Partition of tea saponin with a novel recyclable thermo-pH aqueous two-phase systems

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Abstract

Aqueous two-phase systems (ATPS) have the advantages of environmentally friendly, high mass transfer efficiency, mild extraction conditions. However, it is difficult to recycle these components, which limits the large-scale application of ATPS in industrial production. In this study, a novel recyclable ATPS of P_N/P_ADB4.78 was constructed with a thermo-responsive polymer P_N and a pH-responsive polymer P_ADB4.78 for the partition study of tea saponin. The recovery of P_N reached 98.71% by adjusting the temperature to above its lower critical solution temperature (LCST, 33.0 °C ) and the recovery of P_ADB4.78 was 97.32% by regulating the pH to its isoelectric point (pI, 4.78). Meanwhile, the phase formation mechanism of ATPS was studied by surface tension and low-field nuclear magnetic resonance (LF-NMR). The effects of polymer concentration, pH, temperature, types and concentrations of salt were investigated on tea saponin partition.

In the 1.5% P_N/3.5% P_ADB4.78 ATPS, the optimal partition coefficient (K) and extraction recovery (ER_b) of crude tea saponin were 0.15 and 92.13% in the presence of 1.5 mM KCl at pH=7.6 and 25°C, respectively. The K and ER_b of tea saponin from tea seeds were 0.12 and 94.50% with 7.5 mM LiBr at pH=8.0 and 25°C, respectively.

Keywords: Aqueous two-phase systems; Partition; Polymer recovery; Tea saponin
1 Introduction

Aqueous two-phase systems (ATPS) are composed of immiscible two (or more) components in water which are formed when the systems reach equilibrium. ATPS are a novel separation and extraction technology with higher water content, lower interfacial tension, non-toxic, environmentally friendly comparing with traditional liquid-liquid extraction [1-3]. It has been extensively studied in the extraction and purification of downstream products, for example, nanoparticles [4], proteins [5], antibiotics [6], biological molecules [7].

Traditional ATPS are composed of polymer, salt, organic solvent, inorganic salt, and ionic liquid [8-11], these phase components are difficult to reuse. In order to solve above problems, some polymers responsive to light, temperature and pH have been synthesized in our laboratory, which can be conveniently recycled by adjusting environmental factors and used to construct novel recyclable ATPS [12-14]. Yang, T et al. [15] synthesized pH-responsive polymer ($P_{MDB3.36}$ and $P_{ADB4.05}$), both of which recovery were above 95%. Meanwhile, the ATPS was constructed by using the above polymers. Yang, F et al. [16] purified ε-polylysine by using thermo-responsive affinity polymer ($P_{NHM}$-IDA-Ni$^{2+}$), which recovery was above 95%, and the purity of ε-polylysine increased. The above research have proved that the novel ATPS provides a broad application prospect for the extraction and separation of biomolecules.

Tea saponin, a kind of pentacyclic triterpene glycoside compound, is from the seeds of Camellia oleifera [17]. It has good emulsifying, dispersing and wetting effects.
Besides, it can be widely used in medicine, agriculture, feed, food, textile and other fields [18-20]. Moreover, it plays an important role in antibacterial, anti-inflammatory, anti-tumor, flame retardant materials, and reduce environmental pollution. Traditional extraction techniques of tea saponin mainly includes water extraction, organic solvent extraction and microwave-assisted extraction [21-23]. The above extraction techniques have some disadvantages, for example, complex extraction process, low extraction rate, environmental pollution. Therefore, the development of new extraction technology is very necessary for tea saponin. This study aimed to use a recyclable ATPS for the partition of tea saponin.

In this study, thermo-responsive polymer (Pn) and pH-responsive polymer (PADB4.78) were synthesized by random copolymerization. Meanwhile, thermo-pH responsive ATPS PnPADB4.78 were constructed, which was used to investigate the partition of tea saponin by optimizing the polymer concentration, system temperature, system pH, different types and concentrations of salt. The phase formation mechanism was studied by surface tension and LF-NMR. Moreover, the relationship between the potential difference of two phases and the partition of tea saponin was discussed.
2 Materials and methods

2.1 Reagents

Tea saponin (Purity: ≥98%) was obtained from Shanxi Lebo Biochemical Technology Co., Ltd. Vanillin, absolute ethanol, concentrated sulfuric acid, acrylic acid (AA), sodium bisulfite (NaHSO₃) and ammonium persulfate (APS) were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. N-isopropylacrylamide (NIPA), 2,2-Azobisisobutyronitrile (AIBN) were purchased from TCI Chemical Industry Development Co., Ltd. butyl methacrylate (BMA) and Dimethylamino-ethyl methacrylate (DMAEMA) were purchased from Aladdin Reagent (Shanghai) Co., Ltd. Above reagents were AR.

2.2 Methods

2.2.1 Synthesis of Pₜ and PADB₄₇₈

In this work, ATPS was formed by polymers Pₜ and PADB₄₇₈, which structures were demonstrated in Fig. 1. The Pₜ was polymerized using the monomer NIPA with AIBN as initiator. The specific synthesis method was detailed according to Xu [14], where the reaction temperature was 62 °C. PADB₄₇₈ was synthesized by 0.047 mol DMAEMA, 0.081 mol AA and 0.003 mol BMA in 120 mL deionized water under nitrogen protection. The initiator were 0.24 g NaHSO₃ and 0.24 g APS, the reaction was run under the following conditions: rotating speed 200 rpm, temperature 60 °C and time 12 h. the production was precipitated with ethanol and dried at 55 °C After the reaction was complete. The process of tea saponin extraction in Pₜ/PADB₄₇₈ ATPS was shown in Fig. 2.
2.2.2 Polymer characterization

Fourier transform infrared spectroscopy (FT-IR, Nicolet MagnaIR550 infrared equipment, Thermo, USA). The polymers chemical structure was usually characterized with FT-IR [24].

The viscosity-average molecular weight and intrinsic viscosity of the polymers were determined using the Umbrella Capillary Viscometer 1835 (Liang Jing Glass Equipment Factory, Shanghai) [25]. Varied concentrations of polymer solutions were prepared. Then, the time for deionized water and polymer solutions to flow through the capillary were recorded as \( t \) and \( t_0 \) at 25°C, respectively.

Dynamic light scattering (DLS). DLS was used to measure weight-average molecular weight and size distribution of \( \text{PN} \) and \( \text{PADB}_{4.78} \) at room temperature. 0.1\%(w/v) \( \text{PN} \) and 1\%(w/v) \( \text{PADB}_{4.78} \) were prepared and carried out by DynaPro NanoStar equipment (Wyatt Technology Corporation, USA).

2.2.3 Recovery of polymers

The thermo-responsive copolymer can be recovered by changing the temperature to exceed its LCST. The LCST represented the temperature at which polymer solutions initially become turbid. 2\%(w/v) \( \text{PN} \) solution was placed in a constant temperature water bath and the temperature was increased by 0.1°C every 10 minutes. In addition, a series of salt including KCl, NaCl, \( \text{Na}_2\text{SO}_4 \), \( (\text{NH}_4)_2\text{SO}_4 \), \( \text{K}_2\text{HPO}_4 \), \( \text{KH}_2\text{PO}_4 \) (10 mM) were used to study the influence of polymer recovery. The pH-responsive copolymer could be recycled by changing the pH of solution to its isoelectric point (pI). The pI of \( \text{PADB}_{4.78} \) was determined by zeta potentials. The corresponding zeta potential value is
also zero when the net charge of the polymer solution is zero, and the pH of the solution is the isoelectric point of the polymer.

2.2.4 Preparation of P_N/P_{ADB4.78} ATPS

Firstly, P_N and P_{ADB4.78} were respectively dissolved in deionized water and 0.15% NaOH solution to form 2%-5% (w/v) and 5%-10% (w/v) polymer solutions. Then, the two solutions in equal volume were mixed in graduated centrifuge tube. Finally, these tubes were placed in the water bath until a clear phase interface was observed.

2.2.5 Phase diagram

The cloud point curve was used to determine the phase diagrams of ATPS [24]. Firstly, 10% (w/v) P_N and 10% (w/v) P_{ADB4.78} were prepared and adjusted to the same pH. 1 mL P_{ADB4.78} solution was added to the centrifuge tube and then 100 μL P_N was added drop-wise until the mixed solution changed from clear to turbid, recording the volume of P_N added and calculating the relative parameters, which represented a point on the binodal curve. Then, the deionized water of same pH was used to dilute the mixed solution until the solution was observed to become clear. Finally, more points were obtained by repeating the above steps. In addition, the binodal curves of the ATPS at different pH (6.5, 7.0, 7.5) and temperatures (20 °C, 25 °C, 35 °C) were determined.

2.2.6 Phase formation mechanism

2.2.6.1 Surface tension measurement

Surface tension that used the platinum plate method at constant temperature (25 °C) were measured by surface tension meter (DCAT11, Dataphysics Co., Ltd.). Firstly, 3% (w/v) P_N and 7% (w/v) P_{ADB4.78} were prepared. Then, 2 mL of solution
from the top and bottom phase were taken out for measurement. Duplicate experiments were conducted to obtain the average value of surface tension.

2.2.6.2 Low-field nuclear magnetic resonance (LF-NMR) relaxation time measurement

In this study, the measurement of transverse relaxation time ($T_2$) were conducted on a 21MHz NMR analyser (PQ001, Niumag Electric Company, Shanghai, China). The spin-spin relaxation time $T_2$ was measured by using Carr-Purcell-Meiboom-Gill (CPMG) sequences, a multipulse sequence applied to protons [26]. These data were analyzed by a multiexponential model of MultiExp Inv Analysis software using the inverse Laplace transform algorithm. The relaxation components represented the interaction between polymers and water. In addition, 3\%(w/v) PN and 7\%(w/v) PADB4.78 solutions were prepared to measure the $T_2$ at 25 °C.

2.2.7 Partition of tea saponin

Crude tea saponin and tea saponin from tea seeds were chosen to determine the partition process in PN/PADB4.78 ATPS. The concentration of PN (1%-2.5%, w/v), PADB4.78 (2.5%-4%, w/v), pH 6.0-8.0 and temperature 15°C-30°C were selected to determine the effect on tea saponin partition in PN/PADB4.78 ATPS. Moreover, eight different types and concentrations of salt including KCl, LiCl, LiBr, NH₄Cl, NH₄Br, (NH₄)₂SO₄, Na₂SO₄, MgSO₄ were selected for studying the influence on tea saponin partitioned. Then, 100 µL solutions from each of the two phases were measured the absorbance value at 552 nm with an ultraviolet spectrophotometer and calculated the concentration of tea saponin by using the calibration curve. The tea saponin partitioning was discussed by partition coefficient (K) and extraction recovery (ER). These parameters were calculated as follows eq (1-4):
\[ K = \frac{C_{\text{top}}}{C_{\text{bottom}}} \]  \hspace{1cm} (1)

\[ R_V = \frac{V_{\text{top}}}{V_{\text{bottom}}} \]  \hspace{1cm} (2)

\[ ER_{\text{top}} = \frac{K}{K + \frac{1}{R_V}} \times 100\% \]  \hspace{1cm} (3)

\[ ER_{\text{bottom}} = \frac{1}{1 + KR_V} \times 100\% \]  \hspace{1cm} (4)

Where \( C \) was the concentration of tea saponin, \( V \) indicated the volume of the phase, and the subscripts “top” and “bottom” represented the top and bottom phase.
3. Results and discussion

3.1 Characterization and analysis of PN and P\textsubscript{ADB4.78}

3.1.1 FT-IR of polymers

The FT-IR spectrum of PN and P\textsubscript{ADB4.78} were shown in Fig. 3(a). The analysis results for PN were as follows: 3500-3300 cm\(^{-1}\) (stretching vibration of -NH); 3010-2945 cm\(^{-1}\) (C—H vibration in methyl); 1750-1590 cm\(^{-1}\) (C=O vibration); 1400-1305 cm\(^{-1}\) (stretching vibration of (CH\(_3\))\(_2\)CH—); 1200-1050 cm\(^{-1}\) (C—N vibration) and 710-640 cm\(^{-1}\) (stretching vibration of C—H in polymer chain). The above infrared analysis proved that the PN was synthesized by NIPA.

The analysis of the P\textsubscript{ADB4.78} was as follows: 3795-3100 cm\(^{-1}\) (O-H in hydroxyl and carboxyl groups); 3100-2870 cm\(^{-1}\) (C-H in methylene and methyl); 1870-1680 cm\(^{-1}\) (C=O in ester and carboxyl groups); 1650-1500 cm\(^{-1}\) (C=C in AA, BMA and DMAEMA) and 1330-1080 cm\(^{-1}\) (C-N in tertiary amine group). The results proved that the P\textsubscript{ADB4.78} was polymerized by BMA, DMAEMA and AA.

3.1.2 Intrinsic viscosity of polymers

The intrinsic viscosity was calculated by eq.(5-6) depending on the extrapolation method [16].

\[
\frac{\eta_{sp}}{C} = [\eta] + \alpha[\eta]^2C \quad (5)
\]

\[
\frac{\ln \eta_r}{C} = [\eta] + \beta[\eta]^2C \quad (6)
\]

Both \(\eta_r\) and \(\eta_{sp}\) represented relative and specific viscosity. Relative viscosity (\(\eta_r\)) and specific viscosity (\(\eta_{sp}\)) can be calculated by \(\eta_r = \frac{t}{t_0}\) and \(\eta_{sp} = \eta_r - 1\). C was the
concentration of polymer, $\alpha$ and $\beta$ were the constant parameters. The viscosity-average molecular weight ($M_\eta$) was calculated by viscometer using Mark-Houwink equation as eq. (7):

$$[\eta] = KM_\eta^\alpha$$  \hspace{1cm} (7)

Both K and $\alpha$ were empirical constants depending on temperature and solvent. The K and $\alpha$ of PN were $1.45 \times 10^{-4}$ L/g and 0.65. For PADB4.78, the K and $\alpha$ were $1.78 \times 10^{-4}$ L/g and 0.56, respectively. The calculation results of $[\eta]$ and $M_\eta$ were presented in Fig. 3(b), (c) and Table 1.

3.1.3 The weight-average molecular weight ($M_w$) and particle size of polymers

The $M_w$ and particle size of PN and PADB4.78 were measured by DLS (Table 2). The $M_w$ of PN and PADB4.78 were 92 KDa and 32 KDa, respectively. The particle sizes of PN and PADB4.78 were 4.103 nm and 2.634 nm, respectively. The monomers and initiators used in the synthesis of the two polymers were different, so weight-average molecular weight and particle size of polymers were also different.

3.2 Recovery of PN and PADB4.78

The zeta potential value of polymer solutions was determined and presented in Fig. 4(a). The pI of PADB4.78 was 4.78. The surface charge of PADB4.78 was completely neutralized when pH was adjusted to its pI and the polymer was precipitated from solution. The result was shown in Fig. 4(b). The maximum recovery of PADB4.78 was 97.32% at pH 4.78. The LCST of PN was approximately 33.0 °C. The PN could be easy to precipitate from solution above its LCST. It was mainly due to that the macromolecular chain of PN has both hydrophilic amido groups and hydrophobic isopropyl groups [27], which makes PN aqueous solutions exhibit temperature sensitive
characteristics. When the temperature rised, the polymer molecular chain and the water separated leading to the precipitation of the polymer. The result was shown in Fig. 4(c). The maximum recovery of PN was 98.71% when 10 mM Na2SO4 was added. It may be that the addition of Na2SO4 changed the solubility of PN.

3.3 Phase diagram

The formation conditions and quantitative relationship of ATPS could be illustrated by the phase diagram. The influence of pH and temperature on the phase diagram were investigated and the results were shown in Fig. 5(a) and (b). In the PN/PA DB4.78 ATPS, PN primarily existed in the top phase and PADB4.78 primarily existed in the bottom phase. This area below the binodal curve was single-phase region and the rest was two-phase region. The binodal curves were fitted by eq.(8) [28]. The heterogeneous region decreased with the increasing system pH, which may be due to the presence of the carboxyl group from AA in an ionic state when the system pH was higher [29]. The closer the temperature to LCST of PN, the more hydrophobic. Therefore, the two-phase region was easier to form.

\[
B = \exp(\alpha + \delta A^{0.5} + \mu A + \Phi A^2)
\]  

(8)

Where B was the concentration of PN and A was the concentration of PADB4.78. The \(\alpha\), \(\delta\), \(\mu\) and \(\Phi\) represented fitting parameters.

3.4 Phase formation mechanism study

3.4.1 Surface tension

The measurement of surface tension was helpful to understand the phase formation mechanism of two incompatible polymers. The difference in surface tension between
polymers was one of the factors driving the formation of phase interfaces [30]. The surface tension of PN, P_{ADB4.78} solutions and each of two phases after phase formation were measured. The results were shown in Fig.6 (a) and (b). The surface tension values of PN and P_{ADB4.78} were 49.31 mN/m and 32.27 mN/m at the same temperature, respectively. The surface tensions of PN-enriched phase and P_{ADB4.78}-enriched phase after phase formation were 38.92 mN/m and 38.58 mN/m. Before the phase formation, the surface tension of the two polymers were quite different. It was precisely that two polymer solutions can quickly form aqueous two-phase system after mixing because of the difference in surface tension [27].

3.4.2 LF-NMR T2 relaxation time measurement

In this experiment, the T2 relaxation time was the key to investigate the water mobility in the PN/P_{ADB4.78} ATPS. The result was shown in Fig.7 (a) and (b). T2 relaxation curve of polymers represented a multi-exponential distribution with two states of water. The peaks in the range of 10-100 ms (T21) and 1000-5000 ms (T22) represented tightly bound water and weakly bound water, respectively [31]. Water with a shorter relaxation time was more strongly bound to molecules than water with a longer relaxation time. Therefore, weakly bound water (T22) was chosen to study the interaction between water and polymer molecules. As shown in Fig.7 (a), the T22 of P_{ADB4.78} was shorter than PN, which could indicate that the water mobility of P_{ADB4.78} was weaker than PN and the water-binding capability of P_{ADB4.78} was stronger than PN. The T2 relaxation time of each phase after phase formation was shown in Fig.7 (b). Due to the presence of a small amount of P_{ADB4.78} in the top phase, the T22 relaxation time
of the top phase was shifted to the left comparing with the $T_{22}$ relaxation time of $P_N$ alone. The relaxation time was higher than the $T_{22}$ relaxation time of $P_{ADB4.78}$ alone due to the presence of $P_N$ in the bottom phase. Such difference among the water-binding capability of polymers led to the occurrence of repulsion, reflecting the feasibility in forming aqueous two-phase system [6, 32].

3.5 Partition of tea saponin

3.5.1 Effect of different polymer concentrations

The polymer concentration has a great influence on the TLL in the phase diagram of the ATPS and the difference between the two phases change. In the $P_N/P_{ADB4.78}$ ATPS, tea saponin was mainly partitioned to the bottom phase, the reason may be owing to tea saponin has strong hydrophilicity, and the bottom phase contained more water. The effect of polymer concentration on the partition of tea saponin was shown in Table 3. All of the $K$ were less than 1, the reason was that the viscosity and surface tension of the polymer increased as the polymer concentration increased, which made the mass transfer efficiency of the tea saponin lower in ATPS [24]. The optimal $K$ of crude tea saponin and tea saponin from tea seeds reached 0.46 and 0.42 in 1.5%$P_N$/3.5%$P_{ADB4.78}$ ATPS, which was used to study the partition of tea saponin.

3.5.2 Effect of pH

When HCl or NaOH was added to the ATPS, the change of pH affected the hydrophilicity and hydrophobicity of the pH-responsive $P_{ADB4.78}$ [13]. The pH was too low to form ATPS. Therefore, the effect of pH (6.0-8.0) was investigated at room temperature. The result of crude tea saponin and tea saponin from tea seeds were
observed in Fig. 8(a) and (b). The K decreased slowly with the pH increased. The K of crude tea saponin was 0.57 when the pH was 7.6. When the system pH was 8.0, the K of tea saponin from tea seeds reached minimum, which was 0.49. It could be due to the change of the pH value, which changed the ionization balance of chemical groups such as carboxyl groups and tertiary amine groups on the P_{ADB4.78} surface [6]. In the studied pH range, the hydrophilicity of the P_{ADB4.78} was enhanced, and tea saponin is also hydrophilic, which caused tea saponin transferring from the top phase to the bottom phase. Therefore, pH 7.6 and pH 8.0 were used in the following study on crude tea saponin and tea saponin from tea seeds.

3.5.3 Effect of temperature

Temperature affected the hydrophilicity and hydrophobicity of the P_N and the phase behavior of ATPS [33]. In this study, the influence of temperature on the partition of tea saponin in the P_N/P_{ADB4.78} ATPS was investigated and the range of temperature was from 15°C to 30°C at optimized pH. The result was shown in Fig. 8(c) and (d). The K of tea saponin decreased with the temperature increased, the reason was that the temperature approached to the LCST of P_N, the hydrophobic interaction of P_N increased, so tea saponin tended to transfer to the bottom phase [14]. Compared with 25 °C, the system phase interface was not clear and the extraction recovery of tea saponin decreased when the temperature was 30 °C. Therefore, 25 °C was used for the next exploration experiments.

3.5.4 Effect of inorganic salt species and concentrations

The potential difference between the two phases was one of the driving forces for
the partition of target molecules [34]. When salt ions were added, the phase equilibrium of ATPS and potential difference were changed. In this study, eight different species and concentrations of salt were added into the ATPS in the range from 1.5 mM to 9 mM. As shown in Fig. 9(a) and (b), the optimal K and ERb of crude tea saponin were 0.15 and 92.13 % respectively when the 1.5 mM KCl was added to the ATPS. As shown in Fig. 9(c) and (d), the K and ERb of tea saponin from tea seeds reached minimum which were 0.12 and 94.50% when adding LiBr to 7.5 mM. The main reason for the change of K by the addition of salt ions was explained in 3.5.5.

3.5.5 The interphase potential difference

In this study, the relationship between the potential difference of two phases and the partition was discussed. When the positive and negative ions of salt have different affinities for the two phases, that was, when $K_A^{Z^-}$ and $K_B^{Z^+}$ are not equal, interphase potential difference would generate[24]. The interphase potential difference was calculated by eq.(9):

$$\Delta \phi = U_2 - U_1 = \frac{RT}{(Z^+ - Z^-)F} \ln \frac{K_A^{Z^-}}{K_B^{Z^+}}$$  \hspace{1cm} (9)

Where $\Delta \phi$ was the interphase potential difference, U represented the potential, T was the absolute temperature, R was the universal gas constant, $Z^+$ and $Z^-$ represented the positive and negative ion valances of salt, respectively. F was the Faraday constant. K was the partition coefficient, A and B represented the negative and positive ion in salt solution, respectively.

The relationship between the interphase potential difference values and KCl, LiBr concentrations were shown in Fig. 10(a) and (b). As shown in Fig. 10, the interphase
potential difference and the 1/K were consistent with the changing trend of the KCl, LiBr concentrations. In the 1.5% P_N/3.5% P_{ADB4.78} APS, the corresponding 1/K of crude tea saponin was the largest when the KCl concentration was 1.5 mM, and the interphase potential difference was also the largest. The 1/K of tea saponin from tea seeds reached 8.69 and the maximum Δφ reached 14.3 mV in the presence of 7.5 mM LiBr. The influence of salt ions on the partition of tea saponin was mainly attributed to the effect of salt ions on the interphase potential difference, which resulted in more tea saponin transferring from the top phase to the bottom phase.
4. Conclusion

The recyclable thermo-responsive polymer (PN) and pH-responsive polymer (PADB4.78) were synthesized and characterized in this study. The recoveries of the two polymers were above 95%. Furthermore, the phase formation mechanism was studied by measuring the surface tension and transverse relaxation time of polymer solutions. These results demonstrated that the difference in water-binding capacity between the two polymers was an important factor in the formation of ATPS. The optimal K and highest ER of tea saponin from tea seeds were 0.12 and 94.50% in the presence of 7.5 mM LiBr at pH 8.0 and 25°C, which showed that tea saponin from tea seeds could be effectively separated. Compared with traditional tea saponin extraction methods, such as water extraction [21], organic solvent extraction [22], aqueous two-phase extraction technology improved the extraction recovery of tea saponin and reduced the environmental pollution. Compared with extracting crude tea saponin, the operation procedure was simplified and the extraction cost was saved. Moreover, it provided wide application prospects of ATPS for the separation of industrialized biomolecules.
Ethics Approval

Not applicable.
Consent to Participate

Not applicable.
Consent to Publish

All authors consent to publish the manuscript.
**Authors contributions**

Yanli Wei carried on the data collection and wrote the manuscript.

Xi Chen carried on the data collection.

Ting Yang conducted the data analysis.

Junfen Wan conducted the data analysis, writing and revision of the manuscript.

Xuejun Cao conducted the data analysis, writing and revision of the manuscript.

All authors have read and approved the published version of the manuscript.
Funding

No funding has been received for this work.
Competing Interests

The authors declare that they have no conflict of interests.
Availability of data and materials

Not applicable.
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**Figure captions**

**Fig.1** Synthesis of polymers. (a) $P_N$, (b) $P_{ADB4.78}$

**Fig.2** The process of tea saponin extraction in $P_N/P_{ADB4.78}$ ATPS

**Fig.3** The characterization of polymers. (a) The FT-IR spectrum of $P_N$ and $P_{ADB4.78}$. (b) The intrinsic viscosity of $P_N$. (c) The intrinsic viscosity of $P_{ADB4.78}$

**Fig.4** The recovery of polymers. (a) Zeta potentials of $P_{ADB4.78}$ at different pH. (b) Recovery of $P_{ADB4.78}$. (c) Recovery of $P_N$

**Fig.5** The phase diagrams of $P_N/P_{ADB4.78}$ ATPS. (a) Binodal curves at pH 6.5, 7.0, 7.5. (b) Binodal curves at 20 °C, 25 °C, 30 °C

**Fig.6** Surface tension of $P_N/P_{ADB4.78}$ ATPS

**Fig.7** LF-NMR relaxation time. (a) LF-NMR relaxation time of polymers before phase formation. (b) LF-NMR relaxation time of polymers after phase formation

**Fig.8** Effect of pH and temperature on partition of tea saponin. (a), (b) Effect of pH on crude tea saponin and tea saponin from tea seeds. (c), (d) Effect of temperature on crude tea saponin and tea saponin from tea seeds

**Fig.9** Effect of salts on tea saponin partition. (a) and (c) represent the partition coefficient of crude tea saponin and tea saponin from tea seeds. (b) and (d) represent the extraction recovery of crude tea saponin and tea saponin from tea seeds

**Fig.10** The relationship between the interphase potential difference and KCl, LiBr concentrations. (a) KCl concentrations at 25°C, pH 7.6. (b) LiBr concentrations at 25°C, pH 8.0