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Rapid Extraction of Yam Peel Total Flavonoids in A Cholinium-Based Magnetic Ionic Liquid Aqueous Biphasic System

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ABSTRACT

In this study, for the first time, a magnetic ionic liquid-based aqueous biphasic system (MIL-ABS) was developed to extract total flavonoids from crude extracts of yam (*Dioscorea alata* L.) peel. The effect of various conditions on the separation behavior was systematically examined and optimized. A relatively high extraction efficiency (96.4%) and partition coefficient (K = 33.2) for total flavonoids could be achieved. MI-ABS not only has the advantage of being able to extract quickly in the absence of an organic solvent, but it also responds to a simple external magnetic field. This work has the potential to be a useful reference for practical enrichment or separation of active ingredients.

INTRODUCTION

Yam is one of the major food crops cultivated in many tropical and subtropical countries (Hu et al. 2018, Yeh et al. 2013). As a potential functional food, it has been widely used to promote therapeutic benefits (Xue et al. 2019, Zhou et al. 2018). Modern pharmacological researchers have revealed that yam is effective in promoting immunity (Liu et al. 2009), anti-oxidation (Zhang et al. 2018), anti-inflammation (Chen et al. 2017a), and protecting the heart function (Chen et al. 2017b). Based on previous studies, various active components, including polysaccharide, steroidal sapogenin, allantoin, polyphenol, flavonoids, and protein have been reported in yam (Hsu et al. 2011, Huang et al. 2007, Zhang et al. 2018). Flavonoids are the principal antioxidative component of yam, and they have been shown to have potential benefits on biological activities and human health. Although yam flavonoids have received a lot of attention, there have been few studies on yam peel flavonoids. As a result, given the potential value of yam peel as a waste, the extraction technology for flavonoids in yam peel needs to be researched further. In addition, the crude ethanol-water extract contains a significant amount of non-flavonoids. This factor surely adds to the difficulties of flavonoid separation post-processing. Polysaccharide, protein, and vitamin have been identified as co-existing components with similar solubility and polarity to the target flavonoids. Although polyamide resin (Liu et al. 2017), adsorption (Zhang et al. 2020), high-performance liquid chromatography (HPLC) (Yeh et al. 2013), and two-dimensional reversed-phase liquid chromatography (2D-RPLC/RPLC) (Cai et al. 2020) have already been used to solve the separation post-processing problem, there is scope for exploring new methods for selective extraction and enrichment of flavonoids.

The aqueous biphasic system (ABS) is now one of the most promising technologies for separating bioactive compounds. It is a promising alternative to traditional organic solvent extraction methods because of its short time consumption, mild operating conditions, low cost, ease of scale-up, and relative environmental friendliness (He et al. 2018, Nie et al. 2018, Ren et al. 2018). ABS is also used to separate a variety of bioactive chemicals such as dyes (Penido et al. 2019), antibiotics (Mokhtarani et al. 2008), proteins (Gu & Glatz 2007), and so on. Gutowski et al. (2003) were the first to show that an ionic liquid (IL) may generate ABS in the presence of K_3PO_4 in 2003. In the years since, IL-based ABS has been used for a variety of purposes, including the enrichment of bioactive substances. The high designability of IL due to the variation in different cations and anions is the most appealing feature of IL-based ABS (Dimitrijevic et al. 2020). Thus, IL can be effectively designed with a desired affinity and polarity for a specific application.

Up to now, IL-based ABS has been successfully employed for the effective separation/extraction of many bioactive compounds and drugs (Belchior et al. 2020, Li et al. 2010, Neves et al. 2016, Nie et al. 2018, Ren et al. 2018, Requejo et al. 2019). However, due to the small density difference, IL-based ABS still suffer from inefficient phase separation (Yao & Yao 2017). As a result, the centrifugation step is often used in common IL-based ABS to aid phase separation. Magnetic ionic liquid (MIL) has recently emerged as a new research focus in the separation/extraction fields (Trujillo-Rodriguez et al. 2017, Wang et al. 2014, Zhou et al. 2017). And the related aqueous biphasic system (MIL-ABS) is stimulating interest. Yao and Yao (2017) employed a MIL-ABS system based on guanidinium ionic liquid to detect trace amounts of chloramphenicol in a water sample. The results revealed a considerable enrichment effect, and chloramphenicol recovery was likewise satisfactory in the real water sample. Nie et al. (2018) developed the MIL-ABS for the extraction of berberine hydrochloride in *Rhizoma coptidis* using cholinium-based MIL. This research found that using a simple external magnetic field to separate the phases eliminates the need for centrifugation, making this technique more time-efficient and simple.

For the first time, an ABS method based on cholinium in extraction for flavonoids from crude extracts of yam (*Dioscorea alata* L.) peel was established based on the above. As a result, when the phenomena of aqueous biphasic system emerged, the extraction equilibrium was nearly realized. To aid phase separation, an external magnetic field was used. The single factor experiment was used to systematically adjust parameters, and an acceptable recovery efficiency was attained under optimal conditions. In summary, the findings of this study provided an alternative method for investigating the flavonoids separation procedure.

MATERIALS AND METHODS

Reagents and Materials

All reagents were of analytical pure grade. 4-Hydroxy-TEMPO (4-OH-TEMPO, >98%) and was purchased from Adamas-beta Co. Ltd (Shanghai, China). N,N-dimethylethanolamine, chlorosulfonic acid, 1-bromopropane, 1-bromobutane, 1-bromopentane, and 1-bromohexane were supplied by Aladdin Reagent Co. Ltd (Shanghai, China). The standard of rutin selected as a standard compound for determining the total flavonoids content was provided by Macklin Biochemical Co., Ltd (Shanghai, China). Crude extracts of yam peel were self-made, which was obtained by the extraction of yam peel powder with 50% ethanol according to a previous study with minor modifications (Hsu et al. 2011). And the other reagents/salts were all bought from Macklin Biochemical Co., Ltd (Shanghai, China).

Synthesis of Cholinium-based Magnetic Ionic Liquids

The cholinium-based magnetic ionic liquids (MILs) were synthesized according to the procedure of previously reported literature with a minor modification (Nie et al. 2017). Briefly, two intermediates were prepared first. They were the alkyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,n\,2OH}]Br$, n= 3, 4, 5, 6) and sodium 4-sulfonatooxy-2,2,6,6-tetramethyl piperidine-1-yloxyl ($[TEMPO\text{-}OSO_3]Na$), respectively. $[N_{1\,1\,n\,2OH}]Br$ was synthesized as follows. First, N,N-dimethylethanolamine (0.2 mol) was dissolved in 30 mL ethanol, and then *n*-alkyl bromide (0.22 mol) was slowly added into the solution. The mixture system was refluxed under continuous stirring for 24 h. After the reaction, most ethanol solvents were removed under a vacuum. And the product was recrystallized in diethyl ether for purification and then dried at 50°C. In addition, the synthesis of $[TEMPO\text{-}OSO_3]Na$ was conducted as follows. 4-Hydroxy-TEMPO (0.05 mol) was dissolved in dichloromethane at 0-5°C, and then an equal molar of chlorosulfonic acid was added dropwise within 1 h. The above mixture was reacted under continuous stirring for 4 h at room temperature. After the reaction, the mixture was filtered and washed with dichloromethane 3 times, then dried under vacuum at 50°C. Subsequently, the obtained solid suffered a simple neutralization with NaOH aqueous solution to prepare the $[TEMPO\text{-}OSO_3]Na$ intermediate. Finally, the cholinium-based MILs $[N_{1\,1\,n\,2OH}]$ $[TEMPO\text{-}OSO_3]$ (n= 3, 4, 5, 6) were prepared by the metathesis reaction between $[TEMPO\text{-}OSO_3]Na$ and $[N_{1\,1\,n\,2OH}]Br$ in ethanol. The mixture was reacted at 50°C for 24 h followed by filtration. The obtained product was washed with acetone until no precipitation and dried under vacuum at 50°C. The final products were dark-reddish viscous liquids. The MILs were characterized by FT-IR spectra.

Extraction of Flavonoids with MIL-ABS

A certain volume of crude extracts of yam peel was transferred into a 5 mL sample vial, and a specific amount of MIL was successively dissolved in this solution. Afterward, inorganic salt was added and dissolved in the solution to form MIL-ABS at a constant temperature. After thorough extraction, a NdFeB magnet was employed to assist phase separation. Parameters, including the type of MILs, salts, each mass fraction of MIL + salt + crude extracts, extraction temperature, and time were systematically optimized to obtain higher extraction efficiency (E, %) and partition coefficient (K), which could be calculated according to Eqs. (1) and (2).

$$E(\%) = \frac{C_o \times V_0 - C_b \times V_b}{C_0 \times V_0} \times 100\% \quad \text{...(1)}$$

$$K = C_t/C_b \quad \ldots(2)$$

where C_0 (mg.mL^{-1}) and V_0 (mL) represents the initial concentration of total flavonoids in crude extracts and initial volume, respectively. C_b (mg.mL^{-1}) and V_b (mL) are the concentration of total flavonoids in the bottom (b) phase and volume of the bottom phase, respectively. By subtracting the residual amount in the water-phase from the total amount before extraction, the amount of extracted flavonoids in the MIL phase was estimated.

RESULTS AND DISCUSSION

FT-IR Spectra

Fig.1 exhibits the FT-IR spectra of synthesized MILs. Herein, [$N_{1\ 1\ 6\ 2OH}$] [TEMPO-OSO_3] is taken as an example to analyze the infrared spectrum. The peak above 3000 cm^{-1} is related to the stretching vibration of the hydroxyl group in the cation structure of MIL. Peaks between 2800 cm^{-1} and 3000 cm^{-1} are attributed to C-H stretching. The band 1660 cm^{-1} may be assigned to the skeleton vibration of the anion structure. 1483 cm^{-1} corresponds to C-H asymmetric in-plane bending vibration. The absorption band at 1386 cm^{-1} is mainly assigned to the symmetric in-plane bending vibration of the C-H bond in the gem-dimethyl of the anion (Nie et al. 2017). The band at 1264 cm^{-1} is assigned to the stretching vibration absorption of the C-N bond. In addition, 1095 cm^{-1} corresponds to the stretching vibration of N-O in the anionic structure. The peak at 972 cm^{-1} belongs to the asymmetric stretching vibration of O-S-O in the anion.

Effect of Different MILs

The extraction and partition performance of target compounds can be influenced by the composition of the two phases in the ABS. According to earlier studies, MIL's extraction behavior is mostly determined by its dissolving capacity for the target compound and the cluster characteristics generated by MIL. Thus, the effect of MILs [$N_{1\ 1\ n\ 2OH}$] [TEMPO-OSO_3] (n= 3, 4, 5, 6) on the extraction efficiency (E) and partition coefficient (K) was tested. The condition of 10 wt% IL + 40wt% K_3PO_4 + 2 mL crude extracts was selected and the comparison result was presented in Fig. 2. According to the result, it can be observed that the level of both E and K are simultaneously increased with the prolongation of the alkyl chain. The trend is consistent with the previous report, which mentioned that the capability of MILs phase-separation can improve when the length of the alkyl chain becomes longer (Nie et al. 2018). The hydrophobicity can be reinforced with the carbon chain of MILs getting longer. Thus, the interaction between MIL and flavonoids can be determined by the hydrophobic effect and van der Wall's interaction (Mumcu & Seyhan Bozkurt 2019).

Effect of Weight Percent of MIL

The effect of MIL amount on extraction performance of total flavonoids was tested. Different weight percent of MIL [$N_{1\ 1\ 6\ 2OH}$] [TEMPO-OSO_3] with 2 mL crude extracts and 40wt% K_3PO_4 were investigated (see Fig. 3). It is obvious that a higher MIL amount leads to higher extraction efficiency and partition coefficient until the extraction system becomes saturated. It is well known that the larger amount of MIL was added, the more MIL-phase volume, which suggested that more flavonoids were transmitted into the MIL-rich phase. However, an excessive amount of MIL makes no contribution to the improvement of partition coefficient. This is maybe owing to the increasing volume of the top phase. Thus, 12 wt% MIL shows the optimal effect for extracting the target compound.

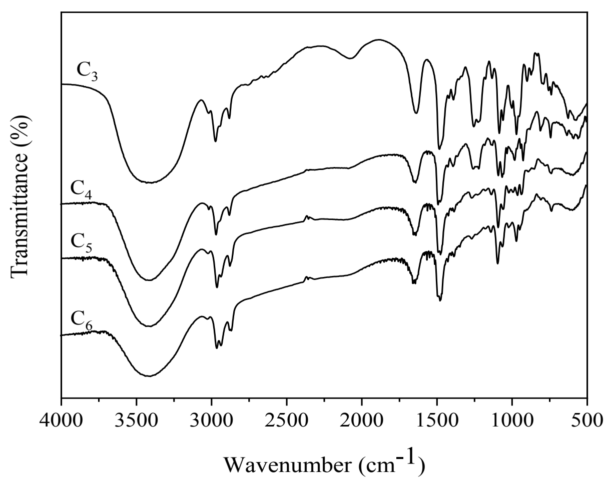

Fig. 1: FT-IR spectra of MILs.

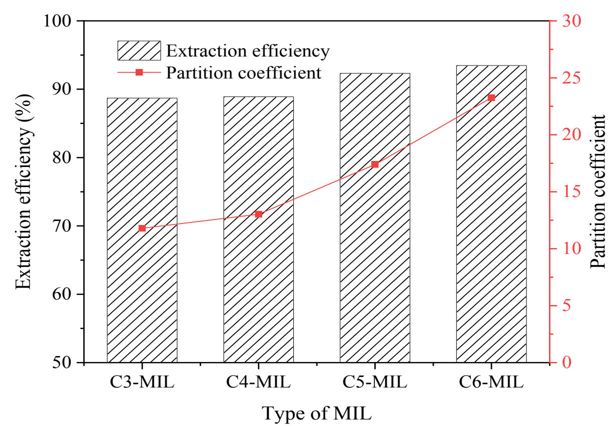

Fig. 2: The effect of different MILs on extraction efficiency and partition coefficient of total flavonoids.

Effect of Weight Percent of K_3PO_4

The weight percent of K_3PO_4 can also play an important role in the system. The weight percent of MIL [$N_{1\ 1\ 6\ 2OH}$] [TEMPO-OSO_3] was maintained at 12 wt% in the following tests, and the explored results were displayed in Fig. 4. It was observed that both the extraction efficiency and partition coefficient increase first, and then decrease with the addition of K_3PO_4 growing from 20 wt% to 40 wt%. The increasing amount of K_3PO_4 boosts the formation of two phases. Flavonoids provide a stronger salting-out effect, which is followed by flavonoids being transferred to the MIL-rich phase. Thus, at a concentration of 35 wt% K_3PO_4, the maximum extraction efficiency and partition coefficient were achieved. Nonetheless, when its concentration is beyond 35% wt%, the values of E and K decline with the further addition of K_3PO_4. Therefore, 35 wt% of K_3PO_4 was selected for further experiments.

Effect of Extraction Temperature

The result of the influence of temperature on extraction and partition performance of target compounds in MIL-ABS was shown in Fig. 5. Obviously, the extraction efficiency decreases slightly with a temperature range of 20°C to 50°C. This may be attributed to the viscosity of MIL reduced with the advanced temperature. The result will bring about the reduced mass transfer resistance of the target compound in the top and the bottom phases, resulting in a slight shrink in the extraction efficiency. As such, to predigest the operation, the room temperature was selected for the experiments.

Effects of Extraction Time

To explore the effect of extraction time on the extraction and partition performance, various durations (10-50 min) were tested and presented in Fig. 6. Clearly, 10 min is enough to extract the target compound. Continuously increasing the extraction time results in a slight decrease in extraction efficiency, but not a significant change. Total flavonoids have a relatively low extraction time. This occurrence could be explained by MIL's high affinity for the target flavonoids. As a result, 10 min was sufficient for complete flavonoid enrichment with a high extraction efficiency and partition coefficient.

CONCLUSION

The MIL-ABS, which was made up of four MILs, was used to design the extraction procedure of total flavonoids from the crude extract of yam peel. [$N_{1\ 1\ n\ 2OH}$] [TEMPO-OSO_3] (n= 3, 4, 5, 6). It was shown that the MIL [$N_{1\ 1\ 6\ 2OH}$] [TEMPO-OSO_3] exhibited a stronger ability for ABS phase formation. Under the optimal extraction condition, the extraction efficiency of total flavonoids is above 96%, which means the method is a relatively effective approach. This research has the potential to provide a useful reference for the enrichment or separation of natural products from complex samples in the real world. The MIL-ABS process could benefit from simplification of magnetic ionic liquid preparation and recycling to be applied at large scales.

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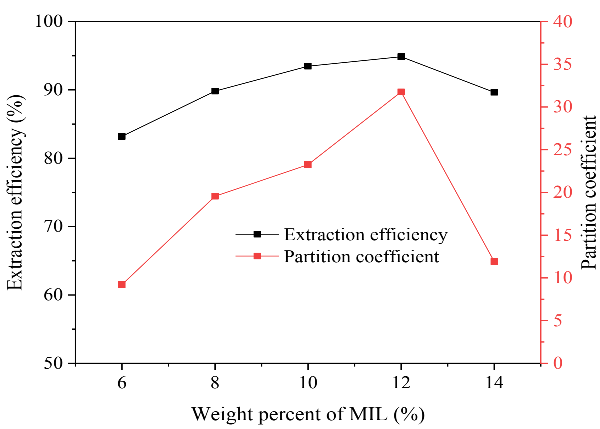

Fig. 3: The effect of weight percent of MIL on extraction efficiency and partition coefficient of total flavonoids.

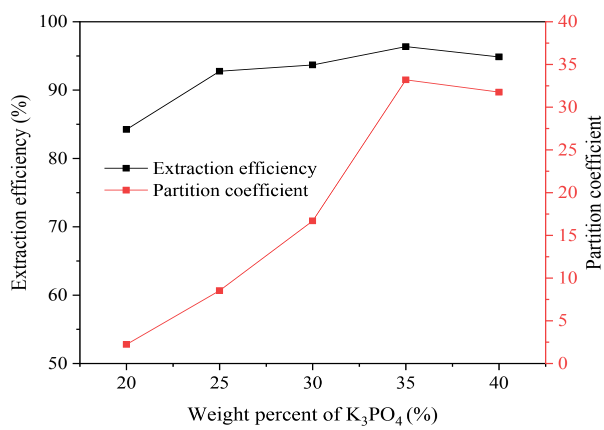

Fig. 4: The effect of weight percent of salt on extraction efficiency and partition coefficient of total flavonoids.

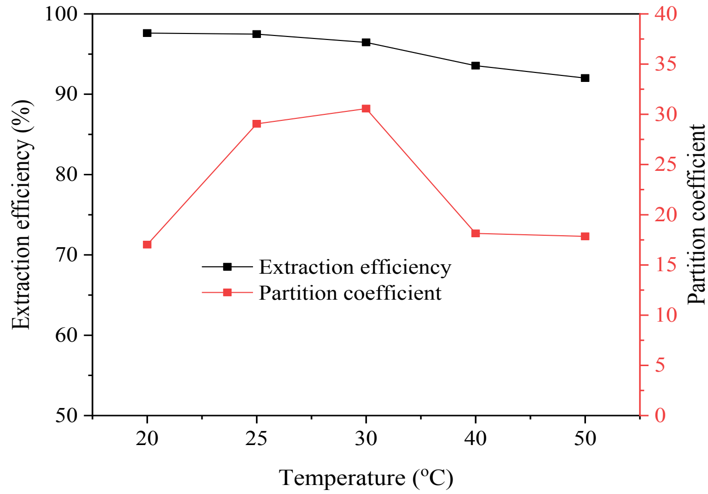

Fig. 5: The effect of extraction temperature on extraction efficiency and partition coefficient of total flavonoids.

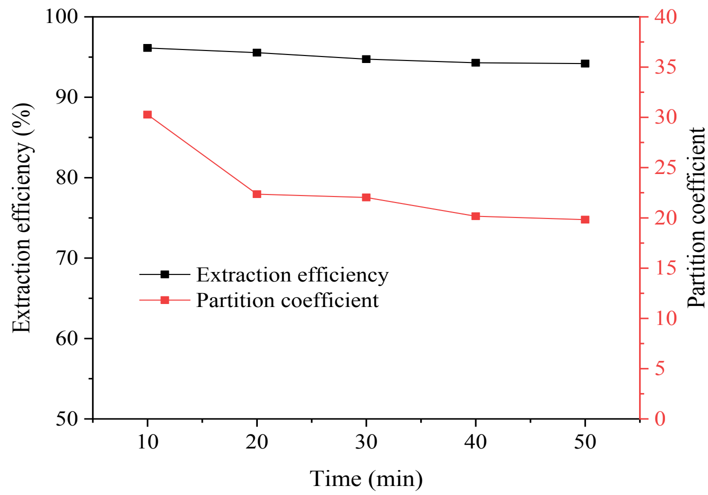

Fig. 6: The effect of extraction time on extraction efficiency and partition coefficient of total flavonoids.

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