




A review on separation and detection of copper, cadmium, and chromium in food based on cloud point extraction technology

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Abstract

Due to its environmental efficacy and economic effectiveness, a unique approach for extracting functional thermally sensitive bioactive components from food was recently developed. Cloud point extraction is one of the most effective alternative methods for extracting a wide range of organic and inorganic components from green surfactants. The extraction method is cloud point extraction by phase separation, which is a quick and easy approach that requires very little solvent and just a small amount of very non-flammable and non-volatile surfactant that is environmentally benign. The theoretical results of cloud point extraction's application in the food industry are summarized in this article. While presenting a series of introductions on how to extract cloud points, the benefits and applications of cloud points have been studied. The method of using cloud point extraction in food samples has been explained in this article. This method is simple, safe, cost-effective, and widely used to measure a variety of tissue samples, and it can detect analytes down to nanograms per millimeter. Spectrophotometric measurement of low levels of cadmium in some vegetables was also developed in the current study as one of the most important applications of the cloud point extraction method in the food industry.

Keywords: food industry; cloud point extraction; cadmium; vegetable.

Practical Application: Cloud point extraction's application in the food industry.

1 Introduction

Due to the technological, scientific, and economic advantages of conventional extraction techniques, the food business has seen a revolution in the development of more green and efficient technology for extracting food bioactive (Singla & Sit, 2021; Zakaria & Kamal, 2016; Molajou et al., 2021). These food bioactives are essential nutrients found in extremely tiny amounts in food (Silva et al., 2017; Rodrigues et al., 2016; Kadbhane & Manekar, 2021). Antioxidant, metal chelator, anti-allergic, antibacterial, and clarifying agent, these components have a wide spectrum of biological action (Gullón et al., 2020; Saini et al., 2020). The most popular techniques for extracting biological molecules from food matrixes include enzyme aided extraction, ultrasound assisted extraction, microwave-assisted extraction, solid-liquid extraction, and liquid-liquid extraction. The degradation of bioactive during extraction, which is susceptible to temperature, light, and oxygen, is a key

issue in these methods (Chemat et al., 2015, 2020; Ekezie et al., 2017; Kipriyanov et al., 2021).

Extraction may be done using novel technologies such as supercritical fluid, solid-phase microextraction, and liquid-phase microextraction, but they are expensive and need specialized equipment. When heated to a critical temperature or above, cloud point extraction (CPE) is a method for extracting organic/inorganic chemicals from chemical or biological systems utilizing benign extractants such as nonionic surfactants that tend to separate from the bulk solution and create clouds (Bagade & Patil, 2021). To extract a chemical species from natural or laboratory samples for analysis, pharmaceutical, food, or industrial applications, other chemical species must be removed from the sample. Extraction methods refer to all physical or chemical processes and processes used in this regard, with micelle systems being one of the most widely used extraction methods (Chemat et al., 2020).

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In recent decades, development, extraction, and concentration steps have been considered to measure very small amounts of compounds in aqueous solutions. A high-sensitivity decomposition, selectivity, and separation method should be used to measure small amounts of contaminants in water, soil, and other biological samples (Azmir et al., 2013).

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are the most commonly used methods for ion extraction and concentration in aqueous samples (McKenna et al., 2021; Wang et al., 2020). Solvent extraction methods use a lot of solvents and don't recycle them very well. As a result, there is a strong trend to replace solvent extraction methods with other methods to reduce sample manipulation and analyte waste and avoid using toxic solvents. However, the issue with both LLE and SPE is the long extraction time and the high manual operation (Huajun et al., 2020). Other developed methods have reduced and eventually eliminated the use of organic solvents (Barros et al., 2018; Canbay, 2017). Solid-phase microextraction (SPME) is a technique for removing organic compounds from biological samples. The analyte equilibrium distribution between the static phase of the polymer and the sample matrix is the basis for SPME. It takes more than an hour to sample using this method (Gómez-Ríos et al., 2017; Khan et al., 2020; Pang et al., 2020).

The use of cloud point extraction for extraction and concentration is a suitable method for most conventional extractions. The aqueous solution of some surfactants was used in this method. Surfactants' high capacity to dissolve different species is one of the most important properties from an analytical standpoint. It allows a substance with low solubility and insolubility in water to be easily dissolved in water. We can concentrate the analyte in one step with a small amount of concentrated surfactant and then measure it with gas chromatography (GC) and high-performance liquid chromatography (HPLC) in the next step. This method is non-toxic and low-cost.

2 Cloud point extraction

The characteristic that a solute contained in the aqueous solution of non-ionic surfactant is dispersed across two phases is the basis for cloud-point extraction (Mohd et al., 2018). It has recently garnered a lot of interest because of its remarkable potential for separating hazardous solutes from various matrices. Micelle-extraction, micelle-mediated extraction, and liquid-concentration techniques are all terms used to describe these techniques (Kori, 2019). Surfactants are often absorbed in the phase interface, where the polar head points to the watery portion

and the hydrophobic tail points to the lipophilic layer (Kwok et al., 2019; Tian et al., 2019). Micelles can range from rough spherical to ellipsoidal in shape, depending on the surfactant and solution conditions (Tan et al., 2020). Degree or aggregation number refers to the number of surfactants present in a micelle (Sun & Bandara, 2019). The number varies depending on the kind of surfactant, the structure of the groups present, the electrolyte's properties and concentration, the nature of the solvent, the temperature, and the pH of the solution. Table 1 compares the cloud point extraction approach to the traditional method (Arya et al., 2019).

Cloud point extraction is a separation and preconcentration method for determining small amounts of metals that have a wide range of applications. The simplicity, low cost, large preconcentration factor, and healthy environment are the main benefits of this method. This includes analytical methods that follow green chemistry principles (Rahnama & Najafi, 2016).

Because the goal of this method of work is to reduce or eliminate the toxic species that are produced (harmful to human health and the environment). It is also devoid of steam and ignition. In chemical analysis, isolation and preconcentration have a lot of value (Citak & Tuzen, 2010). The effect of the matrix is either eliminated or minimized in this method. They use various techniques to lower the detection limit for metals and increase the detection power for the majority of them. Its large preconcentration factor is one of its most significant advantages over solid phase extraction (SPE), liquid-liquid extraction (LLE), and co-precipitation methods (Mousa et al., 2020; Tang et al., 2020; Tang & Lee, 2013).

As a result, organic species and metal ions can be isolated and pre-concentrated using cloud point extraction. One of the major environmental chemistry research projects has been developing a simple, fast, inexpensive, and selectable method for extracting and measuring pollutants in the environment (Baggiani et al., 2007; Ramalhosa et al., 2009).

3 Surfactants

Food surfactants are ingredients used to make gels, suspensions, and emulsions, among other things. The Food and Drug Administration (FDA) must approve these items. Many culinary items, including desserts, dressings, creams, salad, and mayonnaise, include naturally occurring surfactants such as lecithin from different proteins from milk and egg yolk (Nitschke & Silva, 2018). Polar lipids, such as monoglycerides, were later used as food emulsifiers. Synthetic surfactants, including

Table 1. Traditional and cloud point extraction methods are compared.

Cloud point extraction	Solvent extraction
Lower toxicity; use of benign surfactants	Use of toxic and flammable organic solvents
Simple and rapid; reduce extraction time	Longer extraction time
Powerful even at low concentrations of surfactants	Low concentration efficiency for solute
Provides high preconcentration factors even with low concentrations of surfactants	Mass action of extractant is decreased due to dilution
Inexpensive	Expensive
Negligible environmental pollution	Some concerns regarding the environmental pollution caused

ethoxylates and sorbitan esters, as well as sucrose esters, have lately been utilized in food emulsions (Kralova & Sjöblom, 2009). Surfactants (emulsifiers) and hydrocolloids are used in a variety of food compositions. Proteins that act as emulsifiers are found in a variety of foods (Dickinson, 2009). Surfactant is a term that combines the words “surface active agent” and “surfactant.” Organic compounds with hydrophobic groups in the tail and hydrophilic groups in the head are known as surfactants. R, a hydrocarbon chain with 8-18 carbon atoms and X polar and hydrophilic ions, is an R-X surfactant. Surfactants adsorb the air-water interface, lowering the surface tension of water (Myers, 2020). They also adsorb the liquid-liquid interface, which reduces the elongation of the water-oil interface. The nature of their hydrophilic group, which is divided into four groups: nonionic, cationic, anionic, and bipolar ions, is used to classify these materials (Bhattacharya & Samanta, 2011).

4 Micelles

Around 85 years ago, an intriguing relationship was discovered between the physicochemical properties of surfactant solutions and their concentration in the solution, indicating abrupt changes in the physicochemical properties of these solutions over a small concentration range (Pal et al., 2018). The aggregation of divalent molecules was blamed for these changes, and concepts like micelle and critical concentration were proposed to explain it. Surface activating molecules are monomeric in very dilute solutions (Shinoda et al., 2016). They may also be present in the form of dimers and trimers, of course. Aggregation and micelles form spontaneously when the concentration of surface-activating molecules reaches a suitable level. The hydrophobic part of the micelle is in the center, while the polar groups interact with the water surface and are hydrated by a number of water molecules in this accumulation. Micelles can be cationic, anionic, dual ionic, or non-ionic, depending on their chemical structure (Khan et al., 2008).

4.1 Types of micelles

Normal micelles and inverted micelles are the two types of micelles that exist. The hydrophobic groups of the tail direct the activating molecules inward in polar solvents, while the hydrophilic groups direct the head toward the polar solvent, forming ordinary micelle masses. The end groups of surface-activating molecules are directed inward, forming the hydrophilic nucleus of the micelle, while the non-polar sequences (tail groups) interact with the solvent during the aggregation process in non-polar solvents. Inverse micelles are the name for these micelles (Glatter & Salentinig, 2020).

The temperature completely determines the size of the micelles. A decrease in temperature causes an increase in the diameter of the micelle for non-ionic properties in the aqueous medium, which is due to the exotherm of an anvil to the micelle structure at low temperatures. In a non-aqueous medium, light scattering studies for non-ionic properties reveal a direct relationship between temperature and micelle size. Surfactants are used in the cloud point extraction method to extract the analytes, which are then added to the sample solution, and the pre-concentration process is completed (Yang et al., 2018).

5 Cloud point extraction steps

To begin, the surfactant is added to the sample, followed by the addition of salt if necessary to increase extraction. The solution is then kept at a specific temperature for a set period of time until it becomes turbid and reaches a critical temperature, after which centrifugation and extraction are performed. The sample is collected in the aqueous phase, and the supernatant is separated in this manner. Liquid-liquid extraction is similar to this method. Liquid-liquid extraction is similar to this method. The steps of this method are as follows (Wang et al., 2010):

- Surfactant is added to the sample first
- Add salt to increase extraction
- Storage for a specified period of time at a specified temperature, until the solution becomes cloudy (cloud point)
- Centrifuge
- Isolation of the sample on the aqueous phase and collection of the injection supernatant for separation

The benefits of this method can be summarized in four sections (Kori, 2019):

- 1) High concentration factor is available with this method.
- 2) Surfactants are cheap and do not pollute the environment.
- 3) Surfactants are used in small volumes.
- 4) It is possible to work for temperature-sensitive compounds at low temperatures.

6 Application of cloud point extraction

The application of cloud point extraction can be divided into eight general categories (Katsoyannos et al., 2006):

- 1) Separation of metal ions such as aluminum, chromium, etc.
- 2) Separation of polycyclic aromatic hydrocarbons
- 3) Separation of polychlorinated compounds
- 4) Isolation of biological molecules such as proteins (casein, alpha-lactalbumin, etc.)
- 5) Separation of pesticides
- 6) Separation of lanthanides and metal mixtures from aqueous samples
- 7) Separation of fat-soluble vitamins
- 8) Separation of chlorophenols, phenols, and benzyl alcohols from aqueous samples

6.1 Cloud point extraction and spectrophotometric determination of cadmium in some vegetables

Cadmium is a toxic metal that is harmful to all living things, including humans. Water and food are two of the ways this metal enters the body. Given that cadmium is present in some chemical

fertilizers and can thus cause soil contamination, the question is whether this contamination causes cadmium to enter processed vegetables from this soil (Genchi et al., 2020; Sharma et al., 2015).

This paper aims to present a new method for determining cadmium in various vegetable samples based on cloud point extraction and spectrophotometric measurements. Figure 1 depicts the cadmium and dithizone complex structure, while Figure 2 depicts the dithizone absorption spectrum and its cadmium complexation (Ismael, 2014; Moghimi & Yari, 2019). Because this complex is insoluble in water, spectrophotometric measurement requires a solvent extraction step. The resulting complex is extracted from the Triton X-114 surfactant in the mycelial phase after the Dithizone and Cadmium complex has formed, and its amount is determined by absorbing the maximum complex in the area of 100 nm (as shown in Figure 2) (Teodorowicz et al., 2017). The effects of several variables were investigated, including pH, concentrating agent concentration, surfactant content, temperature effect, and reaction time. The results are shown in Table 2 (Souza et al., 2020):

The current method offers a number of benefits, including low cost, high safety, and high extraction power. It also has an acceptable detection and repeatability limit. The method can be used to detect cadmium in a variety of vegetables, and the results have been promising.

6.2 Application of cloud point extraction for separation of protein from cow's milk and separation by mass spectrometry

Polyoxyethylene and isoactyl phenyl ether (Triton X-114) were used as nonionic surfactants in this study, along with NaCl

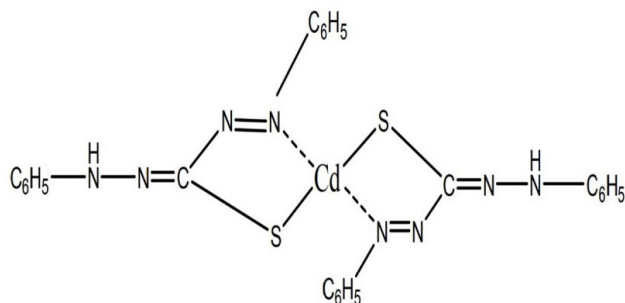


Figure 1. The structure of the dithizone-cadmium complex.

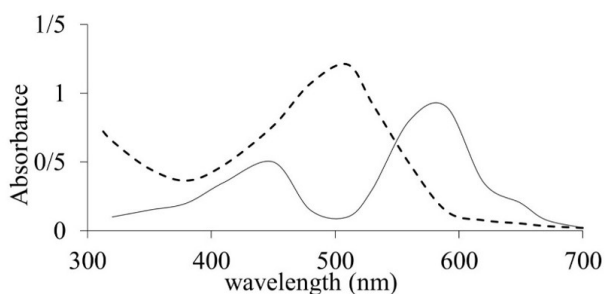


Figure 2. Absorption spectra of dithizone (solid line) and its complexation with cadmium (dotted line) in Triton X-114 solution.

(Lamichhane et al., 2017). All experiments were carried out at 25°C, and after adding NaCl and surfactant to the cow milk sample, the two rich and weak surfactant phases were separated using a centrifuge at 1780 g for 15 min. The effect of factors such as Triton X-114 concentration, sample volume, NaCl concentration, and pH as parameters affecting extraction efficiency was investigated in this study, and then the cloud point extraction method was used to isolate it using MALDI-TOF mass spectrometry (Avadhani & Sen, 2021).

The most important effective factors in this study, according to the findings, were surfactant concentration and sample volume. When the Triton X-114 surfactant concentration was 1% by weight to volume (W/V), the sample volume was 50 l, the salt concentration was 6%, and the pH was 7, the highest effect on extraction efficiency was observed.

6.3 Application of cloud point extraction in the separation of antioxidant compounds (phenols) from the effluent of olive factories

The use of the surfactant Triton X-114, which has been shown to extract phenolic compounds from aqueous media, was investigated in a study (Ray et al., 2018). The effect of surfactant concentration on individual phenolic compounds as well as the total extracted concentration of phenolic compounds in three stages of extraction was investigated in this study. The Olive mill wastewater (OMW) sample was obtained from the Argos (Greek) factory and kept refrigerated at 6 °C until testing.

Other phenolic compounds have extraction efficiency above 90% in the study of the effect of surfactant at a constant concentration of 4% by volume on extraction efficiency (shown in Figure 3), with the exception of gallic acid, which has an

Table 2. The optimal value of effective parameters in the present method.

Parameter	The optimal amount
PH and Buffer type	PH=5 and Acetate buffer
Dietizone	3×10^{-5}
Triton X-114	0.6%
Temperature	65° C
Reaction time	15 Min

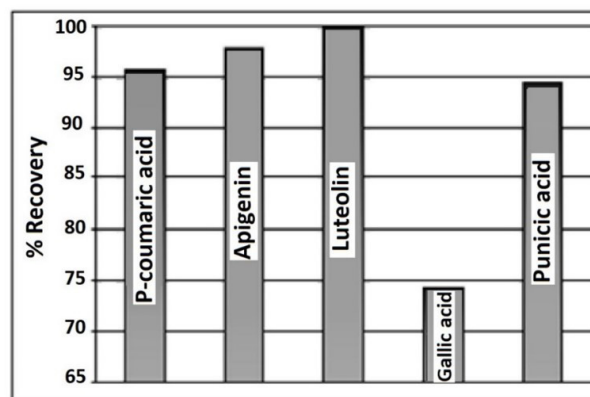


Figure 3. Investigation of the effect of surfactant at a constant concentration of 4% by volume on extraction efficiency.

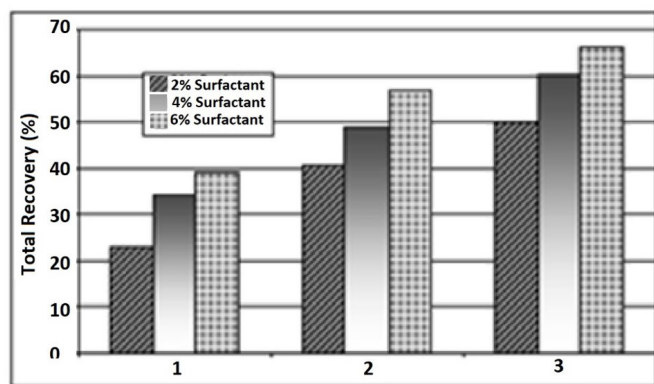


Figure 4. Investigation of surfactant concentrations from 2 to 4 and 6% in 3-step extraction on recovery rate, respectively.

efficiency of 74.2% (Ray et al., 2018). The recovery rate in 3-step extraction increased in the next stage of the experiment, when the concentration of surfactant was increased from 2 to 4 and then to 6% (Figure 4) (An et al., 2017).

The cloud point extraction method developed by Triton X-114 can be used to successfully separate phenolic compounds from aqueous media, with extraction efficiencies exceeding 96% in some phenolic compounds such as Apigenin, Luteolin, and p- Coumaric acid.

7 Conclusions

CPE is a potential technique for extracting bioactive from food, according to our findings. Extraction of bioactive from food processing by-products, as well as separation and purification of proteins, have been the focus of this review. Because of the benefits of CPE over other techniques, its application in food processing is quite broad. Apart from bioactive and protein extraction from food, sample preparation for food constituent analysis has attracted significant focus in the study. As a result, it is possible to conclude that the spectrum of applications for CPE in food processing may be expanded in a number of ways. Cloud point extraction is a powerful analytical technique for improving and increasing the detection of metals, pollutants, biological molecules, and other substances. The use of a micelle system raises the concentration factor, which boosts recovery. This method is also simple, safe, cost-effective, and widely used in measuring a variety of tissue samples, with the ability to detect analytes as small as nanograms per milliliter, such as ions in water.

The spectrophotometric measurement of low amounts of cadmium in some vegetables was developed in this paper as one of the most important applications of the cloud point extraction method in the food industry. Dithizone reagent was used as a complexing agent, and triton Triton X-114 surfactant was used as a micelle phase in cloud point extraction. Several parameters were investigated that were effective in this extraction. For cadmium, a detection limit of 0.2 ng/mL and a linear range of 1.0 to 180 ng/mL were obtained under ideal conditions. The pre-concentration factor was found to be ten. When the method is used to determine cadmium in real vegetable samples, it produces good results.

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