Promising technique, cloud point extraction: technology & applications

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Abstract. Assurance of analysts of various matrices (for example natural or tainted water or soils) at a standard level are often required for identifying the environmental pollution. A couple of used systematic methods are delicate enough for the direct assurance of trace components in samples, therefore, a preliminary step of the analyst prior to analysis is consuming much of the time. One of the best alternative methods that uses surfactant to extract organic/inorganic parts is the cloud point extraction CPE. It is a fast, easy and economical extraction technique includes assembly of monomers of nonionic surfactant to form a hydrophobic core (micelle). At that point, the micelles entangle the hydrophobic bioactive mixe s inside the micellar extraction. This field of extraction is very promising for new and active analytical strategies that are applicable to many systems. This review shows the importance of CPE method, surfactants, procedures used to detect the cloud point, the effect of different parameters on the efficiency of extraction and the possible applications and limitations of the method. It aims to explore the possibility of developing CPE within the approach of green analytical chemistry.

Keywords: cloud point, surfactants, green chemistry, critical micelle, green technology.

1. Introduction

The cloud point extraction CPE is one of the a modern and best alternative methods for fluid extraction that is widely uses surfactant to extract organic/inorganic parts in the separation science 1. This method depends on the clouding phenomena of surfactants 2. It is a strategy to decrease the exposure to a solvent, with low coast and less time 3. CPE technique has been used for extracting and pre-concentrating of metal ions after developing of water-soluble complexes. Any change in the experimental parameters (pH solution, concentration, time and temperature) leads to stage separation. It is useful to isolate the hydrophobic and hydrophilic staff 4. Recently, CPE has effectively used for effectively separating metal elements and thus, has applied in a wide range of applications in the life science and ecological science 5. Watanabe and Tanaka in 1978 were applied the CPE to trace analysis of Ni in the soil and found that surfactants have the same role with organic solvent by dissolving water-insoluble or slightly soluble compounds 6. The method developed then by Hinze in 1992 by making appropriate adjustments to the experimental conditions, such as adding salt and other additives, temperature, extraction time 7. The method has been then used for extraction and estimation of metallic elements 8, removal and estimation of dyes 9,10, estimation of pharmaceutical drugs 11–13 in the food and analysis of organic compounds 14. CPE has many advantages, it is a safe design with no toxic wastes, no cause or reduce potential accidents such as fire, choking or explosions, no need to organic solvents or toxic detectors and environmentally friend. CPE is considered one of the green methodologies because it consists of a little amount of nontoxic surfactant instead of that toxic organic solvents (green analytical method) 15. CEP is a simple procedure system used as pre-concentration to extract and separate micro amount of organic/inorganic substances from different chemical or biological matrices, such as soil 16, water 17, bioactive compounds 18, plasma 19,20, urine 21, milk 22,23, saliva 24, geological samples 25 etc. It gives low limit of detection
(LOD) and good relative standard deviation (RSD), which facilities separation and pre-concentration of micro amounts of generally hydrophobic organic compounds and elements. CPE can use water as a solvent and it works between ~ 30–60 °C. It is also known as liquid-concentration technique or micelle-mediated extraction. This green extract has received a lot of attention due to low concentration required in different chemical and biological systems. The CPE procedure stays away from hazardous organic solvents and therefore, it is an alternative solution for classical techniques such as liquid-liquid extraction (LLE), which needs a large amounts of flammable or toxic solvents with less extraction efficiency.

2. Principle of extraction by the cloud point
The separation of the tested solution into two distinct phases based on heating up to a characteristic temperature called cloud point temperature CPT until becomes a surfactant (only two types of surfactant use in this technique, either nonionic or amphoteric) above its critical micellar concentration.

Figure 1. At a temperature higher than the CPT, micelles shaped from surfactant molecules. Also micelles can shape at room temperature without heating by the effect of added salt such as Na2SO4 that phenomenon reduces the time and called (salting-out phenomenon) or by introduction of electrolytes in process decrease the cloud point temperature, up to room temperature.

Surfactant in CPE plays a similar role of the organic solvents in LLE; the analytics are separated between the micellar phase and watery phase. The particles of surfactants aggregate as a micelle and orientate their hydrocarbon tails toward the middle to make a nonpolar hydrophobic core.

![Figure 1 A diagram of the principle of CPE](image_url)
A synergetic effect of CPT can be achieved with ultrasonic vitality. Modification of CPE by ultrasonic thermostatic-CPE (UTA-CPE) is another approach to increase the efficiency of extraction, where the sample is ultra-sonicated in a boiling water bath, which might accelerate the reaction and improve the extraction pattern by the effect of cloud extraction patterns. This decreases the temperature of extraction and lowers the consumption of reagents (1995). One of these phases has a higher concentration of the surfactant than the other. The phase locates the alate but a little amount of water is called the surfactant-rich phase, which is usually less than 0.5 ml. Oppositely, the other phase (water-rich phase) locates water more than surfactant. Both phases isolate by centrifugation.

3. General considerations: Surfactants

The term 'surfactant' is a truncation for surface active agent, a surfactant characterized by its tendency to absorb molecules at surfaces and interfaces. Surfactant molecules adsorb at the interface or surface when present in a low focus and modifies the interface free energy. The term interface means a limit between any two immiscible phases; (aqueous solution – organic solution) or (aqueous solution – air). Surfactants are amphiphilic molecules consist of a hydrophilic group direct towards the aqueous phase and a long hydrophobic tail. Such amphiphilic molecules scattered in a concentrated solvent under the impact of the hydrophobic chains and self-collect into colloidal-sized clusters, which add further advantage for surfactants.

The surfactant structure consists of R-X, where R is a hydrocarbon tail (8-18 carbon atoms) and X is a polar head group. The tail can be short or long, linear or branched, aliphatic, aryl or alkyl. The group of the surfactant head can be either ionic or non-ionic. Classification of the surfactant depends on the charge of the hydrophilic tail. The surfactants could be cationic (positively charged hydrophilic tail), anionic (negatively charged hydrophilic tail) and nonionic (no charge on the hydrophilic group, but its water solubility drives by polar groups and amphoteric or zwitertionic molecules). Another classification of surfactants depends on the solubility; hydrophilic surfactants are water-soluble, while hydrophobic (lipophilic) surfactants are lipid-soluble. Hydrophilic surfactants are usually ionic; however, the nonionic surfactants are either hydrophilic or lipophilic depending on the balance between the hydrophilic and lipophilic groups. This determine the quantity of the attracting water and the capacity of attracting oil by the surfactant group.

In aqua solutions, surfactants dispersed at low concentration in a monomeic or dimeric state and aggregate to form clusters of colloidal size called micelles. Micelles are distributed in water, either hydrophilic or lipophilic analytes may be chemically or physically associated to each other, which increases their solubility. This makes surfactants phenomenal vectors for solubilization of the analyte in a micellar system into a given surfactant.

Information on solubility is represented in a solubility versus concentration curve or a three segment phase graph that defines the impact of device components (the analyte, surfactant and solvent). The solubilization site within the micelle is closely linked to the analyte's chemical character. The last structure of the molecular aggregates formed by microscopic order (aqueous or vesicular, reversed micelles, bilayers and microemulsions) is estimated by the nature of the surfactant monomer, the nature of the solvent, and the surrounding ions.

The specific structure of micelles enables them to establish chemical or physical interactions with hydrophilic or lipophilic substances. This is useful in pharmaceutical analysis and in separation sciences and chromatographic applications, such as dye solubilization, in micellar liquid chromatography, electrophoresis and electrochemical strategies. Additionally, they are helpful in the studying of the thermodynamic properties and stability of macromolecules such as proteins and enzymes.
4. Strategies to detect Cloud Point
There are several strategies to distinguish the cloud point, such as particle counting method, visual observation, refractometry, turbidimetry, thermo optical strategy and Viscometry

4.1. Particle counting method
When heating a solution to its critical cloud point, a large number of small particles are produced and the solution becomes cloudy. These particles disperse the light beam that is going through. Eliassi et al. 2003 presented a method to measure the number of particles in a solution at different temperatures using laser particle counter with thermocouple to measure temperature. In 2009, Imani et al. prepared solutions of different mass ratios of poly (ethylene glycol) with sulfate salts. They used a spectrex laser particle counter with a laser diode and thermocouple to measure temperature. The number of particles was measured per cubic centimeter and at the cloud point.

4.2. Visual observation
At cloud point, temperature causes a sharp change in number of particles would be watched up by Macroscopic.

4.3. Refractometer
In 2006, Mohsen et al reported the effect of the concentration of salt and polymer and the type of salts on the CPE of ternary systems using refractometer equipped with a digital thermometer. They found that the higher temperature the lower refractive index (RI) of a mixture. However, at the cloud point, RI decreases very slowly due to unexpected appearance of particles in the new phases. After the cloud point, the RI of the systems decreases slowly again.

4.4. Turbidimetry
In 2011, Jimenez and his collaborates used the turbidimetry calorimetry method to measure the CP temperatures and phase separation of the ternary system.

4.5. Thermo-optical strategy
Thermo-Optical analysis (TOA) is a simple, rapid and reliable method to determine the curves of the cloud point of binary polymer-solvent system using a polarizing microscope through a photodiode and a microprocessor. The microscope provided with heating–cooling system to follow the thermal analysis of a mixture.

4.6. Viscometry
At the CPE, number of particles of the new stage rapidly increase, which leads to high viscosity. After increasing the temperature, the molecular interactions increase and then drop down. This change is easily detected by a viscometer equipped with a water bath and a thermostat. The difference between the cloud points measured by visual observation and that measured by other methods should be less than one centigrade.

5. Factors affect the efficiency of CPE

5.1. Type and concentration of the surfactant
As mentioned earlier in this review, there are three main types of surfactant for CPE, nonionic surfactants (polyglycerol alkyl ethers or twents, etc), anionic surfactants (alkyl benzene sulfonates) in addition to zwitterionic (double ionic). The nonionic surfactants are recently widely used due to high efficiency of CPE if hydrophobic surfactants and hydrophobic analytes are supplied. Each surfactant has its critical micelle concentration CMC, the concentration above the threshold, at which surfactants start to form micelle, depends on the surfactant’s structure. CMC decreases as the number of carbon atoms and nonpolar alkyl tail increased. For ionic surfactants, usually halides as a counter-ion attaches to the hydrophilic element. The most frequently used surfactants for CPE are Triton X-114, Triton X-100 and Genapol X-080. Triton X-114 can be used at low temperature (23 °C), absorbed at low wavelength and has high density, which all enable easy separation of the phase. The surfactant enhances the recovery by increasing the concentration up to a certain point. Above that, it dilutes the solution under test and lowers the pre-concentration rate. Increase the recovery of the analyte can be achieved with a concentration of surfactant of 1–9%, ideally 4%. Over this concentration, the concentration of the analyte will decrease.

5.2. Effect of the temperature
The ideal temperature for extraction is 15–20 °C greater than the cloud point of the surfactant. By increasing the equilibrium temperature, the disruption of hydrogen bonds and dehydration of the phase decreases the volume of the surfactant-rich phase. That leads to increase the yield of the extraction process. However, very high temperature may lower the recovery of the analyte due to the decomposition of thermo-labile components, such as vitamins or metal ions extracted by chelating agent. The most commonly used temperatures are between 40–60°C. However, addition of salt might affect the extraction by lowering the temperature of the surfactant below the (15–20°C).

5.3. Effect of pH
The analyte recovery could be enhanced by choosing the required pH for ionizable compounds and/or adding salt, which reduces the solubility of the aqueous phase analyte. An important parameter of CPE is pH optimization. As neutral particles, the deprotonated/protonated particles usually have no strong response to the micellar aggregate. In such a scenario, most extraction is accomplished at pH values where the analyte's uncharged form prevails, and the analyte tends to partition into the nonionic surfactant's micellar phase. The pH of the sample should therefore be appropriate for extraction for a high recovery of the analyte and a sufficient limit of detection (LOD). The presence of potentially toxic substances usually requires screening for toxicity especially for clinical applications. Some of the biological matrices include complex organics with dissociation constants (pKa) or partition coefficients (log P) that affect the extraction process of certain analytes. Therefore, a modified CPE called sequential CPE gives the possibility in the presence of different analytes to screen the biological matrix.

5.4. Effect of salt
Addition of neutral salts, such as CaCl₂ or NaCl affects the value of critical micelle concentration (CMC). When the surfactant is nonionic, the concentration of the electrolyte decreases CMC and reduces the separation efficiency. The ionic surfactants, increase the ionic strength of the solution and accelerate phases separation by increasing the density of the water layer. Addition of electrolytes improve the extraction efficiency for polar substances. It reduces the CPT and improves the effect of hydrophobic interactions between the surfactant and the analyte. Studies validated the effect of the addition of neutral salt in percentage (4–10%) on the final recovery of the analyte. Salt concentration of 1–4% promote the extraction efficiency and the recovery of the analyte to ~10–20% probably due to the salt-out effect, which reduces the amount of solvent (water), but salt concentration of 7–10% decline the recovery by ~10–25% perhaps because substances compete for precipitate proteins at high concentration of salt; this can lower the concentration of analytical components in the solution and negative impact on recovery. NaCl or Na₂SO₄ should be carefully considered since their use is limited with other analytical techniques because they interfere with the high Matrix effect (ME), which is usually coupled with mass spectrometry.

6. The key steps of cloud point technique
The process of CPE includes eight steps: addition of salt, providing suitable environment of pH for solubilizing the analytes in the micellar aggregates, incubation for clouding, centrifugation, cooling (Ice bath), phase separation for analysis, pre-treatment of the surfactant-rich phase and finally instrumental analysis, see Figure 3. Although it is critical to separate aqueous surfactant phase from micellar phase (contains the component to be estimated), after separation, viscosity of the micelles increase by cooling and then supernatant decanted. Traces of water is completely removed by a stream of neutral gas (N₂ gas).
Recently, many efforts have been made to develop and improve classic CPE. These procedures such as Micro-cloud point extraction (M-CPE)\(^{57-59}\), Displacement cloud point extraction (D-CPE)\(^{60-62}\), Rapidly synergistic cloud point extraction (RS-CPE)\(^{51,63-67}\), Dual cloud point extraction (d-CPE)\(^{60,68-72}\), Acid-induced cloud point extraction (A-CPE)\(^{73-78}\) have increased the sensitivity and selectivity of the analytical technique and made it more rapid\(^8\) and Ionic liquid-assisted cloud point extraction (IL-CPE)\(^{79}\).

### 7. Applications

CPE has been widely used to separate rare earth elements and heavy metals such as (arsenic, chromium, cadmium, lead, and mercury, series of f-block elements, etc)\(^{4,8,88,89,80-87}\). It also applied for bioactive extraction from food and medicinal plants, polyphenols from industrial waste, separation and purification of protein, mineral analysis, Food additive analysis, food constituent analysis, pesticide residue analysis, antibiotic residue analysis, mycotoxin analysis and packaging material residue analysis (Plastic additives and residual monomers)\(^{14,17,18,22,23,90,91}\). In forensic science: The extraction of analytes such as alkaloids, organophosphorus (OP) compounds\(^{92}\) and drugs from biological matrices is a challenging and time-consuming task\(^{39}\) or from wastewater\(^{93}\), Separation of nano-particles\(^{94-96}\). Extraction of organic dyes\(^{11,97,98}\) and vitamins\(^{35}\).

### 8. Limitations

Figure 3. A diagram describe of CP system for analytes from complex matrix \(^{39}\)
Limitations of CPE include limit in discovery of analytes because complex nature of surfactants, difficulty of extracting some substances that need to form micellar system, lower efficiency in extracting polar molecules by non-ionic surfactants, the phase separation is limited by temperature while extracting thermally-labile parts, further treatment of some extracts before measurement and difficulty in automation.

9. Future Scope
In spite of impressive CPE technique, it still needs more insights into the potential of extract polar bioactive parts, use cyclodextrin system for extraction, Extract flavour and encapsulation it in simultaneous, extract of heat sensitive biomolecules by zwitterionic surfactant micelle system, automat in sample preparation, separate and purify of enzymes utilizing CPE, improve the extraction efficiency, selectivity and applicability, give more attention to extracting anions and provide commercial CPE systems.

10. Conclusion
Due to its moderate conditions, the technique (Cloud Point) is one of the promising and bright future in analytical applications for the extraction of functional components, which can be linked to other technologies for the efficient extraction of different analytes'. Despite the toxic effect of some of those, giving more attention to using the cloud point extraction for anion extraction is negligent. Therefore, the use of cloud point extraction for preconcentration. The cloud point extraction experiment is widely shown in the principles of green chemistry, removal of organic solvents, incorporation of surfactants in spectroscopic analysis, trace analysis and physico-chemical aspects of phase separation. Thus, it can be widely used in the bioanalysis, due to its simplicity, speed of execution, little impact on the environment and high optimization capability.

Abbreviations
Cloud point extraction (CPE), Cloud point temperature (CPT), Limit Of Detection (LOD), Relative standard deviation (RSD), Liquid - liquid extraction (LLE), Critical micelle concentration (CMC), Ultrasonic-thermostatic-assisted CPE (UTA-CPE), Organophosphorus (OP), Refractive Index (RI), Thermo-Optical Analysis (TOA), Matrix Effect (ME), Micro-cloud point extraction (M-CPE), Displacement cloud point extraction (D-CPE), Rapidly synergistic cloud point extraction (RS-CPE), Dual cloud point extraction (d-CPE), Acid-induced cloud point extraction (A-CPE), Ionic liquid-assisted cloud point extraction (IL-CPE).

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Conflict of interests
Author have no any conflict

Data availability
All data are presented in this review

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