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Review

Cloud Point Extraction as an Environmentally Friendly Technique for Sample Preparation

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Abstract: Cloud point extraction is a sample preparation technique that involves using surfactants that are not harmful to the environment. It is based on micelle formation in which the extracted compound is encapsulated in the hydrophobic core of the micelles, which are the extracting agent. The most commonly used surfactants are nonionic. The others are anionic, cationic, or zwitterionic. The effectiveness of cloud point extraction might be enhanced by the addition of neutral salts, the application of proper pH, as well as acidic conditions and temperature. This sample preparation technique may be applied to extract analytes from the following matrices, such as biological and environmental samples. Cloud point extraction may be combined with various analytical techniques and detectors such as HPLC-UV, HPLC-MS, HPLC-FLD, inductively coupled plasma–optical emission spectrometry, gas chromatography, and flame atomic absorption spectrometry. When it is combined with electrothermal atomic absorption spectrometry, the limit of quantitation is low—even of the order of ng/L. The recovery of the analyte may reach the value of 100%.

Keywords: aqueous matrices; cloud point extraction; green chemistry; optimization; sample preparation

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1. Introduction

Currently, as environmental awareness increases, many industries emphasize using more environmentally friendly methods. Methods in line with green chemistry principles are gaining popularity in the chemical industry. Cloud point extraction (CPE) fits perfectly into this trend. Initially, it was developed by Watanabe and Tanaka and presented in 1978. It extracted zinc (II) ions with polyoxyethylene nonyl phenyl ether [1]. This method is an alternative to classical extraction techniques such as liquid–liquid extraction (LLE), which requires often toxic solvents, and solid-phase extraction, which is expensive and time-consuming. It is based on the ability of surfactants to form micelles in aqueous solutions. When heated, the solution becomes blurry at a specific temperature called cloud point temperature (CPT). It is characteristic of a surfactant [2,3].

The surfactants used in CPE are economical and not harmful to the environment. The amount of surfactant used in the CPE is very low compared with the volume of the solvent in LLE. The surfactants are biodegradable in wastewater treatment plants. However, they may cause foaming, which may reduce the amount of oxygen [4]. The mild conditions make the process suitable for the preconcentration of thermally sensitive analytes, for example, vitamin E [2,5]. Nowadays, it might be applied with various analytes and matrices, such as detecting fluoroquinolones from the environmental samples from wastewater treatment plants [6]. It can also be applied to detect drugs in plasma or vitamins

in solutions, urine, or plasma [7–10]. It might be applied as a first stage of isolation in protein purification [11]. The surfactant-rich phase is compatible with most mobile phases used in analytical systems—they contain the following solvents: methanol, acetonitrile, organic acids, and various buffer solutions [9,12–14]. In CPE, non-toxic, and cost-effective substances such as inorganic salts (for example sodium chloride) are used, which lower the surfactant's CPT [4].

One of the main advantages of this method is the ease of separating the surfactant-rich phase from the aqueous phase after centrifugation. It streamlines the sample preparation process and reduces the risk of analyte loss [3].

2. CPE as Liquid–Liquid Extraction Modification

LLE is one of the most popular sample preparation methods for analysis. It involves using large amounts of immiscible organic solvents such as chloroform, dichloromethane, ether, or ethyl acetate. This process allows the partition of the analyte between the aqueous phase (e.g., blood, plasma, serum, and tissue homogenate samples) and the organic solvent phase [4,15]. The method can increase selectivity by isolating the analyte from interfering matrix substances or by concentrating the analyte from a large sample volume. This technique does not require specialized laboratory equipment but is time-consuming and labor-intensive. The organic layer must be separated from the aqueous layer, transferred to the testing tube, and evaporated, which might take time. It also involves using organic solvents that might harm the environment [4,16].

Unlike most common extraction methods, CPE eliminates the abovementioned solvents, replacing them with non-toxic surfactants. This method achieves a high degree of analyte concentration and significantly increases the selectivity of the analysis while remaining simple and environmentally safe.

On the other hand, CPE also has limitations. The surfactant applied in the process may cause interference with the analyte during the analysis. The absorbance spectra of the surfactant and the analyte may overlap. It implies that a specific analytical method should be applied. The extraction procedure may require heating of the sample. It may lead to the decomposition of the analyte. Applying nonionic surfactant to the polar analytes may result in a low recovery rate. The other challenge is the automation of the process. The necessity of automating the process was also indicated by Hagarová et al. and Halko et al. It could help to speed up the process and save energy [17–19].

3. CPE Mechanism

CPE is a simple, inexpensive, and safe method for both the researcher and the environment. The technique uses surfactants as the extraction medium [15,20]. It is based on micelle-mediated extraction. In CPE, the extracting agents are the micelles. They are formed at a specific concentration called critical micelle concentration (CMC). It is a concentration at which the surfactant's micelles start to form. It is characteristic of a surfactant—each one has its own. In the surfactant's molecule, two parts might be distinguished: the hydrophobic tail and the hydrophilic head. In an aqueous solution, surfactants used at concentrations above the CMC value form a micelle with hydrophobic tails facing inward to form the core and hydrophilic heads facing outward [17,20,21]. The bioactive compounds present in the sample are isolated and encapsulated in the hydrophobic core of the micelles [2,21]. The sample is then heated to a temperature exceeding CPT. CPT is a very important value—at this point, the one-phase solution becomes cloudy, and the surfactant-rich layer starts to separate. CPT, as well as CMC, is characteristic of a surfactant. It should not be too high—it may lead to the decomposition of heat-sensitive compounds [4]. It improves the separation into two distinct phases—one containing surfactant at a concentration less than or equal

to CMC and the other one that is rich in surfactant. Hydrophilic compounds, originally present in the solution and bound to micelles, are extracted into the surfactant-rich phase in which the analyte is preconcentrated [21]. The separation of the phases is mainly due to the dehydration of polar groups in the surfactant molecules due to heating, which reduces repulsion between micelles and facilitates their aggregation [2,20].

Phase separation is usually accelerated by centrifugation. The system is then cooled to increase the viscosity of the surfactant-rich phase. The final step of CPE involves removing the aqueous (surfactant-poor) phase by decantation and dissolving the surfactant-rich phase in a medium suitable for the chosen detector. The sample thus prepared is ready for quantification of the selected analyte [4,21]. The scheme of CPE is presented in Figure 1.

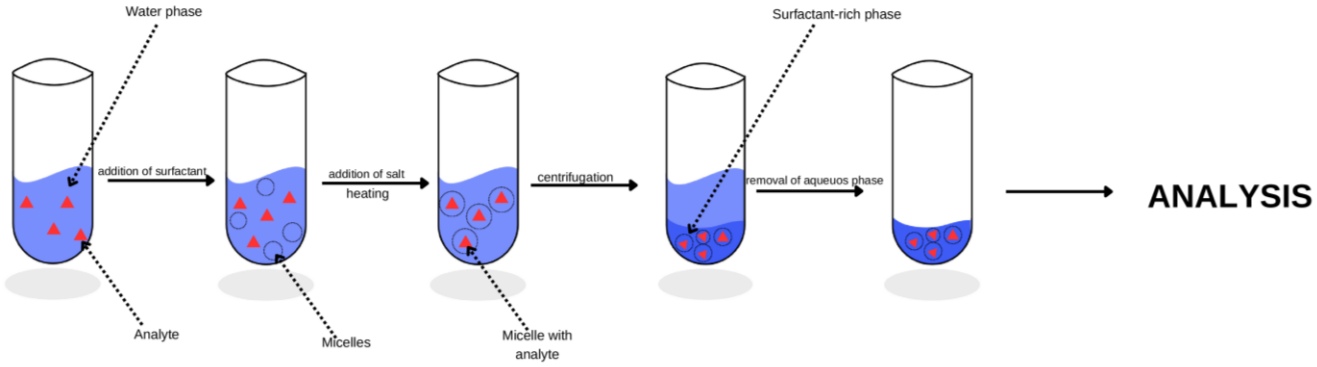


Figure 1. The scheme of CPE procedure.

4. Surfactant Types and Classifications

Surfactants play a key role in CPE, as they are essential for forming micelles that capture material for separation. Based on the type of hydrophilic group, they can be classified into four groups: nonionic, anionic, cationic, and zwitterionic. Table 1 lists the most popular surfactants and their CMC and CPT.

The most commonly used surfactants are nonionic. Their hydrophilic head is uncharged, and they are soluble in water. Examples of nonionic surfactants include Triton X-100, Triton X-114 (TX-114), Triton X-45, and Genapol X-080. The polar chain of the nonionic surfactant contains hydrophilic groups that may interact with water via hydrogen bonds. The CMC of the surfactants may be changed with additives. The phase separation is observed at a temperature above CPT, which is increased with the decrease in molecular mass and branching of the hydrophobic tail [17,22]. The clouding phenomenon occurs in nonionic surfactants when the concentration of the surfactant in solution is above its CMC. TX-114 is preferred for analysis of biological fluids more frequently than Triton X-110 or Genapol-X080 due to its low CPT (Table 1) [8]. Its surfactant-rich phase has a high density, which facilitates the separation of this phase from the water phase by simple centrifugation [23].

Table 1. Comparison of CMC and CPT of the most popular surfactants [2,4,19,24–27].

Surfactant	CMC [mM]	CPT [°C]
Brij-35	0.09	>100
Brij-58	0.077	>100
CTAB	0.9–1.0	24.5 *
Genapol-X080	0.06–0.15	>45
SDS	8.14	>100
Tergitol 15-S-7	0.074	37
Triton X-100	0.2–0.9	65
Triton X-114	0.2	23
Tween 80	0.015	65

* Kraft point.

The CPT value depends on several factors, such as the type and concentration of surfactant used and the presence of other substances, such as salts, acids, bases, alcohols, polymers, or other surfactants. These additives can cause an increase or decrease in CPT. The addition of electrolytes or the presence of polymers with hydrophobic end groups can induce phase separation below CPT. An increase in CPT values occurs when polymers with hydrophilic end groups are present in the sample [4,28–30].

Anionic surfactants have a negative charge on their hydrophilic head, with examples including sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate, or sodium tetradecyl sulfate [2]. In contrast, cationic surfactants have a positive charge in their hydrophilic group. Compounds in this group include alkyl pyridinium halides or alkyl ammonium halides, such as hexadecyl bromide, cetyltrimethylammonium bromide (CTAB), or cetylpyridinium chloride [20,31].

Two-phase separation in ionic surfactant solutions is caused by the salting-out effect, which occurs in the presence of salts such as sodium chloride [32]. Linear alkyl anionic surfactants are commercially available and have a suitable ionic group for the preconcentration of more polar analytes at the cloud point before HPLC determination. Since the clouding of these anionic surfactants is not temperature dependent, thermally unstable analytes from biological and environmental samples can be easily extracted [2,5]. Anionic surfactants, due to the absence of aromatic rings in their molecules, have a low fluorescence signal even at excitation wavelengths below 300 nm. In addition, their polar nature results in short retention times [33].

The last group consists of zwitterionic surfactants with two active groups with opposite charges. The positively charged group is always an ammonium ion, while the negatively charged group is most commonly a carboxylate. Their functional group's charge depends on the aqueous solution's pH. In aqueous high and intermediate pH solutions, zwitterionic surfactants form internal salts of molecules, and their anionic part can attach a proton to itself. In contrast, in low-pH solutions, cations are formed. Most of these surfactants exist in an isotropic phase at higher temperatures, and separate when cooled. The use of these surfactants is limited due to their limited availability on the market [17,32,34]. Examples of this class include 4-(dodecyldimethyl ammonium) butyrate, alkylammonium ethyl sulfates, phosphobetaines, lecithins, or sulfobetaines [20].

Temperature changes cause phase separation in non-ionic and zwitterionic surfactants. In contrast, for ionic surfactants, phase separation is due to the salting-out effect induced by the presence of salts such as sodium chloride [32]. The structures of the chosen surfactants are presented in Figure 2.

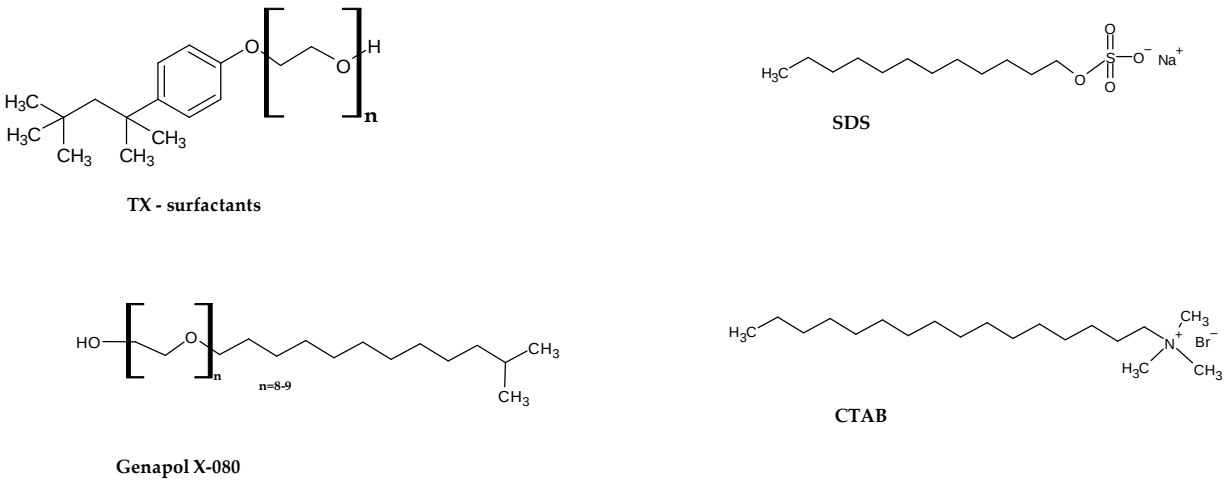


Figure 2. The structural formulas of TX-surfactants, SDS, Genapol X-080, and CTAB.

5. CPE Optimization

The variety of variables that impact the effectiveness of CPE makes optimizing this process a complex issue. However, the optimization may be useful for chemometric models such as Central Composite Design and artificial neural networks. The concentration of surfactant and salt, pH, extraction time, and temperature may be considered independent variables. The recovery will be the dependent variable. Analyzing the parameters in single, as well as in mutual interaction, helps to find the most optimal conditions which result in satisfactory yield of CPE. The optimization process should be preceded by a preliminary study that will establish the ranges of the analyzed independent variables. It is also important to evaluate the feasibility of the process [12,14,35,36].

5.1. Effect of Surfactant Concentration

Surfactant concentration is one of the key parameters affecting the extraction efficiency at the cloud point. Appropriate selection of this concentration is important because phase separation occurs only within a well-defined range of values. Increasing the amount of surfactant can improve the efficiency of analyte recovery, but exceeding the optimal level leads to dilution and weakens the analytical signal. On the other hand, a concentration that is too low results in insufficient analyte capture, which negatively affects the accuracy and reproducibility of the results. According to Kojro et al., the maximum surfactant concentration should not exceed 9%, and the minimum recommended concentration should be at least 1% [4]. Therefore, a key step in method development is to optimize the surfactant concentration precisely, tailored to its specific properties, thus maximizing extraction efficiency and obtaining better analytical results [2,4,20]. To increase the yield of CPE with the application of nonionic surfactant, the addition of ionic surfactant should be considered. Cetylpyridinium chloride or CTAB can be considered as sensitizing agents, which improve the transfer of the complex with metal from the aqueous phase to the surfactant-rich phase. However, the addition of the ionic surfactant should be undertaken cautiously—too high a concentration of sensitizing agent may increase the blank absorbance and reduce the absorbance of the analyte, as was observed for CTAB in the determination of As^{3+} ions [37]. Micelles of the surfactant form clusters at CPT, which is caused by dehydration, and the micelles attract each other [17]

5.2. pH of the Solution—The Impact of Acidic Conditions

The analyzed substance in solution can exist in ionic or non-ionized form. Molecules in ionic form do not interact with micelles as intensively as uncharged forms. pH has an impact on the charge of the analyte's molecule. It may impede the complex formation between the analyte and the micelle [2]. Therefore, optimal extraction efficiency is achieved at a pH that favors the non-ionized form of the analyte, resulting in its preferential transfer to the micellar phase of the nonionic surfactant [4,35].

pH significantly impacts recovery when analyzed as a single factor or in the interaction. The study of Michałowska et al. [38] evaluated the impact of pH and surfactant concentration on the recovery of fluoroquinolones. The study showed that acidic pH combined with a proper surfactant concentration improved the recovery. The growth in pH and low values of TX-114 concentration resulted in decreased recovery. This implies that the application of proper pH may improve recovery. However, it should be considered simultaneously with the concentration of surfactant. This study also indicated that pH is the predominant factor influencing the recovery of ciprofloxacin and moxifloxacin. However, the concentration of TX-114 also plays a crucial role as a limiting agent. For levofloxacin, the critical factor in recovery using CPE is the concentration of TX-114, with the interaction of pH and salt concentration also being significant.

In the case of ionic surfactants, the presence of an acid is crucial to the induction of separation into surfactant-rich and aqueous phases. Unlike nonionic surfactants, ionic surfactants do not require a temperature change to induce the separation of the surfactant-rich phase, allowing the extraction of substances sensitive to higher temperatures [4,32]. Acid is also necessary to induce separation into the surfactant-rich phase and the aqueous phase when an ionic surfactant is used.

An increase in the concentration of hydrochloric acid in the sample leads to a reduction in the volume of the surfactant-rich phase due to a reduction in the amount of water in this phase caused by an increase in the solution's acidity. This, in turn, leads to a higher analyte concentration in the surfactant-rich phase. In addition, it was observed that increasing the volume of hydrochloric acid in the range of 2.0 mL to 4.0 mL increases the sample's fluorescence. However, an acid volume exceeding 5.0 mL negatively affects extraction efficiency [3,4,32].

Xia et al. [6] investigated using acid-induced cloud point extraction combined with spectrofluorimetry to determine fluoroquinolones in environmental water samples. It was demonstrated that it is an effective method for extracting fluoroquinolones from water and other aqueous matrices. The method not only allowed a lower detection limit but also provided a wider range of linearity. A high recovery (up to ca. 94%) was obtained in the samples tested. The method proved simple, fast, and reliable in determining fluoroquinolones in various aqueous samples.

Neukoei et al. [37] indicated the importance of pH in the CPE of As^{3+} ions. The analyzed matrices were different types of water samples (tap, river, well, and lake). The highest yield was observed at pH = 4.0. The extraction yield took the minimum values at lower and higher (up to 8.0) pHs. For these values, the formation of complexes of As^{3+} with amaranth (the chelating agent) was the lowest. In the case of Pb^{2+}, the pH restricted the stability of the complex with the chelating agent (1-phenyl-3-methyl-4-benzoyl-5-pyrazolone)—the extraction depended on the pH at which the complex was formed. In this case, it was stable at pH 5.0–6.0 [23].

5.3. Temperature

Temperature plays a key role in CPE. Optimal thermal conditions usually exceed the CPT of the surfactant by 15–20 °C, with this value depending on the properties and concentration of the surfactant and the presence of additives such as salts, alcohols, or polymers [4,28]. Excessive temperature elevation can reduce extraction efficiency, leading to the degradation of thermolabile compounds such as vitamins or metal complexes with chelating agents and the destabilization of micellar structures of surfactant aggregates [2,4,8]. On the other hand, lowering the temperature below the recommended values can be achieved by adding salt, which reduces CPT, allowing efficient phase separation even under conditions close to room temperature. This can be caused by the application of a suitable salt, known as the salting-out effect [4,20,28]. This effect involves reducing the solubility of many organic substances in water in the presence of dissolved inorganic salts. Adding salt to the solution increases hydrophobic interactions between micelles by increasing dehydration, which, if the surfactant concentration is high enough, causes turbidity and phase separation. Thus, the heating step can be skipped, reducing the time required for the separation process [4,20]. Besides the presence of salt, the CPT value depends on the structure and concentration of the surfactant, as well as the presence of various compounds, such as alcohols, other surfactants, polymers, and certain organic or inorganic compounds, which can raise or lower the CPT [28].

The temperature at which the process is conducted is a significant parameter that should be taken into consideration, at least in the preliminary step. It may enhance

recovery, as in the case of Pb^{2+} [23]. This factor is also important from the point of view of the feasibility of the process. In the study of Michałowska et al. [35], the surfactant-rich phase separation was not observed at temperature below 45 °C. The temperature may also interact with other optimized parameters such as the concentration of surfactant or pH, which may impact the final recovery. The increase in temperature does not always result in an increase in recovery. In the case of moxifloxacin from the previously mentioned study, the increase in temperature led to a decrease in recovery [35].

5.4. Salting out Effect

Adding salts such as NaCl, KCl, KNO_3, $CaCl_2$, or Na_2SO_4 into the solution can significantly affect extraction efficiency, especially for polar substances. The addition of salt improves the hydrophobic interactions between the analyte and micelles. It results in the more effective transfer of the analyte from the aqueous phase to the micelle [17]. They might be considered as co-extracting agents. Adding these substances increases micelles' number and size, as the CMC value decreases with increasing electrolyte concentration. Besides the addition of electrolyte, the cloud point might be influenced by the addition of alcohol, nonionic surfactant, or organic compounds [4,35,37]. In the case of nonionic surfactants, the presence of salts supports phase separation by increasing the density of the aqueous layer. Cations, such as Na^+, can lower the turbidity temperature by dehydrating the surfactant's polyoxyethylene segments, while anions, such as Cl^-, reduce the interaction of water molecules. This results in higher analyte recovery while maintaining high analytical quality. According to Arya et.al., for nonionic surfactants, it is observed that CPT decreases with the addition of neutral salt (for example, Na_2SO_4) and polar organic compounds (for example, aliphatic alcohols). The increase in CPT is observed after adding salting-type salts (for example, nitrates). In the case of zwitterionic surfactants, the situation is the opposite—the addition of sulfate results in the increase of CPT, and the addition of salting-type salt decreases CPT [2,4,17]. The choice of electrolyte might also have an impact on extraction—the yield of extraction of As^{3+} and the absorbance was higher for NaCl than for KCl [37]. On the other hand, Manzoori et al. [23] reported that the addition of the electrolyte (NaF) did not significantly impact CPE efficiency. In the study by Michałowska et al. for fluoroquinolones, the highest recovery for moxifloxacin and levofloxacin was observed at the NaCl concentration ca. 4–6%. In ciprofloxacin's case, no neutral salt addition was preferred [35]. In the other study by Sznek et al., the impact of acidic conditions on the CPE recovery of levofloxacin and ciprofloxacin was evaluated. In this study, the highest recovery was observed for no addition of neutral salt. However, in the case of levofloxacin, 1.5 M HCl was involved in the process. For ciprofloxacin, the concentration of HCl was lower—0.1 M [14].

6. The Application of CPE

6.1. Detection Techniques Versus CPE

CPE is effectively integrated with various spectroscopic and chromatographic detection techniques, such as inductively coupled plasma–optical emission spectrometry (ICP-OES) [38], inductively coupled plasma–mass spectrometry (ICP-MS) [39,40], high-performance liquid chromatography (HPLC) with different detectors [5,6,10,12,13], liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) [8,9], α-spectrometry [41], gas chromatography (GC) [42], flame atomic absorption spectrometry (FAAS) [23,43], electrothermal atomic absorption spectrometry (ETAAS) [44], and spectrophotometry [45]. This method makes it possible to achieve a high preconcentration factor and greatly improves the selectivity of the analysis while keeping the procedure simple and environmentally friendly [3,7,8]. The CPE might also be used with dual detec-

tion, which was applied to analyze Fe^{3+} in blood samples. For ions, spectrophotometric and spectrofluorimetric detection was applied [46]. The examples of CPE with different analytical techniques are listed in Table 2.

Table 2. The examples of the application of CPE in different types of samples.

Substance Determined	Type of Sample	Detection Technique	Analysis Conditions	LOD/LOQ	Reference
Sm	Rock samples, wastewater	ICP-OES	Rf generator power—1200 W Plasma gas flow rate—12 L/min Auxiliary gas flow rate—1.0 L/min Nebulizer gas flow rate — 0.7 L/min Delay time—15 s Integration time—3 s Wavelength—358.160 nm.	LOD 0.06 µg/L, LLOQ 0.20 µg/L	[38]
Pb^{2+}	Tap water, rain water, waste water, urine, liver, hair	FAAS	Pb^{2+} determined as complex with 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone, radiation at λ = 283.3 nm.	LOD 1.49 µg/L	[23]
Ferulic acid (FA), chlorogenic acid (CLA), caffeic acid (CA)	Herb of dandelion	HPLC-DAD	C_{18} column (4.6 mm × 150 mm, 5 µm) at 30 °C. Mobile phase: MeOH:ACN:1% acetic acid (12.5:12.5:75, *v*/*v*/*v*), flow: 0.8 mL/min, detection at λ = 320 nm.	CLA: LOD 0.008 mg/L, CA: LOD 0.005 mg/L, FA: LOD 0.007 mg/L	[12]
1-deoxynojirimycin (DNJ), rutin, isoquercitrin, CLA, astragalin	Mulberry leaves	HPLC-UV	CuroSil-PFP RP column (250 mm × 4.6 mm, 5 µm), colum temperature 30 °C. Mobile phase: gradient elution: eluent A: 0.1% aqueous formic acid, eluent B: acetonitrile. For DNJ: acetonitrile: 0.1% aqueous acetic acid (45:55, *v*/*v*). Detection at λ = 360 nm, and λ = 254 nm (for DNJ); Flow rate: 1 mL/min.	LOD < 0.58 µg/mL, LOQ < 1.87 µg/mL	[13]
Au	Agricultural soil	ICP-MS	Au was collected at m/z 197. Pt m/z 195 was internal standard. Dwell time 10 ms.	LLOQ (calibration curve): 0.05 µg/kg	[39]
Amitriptyline, citalopram, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotline, mianserin, mirtazapine, moclobemide, nortriptyline, opipramol, paroxetine, protriptyline, sertraline, tianeptine, trazodone, trimipramine, venlafaxine	Human plasma	HPLC-ESI-MS/MS	Kinetex C18 (100 mm × 4.6 mm, 5 µm); Mobile phase: gradient elution: Eluent A: 0.1% (*v*/*v*) solution of formic acid, Eluent B: methanol with 0.1% (*v*/*v*) formic acid. Flow rate—0.75 mL/min.	LLOQ 10 ng/mL	[9]
Venlafaxine	Human plasma	RP—HPLC-FLD	Diamonsil C_{18} RP (250 mm × 4.6 mm, 5 µm) at 25 °C; Mobile phase—acetonitrile:phosphate buffer solution (pH 3.0):triethylamine (33.5:66.5:0.4) λex = 276 nm, λem = 596 nm. Flow rate: 1 mL/min.	LOD 2 ng/mL, LOQ 10 ng/mL	[10]
Bisoprolol	Human plasma	LC- ESI-MS/MS	Symmetry C_{18} column (150 mm × 4.6 mm, 3.5 µm) at 40 °C; Mobile phase—gradient elution: eluent A: 0.1% formic acid in grade water, eluent B methanol with 0.1% formic acid. Flow rate: 0.5 mL/min.	LLOQ 0.3 ng/mL	[8]
Vitamin E	Aqueous solution	HPLC-DAD	Waters Nova-Pack column C_{18} (150 mm × 3.9 mm) Mobile phase: 100% acetonitrile at λ = 220 nm. Flow rate: 1 mL/min.	LLOQ 0.1 µg/mL	[5]

Table 2. *Cont.*

Substance Determined	Type of Sample	Detection Technique	Analysis Conditions	LOD/LOQ	Reference
Pericyazine Chlorpromazine Fluphenazine	Human serum	GC	Non-polar fused-silica wide-bore capillary column (15 m × 0.53 mm, film thickness 1.5 µm; column temperature: 280 °C, injector and detector temperature: 290 °C.	LOD 1.5 × 10^{-6} mol/L	[42]
Paracetamol	Human urine	Spectrophotometry	λ = 580 nm (for direct spectorphotomeric determination); λ = 590 nm (for preconcentration procedure).	LOQ 0.13 µg/mL	[45]
Norfloxacin, ciprofloxacin, sarafloxacin, gatifloxacin	Water (lake, river, wastewater)	HPLC-DAD Fluorescence analysis	Chromatographic analysis: Agilent TC-C18 column (150 mm × 4.6 mm, 5 µm). Fluorescent spectra: the wavelengths for excitation/emission were ($\lambda_{exc}/\lambda_{em}$): 276/444 nm for norfloxacin, 278/449 nm for ciprofloxacin, 291/496 nm for sarafloxacin, and 288/486 nm for gatifloxacin.	LOD 0.007 µg/mL (norfloxacin), 0.010 µg/mL (ciprofloxacin), 0.009 µg/mL (sarafloxacin), and 0.013 µg/mL (gatifloxacin)	[6]
Se	Water (drinking, river, well, surface runoff, and spring)	ICP-MS	The surfactant-rich phase was mixed with concentrated HNO_3 and H_2O_2 and irradiated at 120 °C (1600 W) for 5 min and heated to 190 °C. $^{78}Se^{16}O^+$ was monitored with ICP-MS (triple quadrupole).	LOD 0.03 µg/L, LOQ 0.1 µg/L	[40]
^{231}Po	Urine, water, digested samples	α-spectrometry	α-spectrometer—^{231}Po was deposited on 1.0 mm silver disks.	LOD 3.5 mBq/L, LOQ 12 mBq/L	[41]
Te	Soil, water, sediment samples	ETAAS	The extractant phase was diluted with 50 µL of absolute ethanol and injected into the graphite furnace for analysis.	Te (IV) LOD 1.1 ng/L Te(VI) 1.7 ng/L	[44]
Bisphenol A	Milk	FAAS	Bisphenol A—Mn^{3+} complex was extracted to the mixture of CTAB and TX-114. The extract was diluted with methanol and analysed.	LOD 0.23 µg/L LOQ 0.76 µg/L	[43]

6.2. Determination of Xenobiotics in Biological Matrices

CPE can be used as a sample preparation method for drug analysis in biological matrices.

In the study conducted by Gniazdowska et al. [9], CPE was used to prepare the sample for analysis by LC-ESI-MS/MS. Twenty-one antidepressants were analyzed, including amitriptyline, citalopram, and clomipramine. Recoveries exceeding 80% were achieved for 85% of the compounds tested.

A study by Qin et al. [10] shows the determination of venlafaxine in human plasma by HPLC with cloud point extraction and spectrofluorimetric detection. The average extraction efficiency for venlafaxine always exceeded 90%.

Giebułtowicz et al.'s [8] study showed that extraction at the cloud point was compatible with LC-ESI-MS/MS for the determination of bisoprolol in human plasma. Plasma bisoprolol concentrations obtained by CPE and LLE methods were comparable in the 1.0–70.0 ng/mL range.

The study by Ohashi et al. [42] shows the use of cloud point extraction and preconcentration for gas chromatography of phenothiazine tranquilizers (pericyazine, chlorpromazine and fluphenazine) in spiked human serum. The recoveries of pericyazine, chlorpromazine, and fluphenazine were 95.1%, 87.1%, and 84.7%, respectively.

In a study by Filik et al. [45], a spectrophotometric method for determining paracetamol in urine was developed using tetrahydroxycalix[4]arene as a coupling reagent and preconcentration with TX-114 through cloud point extraction. The proposed CPE spectrophotometric method proved to be specific for p-aminophenol. It can be applied

to determining p-aminophenol or paracetamol in various pharmaceutical, environmental, and biological matrices (urine). The achieved preconcentration factor was 10.

In the study of Taha et al. [47], cloud point microextraction was used to determine mesalazine. The determination of mesalazine was based on forming a blue indophenol dye in an alkaline medium. In this case, the nonionic surfactant Triton X-114 and Na_2SO_4 were used. The developed method was applied to determine mesalazine in the pharmaceutical formulation and the biological matrix (serum). The developed method was characterized with a complete recovery of analyte from a matrix close to 100%.

6.3. Application of CPE in Environmental Samples

CPE is also used in various technological processes and in environmental samples. In a study by Ji et al. [12], this method was used to isolate and analyze phenolic acids from dandelion. The recovery of compounds, such as CLA, CA, and FA, at three different concentration levels ranged from 80% to 100%. Guo et al. [13] extracted DNJ, CLA, rutin, isoquercitrin, and astragalin from mulberry leaves. The compounds were extracted with TX-114. The applied technique was HPLC-UV. The average observed recoveries for spiked samples for the analyzed compounds were within the range of 92.28–98.81%.

Cassero et al. applied the acid-induced CPE for the analysis of vitamin E as an example of a thermal labile compound [5]. The analyte was extracted with sodium dodecanesulfonic acid at a low temperature (10 °C). The observed recovery was high—80–85%— which proves that CPE is a suitable technique for the extraction of thermal-sensitive compounds.

Xia et al. [6] used CPE to determine fluoroquinolones in samples from wastewater treatment plant. The recovery of fluoroquinolones from the water samples (lake, river, wastewater) ranged from 83.0% to 93.6%. Nekouei et al. reported the application of CPE in the determination of arsenic ions in water samples from different areas such as rivers, tap water, well water, and lake water [37]. The recovery value exceeded 95%. The other elements that may be extracted from drinking water were iron, lead, and cadmium using TX-114 [48].

In the study by Mortada et al. [38], CPE combined with ICP-OES, was used to analyze samarium as Sm^{3+} in environmental samples—wastewater and rocks at trace levels. The procedure showed favorable analytical properties for Sm^{3+}, such as a low limit of detection (LOD), wide dynamic range, and high enrichment factor of 102. The observed recovery was high—up to 99%.

Manzoori et al. applied FAAS in the determination of Pb^{2+} in various matrices (urine, tap water, rain water, wastewater, liver, or hair). In this study, the optimized parameters were pH, concentration of chelating agent and surfactant, equilibration temperature, and incubation time. The enhancement factor was 110 for the optimal conditions, which provided a low LOD of 1.49 μg/L [23].

Yang et al. applied CPE in the Se analysis [40]. In this method, Se(IV), Se(VI), and Se nanoparticles were determined in water samples such as drinking water, river water, well water, surface runoff water, and spring water. The observed recoveries were within the following ranges: 65.5–113% (for Se(IV)), 80.3–131% (for Se(VI)), and 61.1–104% (for Se nanoparticles). The applied method of analysis was an ICP-MS.

The CPE-aided analysis of trace elements was applied in the case of ^{210}Po. The observed preconcentration factor reached 78. The measurements were conducted with an α-spectrometer, and the analyzed matrices were urine, water, and digested samples. The recovery for reference materials was high and it ranged for solid samples from 87 to 104%. In the case of spiked aqueous samples, it was 95–107% for water and 93–108% for urine [41]. Llaver et al. analyzed total Te, Te (VI), and Te(IV) concentrations in soil, water (tap, underground, and seawater), and sediment samples [44]. In this study, the

following parameters were optimized: surfactant concentration, time and temperature of the CPE procedure, and the conditions of ETAAS, which was a detection technique. In this analysis, TX-114 was applied with the addition of 1-octyl-3-methylimidazolium chloride for extraction improvement. The observed extraction efficiency was high—90%—and the enhancement factor was 87. The determination of total Te required the pre-reduction step where all Te(VI) was reduced to Te(IV). Te(VI) was calculated as a difference between total Te and Te(IV).

The other interesting application of CPE combined with ICP-MS was the analysis conducted by Wei et al. [49]. This was an analysis of nano-silver sulfide, which results from the transformation of silver nanoparticles, which are an emerging pollutant. The sulfide was identified in water samples with an extraction rate within the 76–106% range. The same analytical technique was applied by Hadri et al. [39] for the determination of gold nanoparticles from soil. The extraction process yielded very high recovery, which exceeded 90%, with an enrichment factor of about 10 times. The extraction was enhanced with the addition of HNO_3, NaCl, citric acid, or EDTA. Lai et al. [50] used Au-nanoparticles for labeling various nanoplastics of common plastic types (PS, PP, PE, PET, PMMA, and PVC) in water samples. The sucrose density gradient centrifugation separated the Au-labeled nanoplastics The applied method was ICP-MS. The observed spiked recoveries were within the range of 72.9–92.8%.

CPE was also useful in the analysis of bisphenol A in a milk sample [43]. Bisphenol A was determined as a Mn^3+-chelate complex by FAAS in this method. The complex was formed at pH = 5.0 and extracted to the micellar phase, consisting of a nonionic and ionic surfactant. The preconcentration factor was 70 with 41-fold sensitivity improvement. In this analysis, an indirect Mn^{3+} response was observed.

CPE may be successfully applied in food processing [17]. It can be useful in the extraction of bio-actives that are present in food. The following substances were extracted using this technique: lycopene, olive mill, betaine, and wine sludge. The applied surfactants were Tween-80, Span-20, and TX-114 [51–56].

7. Conclusions

Cloud point extraction is a promising technique for preparing samples for analysis, distinguished by its wide compatibility with various substances, matrices, and analytical methods. It is successfully applied to analyzing food, drug, metal, and environmental samples. Its simplicity, low cost, and minimal toxicity make it an operator- and environmentally-safe method. The cost may also be reduced when the CPT of the surfactant is low due to the addition of neutral salt—there might be no need to heat the sample. It will also save time.

This technique can be combined with various analytical techniques, as shown in Table 2. The promising direction in the development of CPE is the forensic analysis of psychotropic substances. Many techniques of sample preparation have been reported. However, CPE is not widely used to analyze these substances, and other techniques of sample preparation still dominate [57]. The different types of applied detection prove that this technique is universal for analyzing different types of samples, such as biological, food, and environmental ones. The simplicity of CPE makes this technique useful also in the application of therapeutic drug monitoring. The sample preparation is fast, which is a very important issue. CPE might also be successfully applied in industry and quality control. The other promising direction for development is trace analysis. Recent studies proved that it might be useful in the analysis of such elements as Po, Se, Te, nanoparticles of Ag, nanoplastics, and chemicals such as bisphenol A [40,41,43,44,49,50]. According to Wei et al. [49], determining Ag as Ag-sulfide nanoparticles provides an idea for the determination of other metal sulfide nanoparticles.

With multiple factors to optimize recovery, CPE can be tailored to the specific properties of the substances being analyzed, increasing its versatility. In the context of optimizing the extraction process, important parameters include surfactant concentration, temperature, centrifugation time, and the presence of salt. These factors can be precisely controlled to maximize analyte recovery efficiency and minimize losses. In addition, modifications in the extraction technique, such as using different surfactants or supporting additives, make it possible to expand the list of substances that can be efficiently extracted using CPE. The effectiveness of CPE might be improved by applying an optimization procedure involving chemometric models such as Central Composite Design [35]. These models may be applied interchangeably with the model of machine learning, such as artificial neural networks [14].

The eco-friendliness of CPE is due to the use of small amounts of non-toxic surfactants and salts, which significantly reduces the environmental burden compared to traditional extraction methods, which often require large amounts of toxic solvents.

Further research into optimizing this technique could lead to even wider application in the analysis of a variety of substances.

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Abbreviations

The following abbreviations are used in this manuscript:

CA	Caffeic acid
CLA	Chlorogenic acid
CMC	Critical micelle concentration
CPE	Cloud point extraction
CPT	Cloud point temperature
CTAB	Cetyltrimethylammonium bromide
DNJ	1-deoxynojirimycin
ESI	Electrospray ionisation
ETAAS	Electrothermal atomic absorption spectrometry
FA	Ferulic acid
FAAS	Flame atomic absorption spectrometry
FLD	Fluorescence detector
GC	Gas chromatography
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma–optical emission spectrometry
LC-ESI-MS/MS	Liquid chromatography–electrospray ionization–tandem mass spectrometry
LLOQ	Lower limit of quantitation
LLE	Liquid–liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation
SDS	Sodium dodecyl sulphate
TX-114	Triton X-114

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