	50	50	104.51 \pm 0.11	79.00 \pm 0.10	182.90 \pm 0.12	98.81	99.52	99.67	2.8	2.5
	100	100	156.11 \pm 0.070	129.16 \pm 0.07	285.50 \pm 0.30	100.55	99.83	100.08	3.3	2.8
River water ^c	–	–	22.15 \pm 0.13	18.22 \pm 0.10	40.37 \pm 0.07	–	–	–	2.9	3.1
	50	50	72.01 \pm 0.13	68.54 \pm 0.11	140.70 \pm 0.11	99.80	100.47	100.03	2.3	2.4
	100	100	123.70 \pm 0.17	118.11 \pm 0.08	242.15 \pm 0.14	99.90	101.70	100.28	3.5	3.2
Tap water	–	–	10.12 \pm 0.10	7.39 \pm 0.04	17.51 \pm 0.08	–	–	–	3.3	2.9
	50	50	59.64 \pm 0.13	55.55 \pm 0.02	113.19 \pm 0.05	99.20	96.79	98.26	2.5	2.7
	100	100	112.21 \pm 0.11	108.30 \pm 0.06	223.11 \pm 0.06	101.90	100.85	101.18	2.1	2.4
Well water	–	–	25.21 \pm 0.07	15.31 \pm 0.07	40.52 \pm 0.13	–	–	–	3.9	3.6
	50	50	73.25 \pm 0.15	62.61 \pm 0.15	132.86 \pm 0.05	97.39	95.86	97.79	3.8	3.3
	100	100	122.40 \pm 0.06	112.05 \pm 0.12	225.17 \pm 0.05	95.87	96.53	96.04	3.1	3.0
Lake water	–	–	25.16 \pm 0.09	17.01 \pm 0.04	42.17 \pm 0.11	–	–	–	2.5	3.1
	50	50	75.10 \pm 0.11	65.00 \pm 0.12	139.30 \pm 0.40	99.92	97.00	99.43	3.2	2.7
	100	100	123.16 \pm 0.09	115.97 \pm 0.10	237.15 \pm 0.08	98.40	99.11	99.17	3.1	3.5

^a $\bar{x} \pm ts\sqrt{n}$ at 95% confidence ($n = 5$). ^b Karoon river (located in a industrial area), Ahvaz city. ^c Pol-e-Zohreh (located in a non-industrial area), Gachsaran city.

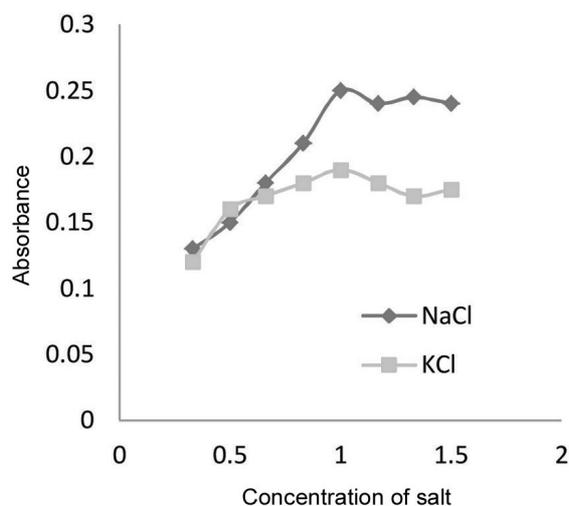


Figure 6. Effect of salt concentration on CPE. (Experimental conditions: pH 4; 1.5×10^{-5} mol l^{-1} of amaranth; 5×10^{-5} mol l^{-1} of CTAB; 0.3% (v/v) of Triton X-114.)

of CTAB concentration and decrease at higher concentrations. Thus, 5×10^{-5} mol l^{-1} of CTAB in the final solution was chosen as the optimum concentration for As(III) extraction process. It must be mentioned here that higher concentrations (more than 5×10^{-5} mol l^{-1}) of CTAB in the final solution cause an increase in the blank absorbance and decrease in the absorbance of the complex.

Three non-ionic surfactants, including Triton X-114, Triton X-100 and Triton X-45 (0.1–0.45% (v/v)) were applied to CPE. Triton X-114 was selected due to its

higher extraction yield. The results are given in Figure 5. As can be seen from Figure 5, the best extraction yield was obtained in the presence of Triton X-114. So, it was chosen for further studies. Triton X-114 was examined in the range 0.1–0.45% (v/v). By increasing the concentration of the surfactant up to 0.3% (v/v), the signal increased, after which it remained nearly constant at higher concentrations. Thus, 0.3% (v/v) Triton X-114 was used as optimum concentration. At lower concentrations of the surfactant, the extraction yield of the complex was slightly lower probably due to inadequacy of assemblies to entrap the hydrophobic complex quantitatively²⁰.

In this study, NaCl and KCl were used as the co-extraction agents. The cloud point of micellar solutions can be controlled by addition of alcohols, salts, non-ionic surfactants and some organic compounds (salting-out effects)²¹. The effect of electrolyte concentration on the extraction of As(III) was examined using various amounts of 0.1 mol l^{-1} NaCl and KCl solutions. The results showed that the absorbance increased with the increase in both NaCl and KCl concentrations, but NaCl had a greater effect on the absorbance of the solution and yield of the extraction. Therefore, it was chosen as the electrolyte for further studies. By increasing the concentration of electrolyte up to 1×10^{-2} mol l^{-1} , the signal increased. Thereafter it remained nearly constant at higher concentrations (Figure 6).

Often it is necessary to pre-concentrate trace amounts of metals or metalloids with high yield within a minimum time. The results demonstrate that centrifuging for 15 min at 3500 rpm leads to the highest extraction of As(III).

Additionally, the influence of time on the extraction of As(III) was surveyed in the range 5–20 min; maximum absorbance was acquired 15 min. The influence of equilibrium temperature in the range 40–90°C was investigated. It was established that 60°C is sufficient for quantitative analysis.

The analytical performance of conventional spectrophotometer in the range 30–1500 $\mu\text{g l}^{-1}$ was studied. The calibration curve for As was linear. The limit of detection (LOD) was calculated as three times the standard deviation of a blank solution. LOD was found to be 2.8 $\mu\text{g l}^{-1}$. The regression equation was acquired by the least square method: $A = 2.05 \times 10^{-3} C_{\text{As}} + 1.21 \times 10^{-2}$ for 30–1500 $\mu\text{g l}^{-1}$ of As(III) with a correlation coefficient of 0.997 ($n = 12$), where A is the absorbance and C_{As} the concentration of As(III) ($\mu\text{g l}^{-1}$). The relative standard deviation (RSD) at 200 and 600 $\mu\text{g l}^{-1}$ was 2.23 and 1.73 respectively ($n = 10$).

Usually coexisting ions may influence determination of As(III) by CPE. To verify this assumption, solutions containing 200 $\mu\text{g l}^{-1}$ of As(III) were taken with different amounts of foreign ions and recommended procedure was followed. These results indicate that the extraction yield for As(III) is not affected by the presence of foreign ions under the tested conditions. Tolerance limits were as follows: Fe^{3+} , Cr^{6+} , Al^{3+} , Sb^{3+} (50-fold), Co^{2+} , Sn^{2+} (100-fold), Cd^{2+} , Ni^{2+} , HPO_4^{2-} (250-fold), Ca^{2+} , Ag^+ , HCO_3^- , Br^- (500-fold), Cu^{2+} , Pb^{2+} , Li^+ , Cl^- (800-fold), Na^+ , K^+ , NH_4^+ , NO_3^- (1000-fold).

The results of the present study are shown in Table 1. LOD of the present work is comparable or better than some previous studies for the determination of As(III) ions^{22–26}.

The proposed method is a simple, rapid, reproducible and highly sensitive one for As pre-concentration that leads to good extraction yield. Triton X-114 was chosen as a non-ionic surfactant due to its excellent physico-chemical characteristics, including lower cloud point temperature and higher density, which are easy for phase separation. The LOD of the proposed method seems to be satisfactory. Furthermore, in contrast to solvent extraction methods, it is much safer because only a small amount of the surfactant, which has a low toxicity, is used. The results indicate that the concentration of As, in water samples of rivers located in industrial areas is considerably higher than in non-industrial areas.

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Early Eocene *Annona* fossils from Vastan Lignite Mine, Surat district, Gujarat, India: age, origin and palaeogeographic significance

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The family Annonaceae has Gondwanan affinity and is being reported from the Cambay Shale of Vastan Lignite Mine on the basis of well-preserved fruit (in counterpart), leaf and pollen grains. This finding is significant because it serves as yet another example of an angiosperm family found in South America and Africa that may have boarded the Indian raft when India was attached to Madagascar, reported on the basis of pollen from Kutch. The Vastan occurrences represent a continuous record from the Indian latest Cretaceous, through the Palaeocene, based on multiple vegetative entities. The well-preserved fruit is morphologically similar to *Annona palustris* L. At present the dispersal history of the family into India represents an origin in the Lower Cretaceous of North America with later dispersal to South America and Africa and then onto India, as it is recorded from the sedimentary beds associated with the Deccan Volcanics. Another angiosperm family, Dipterocar-

paceae, is also found in Vastan, with a similar phyto-geographic distribution.

Keywords: *Annona*, fossil leaf, fruit and pollen, lignite mine, phytogeography.

A well-known fossil hotspot, Vastan Lignite Mine is situated about 29 km northeast of Surat town (21°25'47"N, 73°07'30"E) in Surat District, Gujarat, India. The Cambay Shale stratigraphy, floral and faunal remains have been described in detail^{1–11}. The present fossil material was collected from the Cambay Shale Formation of Vastan Lignite Mine. The rock is a claystone rich in fossil plants and mammals. It occurs about 1/2 m above the base of lignite seam 2. Cambay Formation of the Vastan Lignite Mine section is 20–145 m thick and consists of multiple lignite, carbonaceous shale and grey shale horizons (Figure 1). Fossil fruits, seeds, leaves and pollen grains of *Annona* and other associated fossils were recovered from the exposed section of the mine (Figure 1). The age of the fossiliferous succession in the Vastan Mine is considered as Early Eocene (~53 Ma) based on recorded index foraminiferal fossils assemblages, as well as age diagnostic dinoflagellate cyst assemblage^{12,13}. Fossil fruit is small in size, measuring 2.8 cm width and 3.5 cm in height. In Indian geological record, Annonaceae has been found to appear first in the Late Cretaceous¹⁴ and continued into the Palaeocene¹⁵, spread in the Eocene and became dominant in the Miocene and there are also reports in archaeological sites¹⁶.

The recovered fossil assemblages of fruits, leaves and pollen from Vastan are assigned to form species, *Annona eocenica* sp. nov., *Annona vastanensis* sp. nov. and *Matanomadhiasulcites (Lilicidites) maximus* Saxena respectively. Their systematic descriptions are as follows.

Fossil leaf

Order: Magnoliales, Family: Annonaceae.

Genus: *Annona* Linn., *Annona eocenica* n. sp. (Figure 2 a–d).

Material: Leaf impressions recovered from the grey coloured soft shale and devoid of the cuticles. BSIP Museum specimen nos 40018 (holotype), 40019, 40020 (paratype).

Locality: Vastan Lignite Mine, Cambay basin, Gujarat, Western India.

Horizon and age: Cambay Shale Formation, Early Eocene.

Etymology: This species is named after the age of the Cambay Shale Formation from where the specimens were recovered.

Diagnosis: Leaves asymmetrical; base obtuse; margin entire, venation pinnate, eucamptodromous; 4–5 pairs of secondary veins, alternate, branched, angle of divergence of secondary veins 55–60°; angle of origin of tertiary

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