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Separation of purine alkaloids with magnetic silicone microspheres from the extract of *Camellia sinensis (L.)* O.Kuntze

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ABSTRACT

Under the controlled magnetic field, an efficient separation method has been developed for separation of purine alkaloids from *Camellia sinensis (L.)* O.Kuntze with magnetic microspheres with sulfonic acid groups. Contact time, temperature, solid-liquid ratio and initial concentration of the extracts have various influence on the separation performance of related bioactive constituents. After the desorption with HCl aqueous solution, the magnetic microspheres could be recycled and reused several times without significant change. The application of this new method is expected in the preparation of similar bioactive natural products and functional constituents from various sources. © 2016 Trade Science Inc. - INDIA

KEYWORDS

Separation;
Purine alkaloids;
Silicone microspheres;
Camellia sinensis;
Magnetic field.

INTRODUCTION

The past decade has witnessed a tremendous development in the interest and use of medicinal plant products and functional foods in which phytochemical constituents can have medicinal qualities or long-term health promoting in the whole world. For many of the phytomedicines of current interest, a primary focus of research to date has been in the area of comprehensive and systematic separation for a large number of bioactive compounds. Besides traditional techniques (e.g. partition, crystallization, ultrafiltration, distillation, flocculation, precipitation and chromatography, etc), many simple and robust methods for these complex systems are being developed and employed for separation of the target constituents from extracts, preparations, blood or excrement.

In recent years, magnetic separation is attracting more and more attention of researchers from different fields, and it is a process in which magnetically susceptible material is extracted from a specific system using a extranal magnetic force. This new technique has become an important driving force for the innovation in chemistry, physics, medicine, life science, enviormental and their interdisciplinary. Especially, as a new kind of multifunctional material developed in recent years, micron- or nano-scale magnetic microspheres have received much concern and been used in catalysis[1,2], controlled drug release[3], enzyme immobilization[4], nanobiosensor[5], pollution treatment[6], ligand fishing[7] and so on. However, it is barely used in the preparative separation of bioactive natural products.

Camellia sinensis (L.) O.Kuntze is a major resource of tea, which is native to East Asia, the Indian Subcontinent and Southeast Asia. But it is today cultivated across the world in tropical and subtropical regions, and the bioactive constituents in it have many health-care functions. One of them is purine alkaloids, which is mainly composed by caffeine, theophylline, theobromine, xanthine, hypoxanthine, paraxanthine, etc (see Figure 1)[8]. Recently, Du et al[9] used acidic cation exchange resin to concentrate the extracted fluid of caffeine from industry. On the basis of above research status been succsefully used, this paper is ready to introduce the development of a rapid and effective magnetic microspheres-based separation approach for the preparation of purine alkaloids from the extracts of *Camellia sinensis*, which to our knowledge has not been reported by now. Therefore, it is necessary to investigate the effects of related conditions on the separation of purine alkaloids. In the end, the recovery of magnetic microspheres was also discussed.

EXPERIMENTAL

Chemicals

All chemicals involved in this study were at least of analytical reagent grade and purchased from KeLong chemical factory (Chengdu, China) if not specially specified. Water is redistilled. Standard caffeine used for quantitative analysis was purchased from Tauto Biological Technology Company (Shanghai, China). Sulfo silicone magnetic adsorption microspheres (4-5μm) were purchased from Zengzhun Biological Technology Company (Shanghai, China). Raw materials of *Camellia sinensis (L.)* O.Kuntze were purchased from local food store. And all samples were milled and dried. And the sample practice size was controlled in 0.45~0.90 mm by passed through a stainless steel sieve.

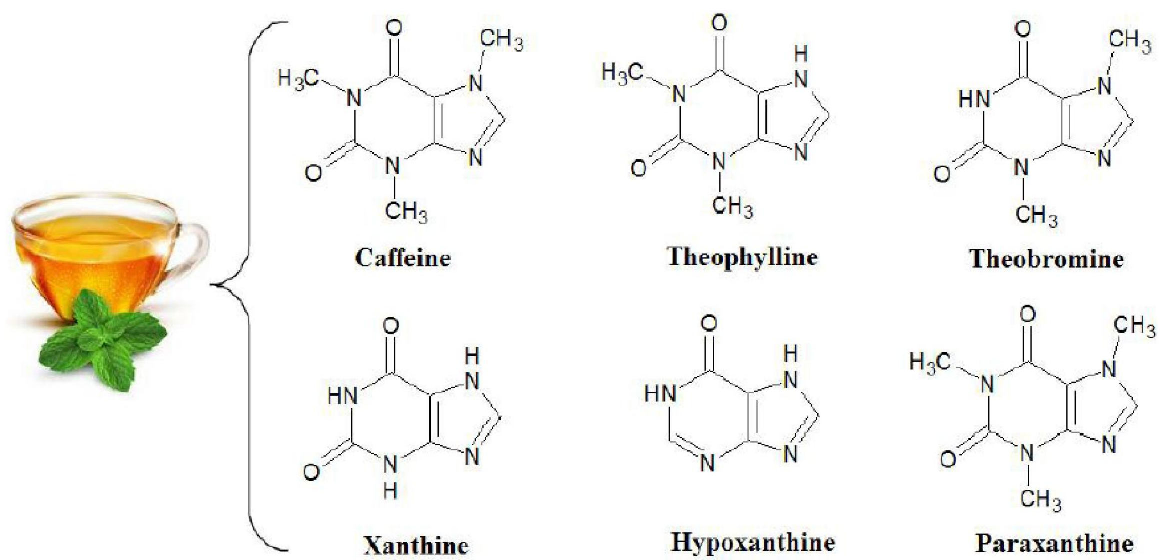

Figure 1 : Purine alkaloids in the extracts of *Camellia sinensis (L.)* O.Kuntze

The same batch of sample was used through this study and stored in closed desiccators until use. Every measurement was repeated three times and then the average value was calculated.

Apparatus

Constant-temperature water bath pot (Yuhua Instrument Co., Ltd., Gongyi, China) was used in the extraction step. Moreover, AL104 electronic balance (±0.0001 g, Mettler Toledo International, Inc., Zurich, Switzerland) and TU-1810 UV-Vis spectrophotometer (Purkinje General Instrument Co., Ltd, Beijing, China) were used for quantitative analysis.

Extraction of purine alkaloids

2.0 g of dried sample was placed in a beaker and mixed with 100 mL of redistlled water, and then the suspension was boiled for 15 min. After the cooling and filtration, 1mL of saturated sodium chloride solution and 2 mL sodium hydroxide solution (1 mol/L) were added into the filtrate, succesively. Then they were fully mixed and extracted three times by chloroform with the same volume. The extracts were concentrated under vacuum and stored in refrigerator prior to use.

Separation of purine alkaloids

The appropriate amount of sulfo silicone magnetic microspheres were immersed into the conical flasks containing chloroform solution of the extracts (the initial concentration of total purine alkaloids=C_0 mg/mL) with continuously stirring for desired time. Then the magnetic microspheres were recovered with the strong external magnetic field. The residual solution was filtrated and the volume of filtrate was supplemented to initial volume. The concentration of total purine alkaloids in the filtrate was measured as C (mg/mL). So the yield (%) can be calculated as the following equation:

Yield of purine alkaloids (%) = $[(C_0 - C) / C_0] \times 100\%$

UV analysis

The concentration of total purine alkaloids was determined by ultraviolet spectrophotometry and the absorbance at 275 nm of all the samples was measured. Caffeine was used as the standard sample for UV determination. Its standard curve was made for the determination of extracts: y=0.5723x-0.0018, R^2=0.9995. The methodology validation has proved above method exhibited good performance, and the recovery of purine alkaloids was 97.3±0.6% (n=6), and the RSD for repeatability was 1.98±0.5% (n=6).

RESULTS AND DISSCUSSION

Effect of magnetic field intensity

In order to recover the magnetic microspheres (4-5μm) as more as possible, enough intensity of external magnetic field. Magnetic microspheres have obvious core-shell structure, and their silicone surface is bonded with large amount of sulfonic acid groups. The internal core of these microspheres is Fe_3O_4 magnetic particles, so their magnetic is relatively strong. Various intensity (0.1~0.6 T) of external controllable magnetic field was used to attract microspheres after the full interaction them and alkaloid moleculars in the solution. Residual microspheres in solution would be filtered and weighed after dryness. According to the results (see Figure 2), 0.5 T could achieve ideal recovery of the magnetic microspheres, which was chosen in the following experiments.

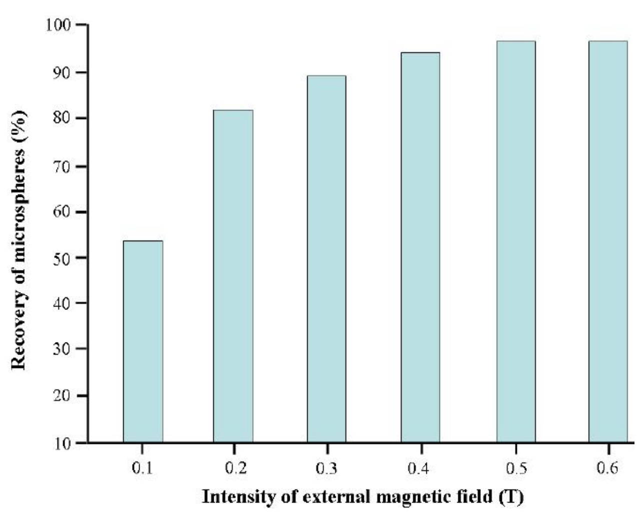

Figure 2 : Effect of magnetic field intensity on the recovery of microspheres

Effect of contact time

Since the contact time between the purine alkaloids and magnetic microspheres is a key parameter for the separation process, the contact time required for the adsorption equilibrium experiments was first determined at room temperature, which were carried out for contact times ranging from 10 to 180 min. As seen from Figure 3, the adsorption rate was very fast and the adsorption efficiency increased rapidly with the increasing time. These results showed that the adsorption efficiency almost reached a plateau above 40 min, when most of purine alkaloids was removed from the extracts solution and the adsorption process reached saturation. It indicated the adsorption equilibrium was achieved. So in the following experiments, the adsorption time was set at 40 min.

Effect of initial concentration

The adsorption efficiency was also influenced by

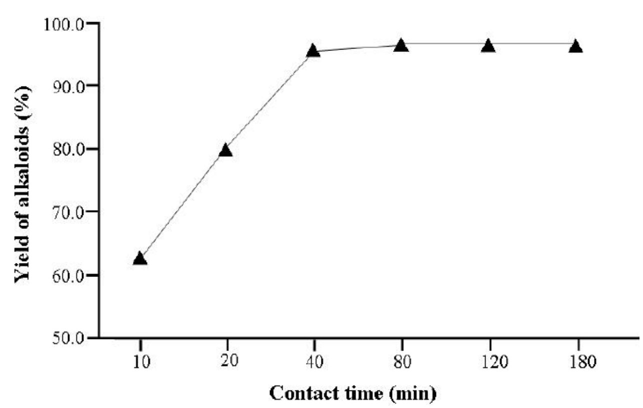

Figure 3 : Effect of contact time on the yield of purine alkaloids

the initial sample concentration of purine alkaloids. The magnetic microspheres were placed in 10 mL of different solutions of the extracts with various initial concentrations. After shaken for 40 min at 25 ± 1°C, the concentrations of free purine alkaloids in solutions were measured. The results are shown in Figure 4, and it is obvious that the adsorbed percentage firstly increases with the increase of initial concentration. When the initial concentration is more than 80 mg/L, the adsorption capacity begin to decrease slowly, indicating that the adsorbent gradually tend to adsorption saturation. Because for a given microspheres dose, the total number of available active groups on them are fixed, while the relatively large number of active groups is required for the much higher initial concentration of alkaloids, they are insufficient. In this condition the number of free purine alkaloids in solutions will become more and more.

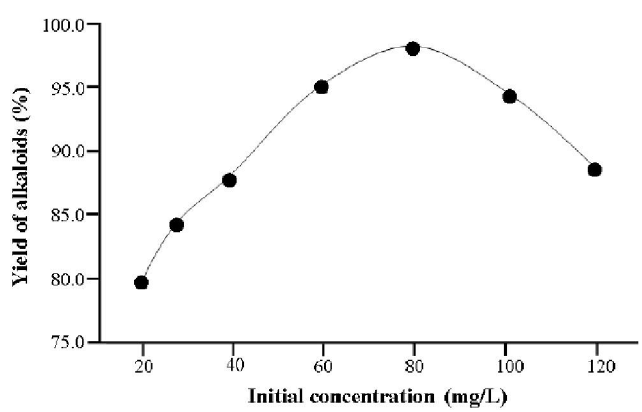

Figure 4 : Effect of initial concentration on the yield of purine alkaloids

Effect of temperature

Adsorption equilibrium and behaviors of a specified object are usually different at various temperatures. So the effect of temperature on the separation of purine alkaloids by magnetic microspheres was investigated within the temperature range of 10~50°C in a thermostatic waterbath, and the results are shown in Figure 5. According to the results, the adsorption efficiency decreased with the rising temperature slightly, indicating that the adsorption process was exothermic. Meanwhile, the adsorption efficiency maintained at a high level under the test temperature range basically, which demonstrating that temperature was not a major effect on the adsorption process under normal conditions. The separation could be preceded within a wide temperature range, and room temperature was selected in the following experiments.

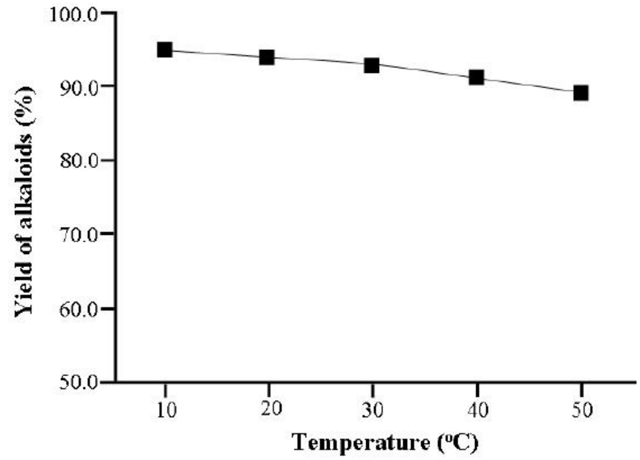

Figure 5 : Effect of temperature on the yield of purine alkaloids

Effect of solid-liquid ratio

Solid-liquid ratio is a crucial parameter in adsorption, which can significantly affect the separation efficiency in most cases. For a given amount of adsorbed object, redundant sorbent is unnecessary. It was observed that the yield of purine alkaloids remarkably increased with the increasing amount of magnetic microspheres. This observation can be explained by the greater number of interaction sites and chances available for target molecules at larger microspheres dosage added in the solution. When the solid-liquid ratio was increased to 5:10 (mg/ml), no significant change in adsorption efficiency was observed. So 1:2 (mg/ml) was seemed as the optimal solid-liquid ratio.

Kinetics study in separation process

The kinetics process of separation for alkaloid molecules with magnetic material can be expressed with related kinetic models. Generally, at the first stage, the adsorption of target constituents occurs on the surface of microspheres; secondly, these molecules interact and combine with active sulfonic acid groups. In order to investigate related absorption behavior, the adsorption kinetics curve was studied for purine alkaloids at different initial concentrations of 60, 80 and 100 mg/L under room temperature. The kinetics experimental data were correlated with the equations of the pseudo first- and second-order kinetic models. According to the comparison of the two adsorption kinetic and correlation coefficients (see TABLE 1), the pseudo second-order model was most suitable to describe the adsorption kinetic data than the pseudo first-order model for all the investigated concentrations. It indicates that the adsorption rate is controlled by chemical adsorption mechanism.

TABLE 1 : Kinetic model parameters from equation fittings

Kinetic order	Kinetic model parameters	R^2	SD
pseudo-first-order	K_1 (1/min) = 0.1673	0.9783	0.1102
pseudo-second-order	K_2 (min·g/mg) = 0.0268	0.9900	0.0831

Recovery of purine alkaloids

Recovery of purine alkaloids from microspheres is very important for the economic benefit and repeated utilization of this kind of magnetic separation material. Through preliminary experiments, the performance of different acidic solutions was compared for the static desorption experiments. It was evidently found that the efficiency of 5% HCl aqueous solution was clearly higher than that of others. And 98.9 % of adsorbed alkaloids could be desorbed after the interaction with 5% HCl within 60 min. In addition, the magnetic microspheres could be recycled and reused at least up to seven times without significant reduction in its separation efficiency, and the obvious change of the status and magnetic of these microspheres was not observed. In conclusion, the results could reveal that the sorbent was stable and recyclable under above experiment conditions.

CONCLUSIONS

An efficient separation method has been developed for separation of purine alkaloids from *Camellia sinensis (L.)* O.Kuntze with magnetic microspheres. Contact time, temperature, solid-liquid ratio and initial concentration of the extracts have various influence on the separation performance of related bioactive constituents. Moreover, the magnetic microspheres could be recycled and reused several times without significant reduction in its separation efficiency. It can be expected that, based on the advantages of magnetic separation, the new method will have great potential and broad space when it is applied in the natural products and pharmaceutical industry as an environmental friendly approach.

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