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Article

A new method for extracting microfluidics by combining two droplets into single droplet using a mixing and diffusion-based droplet system mechanism

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Abstract: A new and highly efficient method for extracting micro-sized liquids using droplet system is presented by mixing two microdroplets to form a single droplet by utilizing mixing and diffusion mechanism. This working system has the potential It is applied to a wide range of chemical and biological analysis. It is very suitable for microfluidics, has short operation time, no environmental pollution, and has a simple assembly design that can be installed anywhere in the laboratory. The main working design consists of several main components: a two-line micropump device to pump or withdraw the aqueous and organic layer through two rubber microtubes connected to two microneedles (with specific size) to generate aqueous and organic droplets. The two needles are fixed by two magnetic holders based on two graduated holders, and then the microextraction is enhanced by a micromixer to complete the mixing process. by diffusion. Several key variables affecting this microextraction were studied, including the optimal concentration of copper (II) chloride (10-4M) representing the metal ion, and the optimal concentration of diathazone solution (10-4M) representing the organic reagent solution. The effect of micropump speed (200 µl/30 s), micromixer speed (maximum), micropump type rubber tubing (Tygon E-Lab tubing 1.2 mm) was studied, and under these optimum conditions the extraction degree was (97±2%).

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1. Introduction

Liquid-liquid extraction is one of the most widely used sample pretreatment techniques for chemical and biological analyses[1] .However, it has many drawbacks and disadvantages[2], such as taking a long time, consuming large quantities of organic solvents, and the need for employees trained in these traditional methods. To solve these problems, new techniques have been proposed with distinctive properties, including microextraction[3]. Which is characterized by high sensitivity, small sample size, and rapid extraction and the small volume of solvent[4]. It is divided into:

solid phase microextraction (SPME) And liquid phase microextraction (LPME), from which the single-drop microextraction technique emerged, which is an advanced and distinctive technology with high analytical capabilities and can be applied to liquid samples that contain non-polar or moderately polar analytical products. This technique was characterized by simple experimental setup, reduced equilibration time, enhanced extraction, reduced contaminants and interference, and drop stability. The extraction efficiency in this technique depends on both the distribution coefficient (K) of the target analytical product and the initial concentration of the analytical product in the sample (C) [5]. Both extract volume and sample volume play an important role in determining the equilibrium concentration that results in decomposition (extraction efficiency). So that every drop in the system of this technology is considered a tank and a vessel for the reaction. . Each drop of the extract is subjected to a drop of sample containing target materials in the process of microfluidic extraction between two phases, the organic phase and the aqueous phase[6]. The formation of these drops depends on a process called (the typical solvent exchange process), and a mixing zone is formed between the liquids, thus becoming The concentration of the organic phase in the mixing zone is too much[7]. The extraction efficiency in this technique depends on. Diameter of the nozzle or needle. The extraction rate increases as the diameter of the needle or nozzle decreases, which in turn leads to a decrease in the surface area of the droplet obtained. The chemical reaction will take place at the interface of the organic-aqueous phase. [8]

2. Materials and Methods

All solid and liquid chemicals used in this study were provided by BHD and Fluka and are of high purity. Dithizone ($C_{13}H_{12}N_4S$) (Fluka) solution at a concentration of (10-2M) was prepared by dissolving (0.0256 g) in (10 ml) of $CHCl_3$ (BDH). . For the requirements of the study, another set of dithizone solutions with a concentration of (10-2M) was prepared by taking (5 ml) of the previous dithizone solution (10-2M) and diluting it in (50 ml) of $CHCl_3$ in a standard volume. bottle, and store the solution in a dark bottle away from light. An aqueous solution of copper (II) chloride ($CuCl_2.2H_2O$) (Fluka) was prepared at a concentration of (10-2M) by dissolving (0.42625g) in a small volume of distilled water, then completing the volume to (250 ml). of distilled water in a standard volume vial and save the solution for use. For the requirements of the study, another set of $CuCl_2$ solutions with a concentration of (10-4M) was prepared by taking (0.5 ml) of the previous copper (II) chloride solution (10-2M) and diluting it in (50 ml) of the previous copper (II) chloride solution (0.01M). Distilled water in a standard volumetric flask, when the concentration is standard Cu(II) (0.635 ppm). The acidity of the $CuCl_2$ solution is adjusted to pH = 1, and the pH is determined by the pH meter by adding a small amount of hydrochloric acid (BDH).

Equipment

The system requires several devices to operate in a suitable microenvironment. Ismatec two-line micropump pumps or withdraws the reagent liquid (organic) and the metal ion liquid (aqueous), a specially designed rechargeable micromixer, WTW - SERLES Germany pH meter, to determine the pH of the microfluid, P.G-instrument atomic absorption device, which is used to measure the residual copper (II) content in the organic layer..

How to make the method

This method of work involves: generating micro-droplets by pumping a dithizone solution with a concentration of (10-4M) [9] using a peristaltic micropump, across the rubber microtube connected to a microneedle to generate drops of the organic and aqueous phases at a rate of (1drop /12 seconds) and mix it simultaneously during its formation by directing the two needles perpendicularly so that the aqueous and organic drop becomes one mixed drop and then descends on mixer, s surface to complete the extraction process, after setting the pumping speed at (200μl /30 s)for a specific volume to a specific volume of Cupper chloride(II) after determining The optimal conditions for completing the microextraction process are: the temperature (25-27°C), pH = 1, and the speed of the micromixer is max. [10-12]

3. Results and discussion

Microdroplet Extraction System Design

new design of micro-extraction for generating and mixing aqueous droplets (for the metal ion solution) and organic (for the reagent solution) and mixing them simultaneously through the surface tension property of the droplets, followed by using a micro-mixer that works with a special mechanism for spreading the droplets, which completes the extraction process. This system provides a mechanism for repeating the extraction process a large number of times in a short time for the extraction process. The components of this design for the operation of the droplet system consist of the following:

1. Two micro-needles for generating the aqueous droplet and the organic droplet. Each needle is fixed in a specific way by a small magnetic holder and moves on a graduated holder (1-30 cm).
2. A two-line micro-pump device, to transfer the reagent solution and the sample by passing it through the micro-rubber tubes (special) to the two micro-needles.
3. A micro-mixer (works with a specific mechanism) at three different speeds (slow, medium and high).
4. Beaker(20 ml) in which the micro-extraction process is carried out.

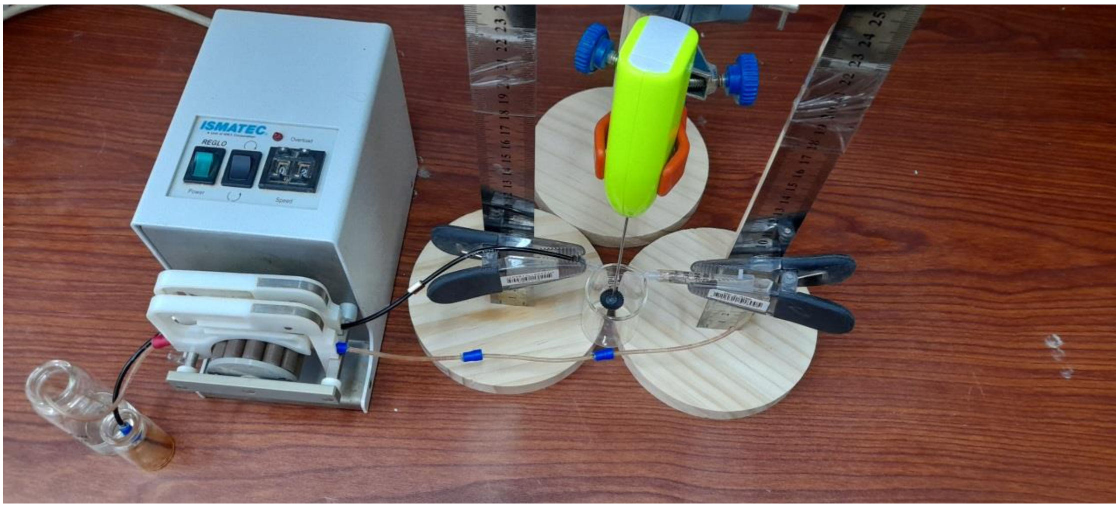

Figure 1. . Design of a microextraction system with pre-mixed droplets.

Stages of a microextraction system with pre-mixed droplets

The basis of the reliability of this new method of micro-droplet extraction is the generation of aqueous droplets and organic droplets of micro size at a rate of (1droplet/ 12 sec). The micro needle generates an aqueous droplet and the other micro needle generates an organic droplet. The two needles are perpendicular in direction, which allows the two droplets to mix during their formation period to generate a single mixed droplet (aqueous - organic) [13]. Here the first features of (complexation) appear, represented by the micro-extraction by droplets. At the beginning of mixing, we will see the color of the two droplets change from a light red droplet to a dark red gradually, as the aqueous droplet of copper(II) chloride will react with the diathazone droplet represented by the organic reagent to form (the resulting complex). When the mixed droplet falls down vertically due to acceleration, it will fall on the surface of the micromixer, whose working mechanism has been studied to be compatible with the droplet system. The single falling droplet will spread into very small particles and will be distributed in the form of a dark red circular halo floating on the inner surface of the baker[14]. This system will be repeated after each other droplet, which achieves a very high-efficiency micro-extraction. After the micro-extraction is completed by the droplets and the two layers are clearly and stable, the aqueous layer can be withdrawn in reverse from the micro-pump and a sample taken from it for the atomic absorption device to measure the remaining copper percentage and thus calculate (K) value and the efficiency of the microextraction[15].

Figure 2. Stages of micro extraction for droplets process

study of the mechanics of droplet transport and their integration in single-drop microextraction

Initially, equal micro-volumes of copper (II) chloride solution and dithiazone solution are determined to be compatible with microfluidics within the limits of (1000-100000 µl) because the design of this method is consistent with the principles of microfluidics. After that, the droplet height is determined, preferably (12 cm) or higher to ensure high mixing and obtain high efficiency for the extraction process[16,17]. The temperature is fixed because it directly affects the droplet system, and the micropump speed is fixed at a medium speed (200 µl / 30 seconds) because the micro-extraction process with drops requires a state of equilibrium and mixing between the mixed droplet of the organic reagent and the aqueous metal ion. Finally, the micro-mixer is turned on and the high speed is chosen to obtain a high extraction result through the wide diffusion process of the micro-particles of the organic and aqueous droplets and the appearance of the floating halo, which is the basis of the micro-extraction process in a dark red color similar to the violet color, indicating that the micro-extraction process is carried out quickly and excellently. This is evident from the beginning of mixing the first drops of the first organic droplets of the reagent with Metal ion water droplets[18].

The effect of the conc. of cupper chloride(II) and dithizone on the extraction.

Studies of thermodynamic equilibrium and kinetics involving microextraction processes have confirmed that the concentration of metal ions has a significant and direct effect on the composition and stability of the extracted compound, which is important in obtaining the best values of the distribution constant D and the degree of extraction %E. Therefore, the effect of different concentrations (10^{-2}M,10^{-4}M) of copper (II) chloride was studied by taking samples with micro-sized equivalents to the micro-sized equivalents of dithizone (the organic reagent). It was found that the optimum concentration that gives high extraction values is (10-4M), Because the extraction process by droplets in this way is compatible with dilute concentrations. while fixing the rest of the optimum conditions[19].

Table 1.Optimal copper chloride (II) concentration for the droplet extraction

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sample	Copper chloride (II) conc.	D	E%
1	10^{-2}M	10	70
2	10-4M	15	90

The effect speed of the micropump on the microextraction

The system of uniting two droplets into a single droplet is mainly based on the generation and mixing of droplets, so the prominent and fundamental role is for the micropump in this system, as the formation of organic and aqueous droplets and their mixing process depend on the speed of the micropump. The experimental results indicated that the average speed(200µl/30s)of the micropump achieves the best efficiency for microextraction due to the sufficient time for the formation of droplets and the completion of the mixing process of organic and aqueous droplets during their formation. Changing in the speed of the micro pump was studied to four speeds: (µl/10s), (µl/20s), (µl/30s), and (µl/40s). The reason is that the generation and mixing of droplets requires a special mechanism that is compatible with the micro-sized environment, so that each water droplet mixes with an organic droplet to form a single mixed droplet and will descend vertically on the surface of the special micro-mixer at a rate of one falling droplet per 12 seconds. This system requires the micro-pump speed to be fixed at a moderate speed (200 µl) to mix the droplets well, and the pump speed when it is high does not allow the extraction and mixing process to be sufficient and effective. With the rest of the main conditions fixed in terms of moderate concentrations of the reagent and the metal ion and the selection of the appropriate micro-rubber tubes(Tygon E-Lab Tubing1.2mm) ,and the selection of the appropriate droplet height(12cm) [20-22].

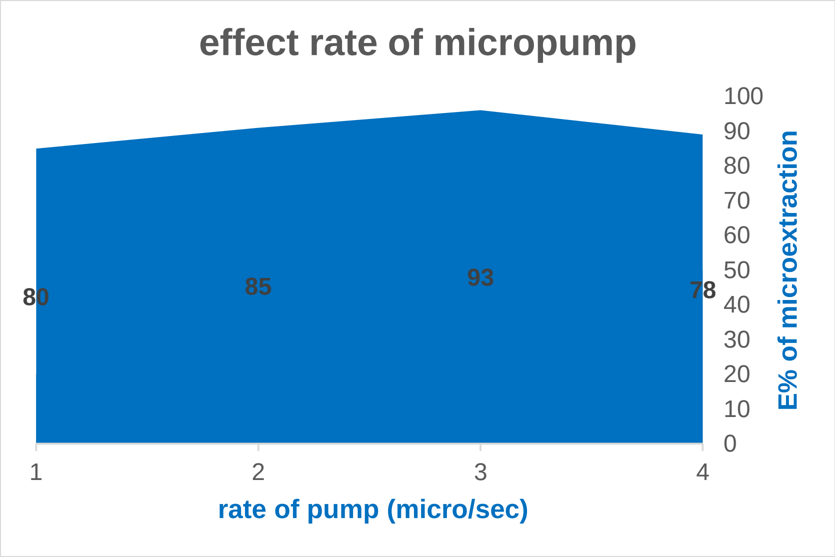

Figure 3. Effect of change the rate of pump speed

Study of equal and different volumes

The design of a mechanical single-droplet two-droplet fusion system mainly requires equal and precise volumes of both the reagent and the metal ion, where each organic droplet of the reagent should be generated with the same volume as the aqueous droplet of the metal ion as much as possible to achieve a high state of equilibrium, because each droplet is a platform for the reaction, whether this reaction is a complex reaction or any other reaction. The microdroplet extraction process requires precise volumes that are compatible with the principles of microdroplet microextraction. In this study, four equal volumes of the reagent solution (dithiazone) and the metal ion solution copper chloride(II) were injected with equal volumes (1000, 2000, 3000, 4000 µL) respectively [23]. The optimal pump speed (200 µL/30 s) and the maximum speed of the micromixer were selected. The study showed that the extraction efficiency using this technique increases depending on the equal volumes of both the organic and aqueous phases, as equal volumes allow for the best completion of the complexation, which allows for achieving a state of balance in the precise extraction process. However, when using different volumes, the balance process will not be achieved and the complexation will be low.

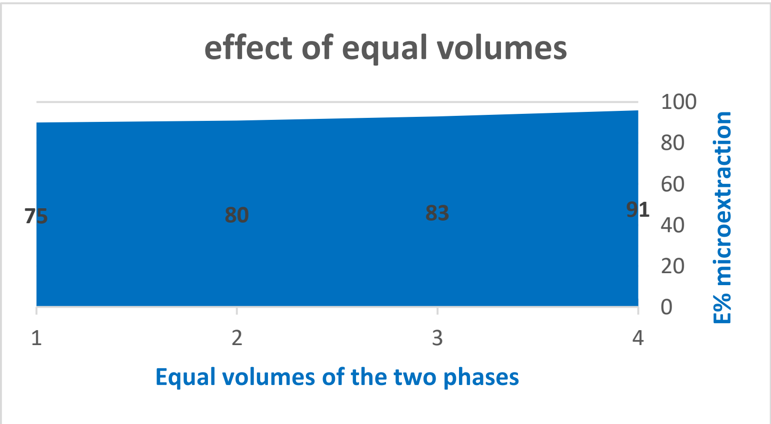

Figure 4. Study the effect of equal volumes

Study the effect of micromixer speed on microextraction

The micro-extraction process with the droplet fusion system achieves a fairly good percentage of extraction efficiency, but there are several factors that affect the nature of good mixing of the droplets, including the speed of the micropump, the design of the droplet formation, the difference between the organic droplet and the aqueous droplet in size and density, with the effect of temperature[24], which in turn affects the surface tension of the droplet. The operation of the micro-mixer depends on a solid foundation, which is to ensure the complete mixing of the descending droplet resulting from the union of the two droplets. In this study, equal volumes of copper chloride solution and dithizone solution were pumped, and the effect of different speeds of the micromixer (slow, medium, high) on the degree of extraction was studied, as the pump speed was fixed at (200μl/30s), the droplet height (12cm), the temperature (25 C^{o}), the acidity (PH=1), and the type of pump tube (Tygon E-Lab Tubing1.2mm). The results showed that the %E values increase with increasing the speed of the micromixer until reaching the maximum speed, which represents the best result, which gives a larger surface area for accurate extraction, because each descending droplet is two combined droplets, and a high percentage of extraction has actually occurred. However, when the descending mixed droplet touches the surface of the micromixer, it will spread into very small particles as a result of the speed of the mixer, the central rotation, and the high diffusion on the surface of the vessel, and thus the extraction reaches a very high percentage and is renewed with each other descending droplet.

Study effect of pump microtube type on microextraction efficiency

Working on a micro-analytical environment requires studying most of the analytical conditions and tools that would create sufficient accuracy and high productivity for such a precise system. In this study, several rubber microtubes were tested to show the best tubes in terms of accuracy and speed for micro-analytical extraction, where the number of generated droplets and their speed were calculated during a short period of time for each tube, as we need there to be compatibility between the accuracy of the analytical work and the speed prepared for measurement, where the type of micro-tube (Tygon E-LabTubing1.2mm) was chosen, which is the best for generating the aqueous micro-droplet for the metal ion solution (copper (II) chloride) and the organic micro-droplet for the organic reagent solution (diathazone). [25,26]

The effect of the height of the droplet resulting from the union of two droplets

Within the mechanism of micro-extraction of the union of organic and aqueous droplets, a graduated stand was designed with a height of (one to 30 cm). To know the effect of the height of the falling droplet after its union from the organic and aqueous droplets. In addition to the factors that affect the droplets such as the speed of the micro-pump and the speed of the micro-mixer and the selection of rubber micro-tubes, this study was conducted to know what is the best height for the falling droplet that achieves the best extraction rate. The result was that the appropriate and best height is (12 cm) as it would complete the mixing of the droplet after its union from the aqueous and organic droplets and its descent by the effect of the ground acceleration vertically on the surface of the micro-mixer to obtain a result with a high percentage of micro-extraction[27,28].

4. Conclusion

this method is considered a new method of separation and micro-extraction and depends on pre-mixing the droplets, as many variables have been studied, including: the optimal concentration, equal and different volumes, droplet height, micro-needles for generating the droplets, pump speed, and micro-mixer speed. With the optimum conditions of temperature and acidity being fixed.a new micro-extraction method that works with a pre-mixing system for the droplets by generating organic and aqueous droplets and mixing them simultaneously during their formation period to obtain a single mixed droplet and then enhancing the separation efficiency using a special micro-mixer that performs the process of spreading the droplet and distributing it in the form of a floating the circular halo.

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