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Temperature swing adsorption of melamine on thermosensitive poly(*N*-isopropylacrylamide) cryogels

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Abstract In the past few years, polymer-based adsorbents have been emerging as highly effective alternatives to activated carbons for pollutants removal and recovery from industrial effluents. In this article, novel thermosensitive poly(*N*-isopropylacrylamide) (PNIPAAm) cryogels adsorbents were directly prepared with *N*-isopropylacrylamide (NIPAAm) as a thermosensitive monomer and PEG-20,000 as a porogen at −12 °C by means of an in situ free-radical redox cryopolymerization. Subsequently, PNIPAAm cryogels were further employed to adsorb and desorb melamine through temperature swing adsorption (TSA) between 25 and 50 °C. The adsorption isotherms were correlated to Langmuir and Freundlich isotherm models. Moreover, the result indicated that the developed PNIPAAm cryogels adsorbents could be utilized effectively to concentrate melamine from aqueous solutions and spiked liquid milk. The cycle of the adsorption and desorption could be repeated without much loss of the melamine adsorbing ability.

Introduction

Melamine, known as tripolycyanamide, is an important industrial chemical in the production of melamine resins, and byproducts from the manufacture of pesticides, plastics, plant fertilizers, or building materials [1]. The chemical structure of melamine is displayed in Fig. 1. Because melamine has a significant nitrogen content (67% by mass) and has a low cost, it was illegally adulterated to dairy products and animal feed in order to masquerade as nitrogen in proteins. The 2007 Pet Food Recalls and the 2008 Chinese Milk Scandal have revealed that ingestion of a high melamine level may lead to kidney stones, renal failure and even death in humans especially babies and children [2].

During the past few years polymer-based adsorbents have been emerging as potential alternatives to activated carbon to concentrate ubiquitous organic pollutants from different sources in terms of their vast surface area, adjustable surface chemistry, pore size distribution, and feasible regeneration under mild conditions [3]. Recently, the introduction of cryogels is one of the most significant breakthroughs in the area of macromolecules. Cryogel possessing is a specific category of continuous hydrogel with a characteristic porous structure formed during cryogenic treatment [4].

Nowadays, *N*-isopropylacrylamide (NIPAAm) has been extensively investigated as the best-known thermosensitive hydrogel monomer in the intelligent polymers subdiscipline [5, 6]. Poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits a reversible phase transition induced by a small external temperature change below and above its unique lower critical solution temperature (LCST) at approximately 32 °C [7, 8]. When the environmental temperature is below the LCST, the PNIPAAm hydrogel is swollen and hydrophilic. Nevertheless, above the LCST, the hydrogel presents a shrunken and hydrophobic state as a result of the disruption of the delicate hydrated/dehydrated balance along with its conformation transition [9]. Nevertheless, the conventional PNIPAAm hydrogel is often restricted in its applications, such as separation processes, due to

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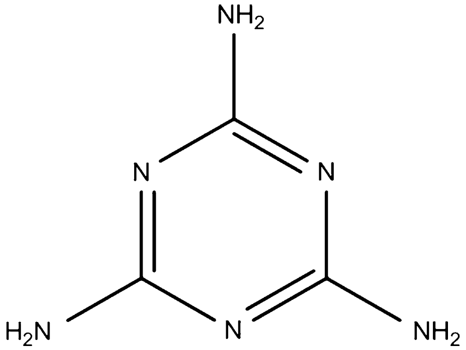

Fig. 1 The structure formula of melamine

inferior sensibility in response to a temperature stimulus. For the sake of accelerating the thermo-sensitive rate, either cryopolymerization or the use of polyethylene glycol (PEG) as a porogen during the polymerization reaction has been proposed as a solution to this problem [10, 11].

In the present article, PNIPAAm cryogels, as reported in our recent article [12], were obtained by in situ free-radical redox cryopolymerization (−12 °C) in a stainless steel tube, using NIPAAm as the functional monomer and PEG-20,000 as the porogen. This facile and direct synthesis route averted the demerits as a result of the surface modification or functionalization, such as complex multistep approaches and decreased of the pore size and surface area [6]. Subsequently, the novel PNIPAAm cryogels were first used as thermosensitive adsorbents to adsorb and desorb melamine from aqueous solution and spiked liquid milk via temperature swing adsorption (TSA), i.e., the control of adsorption and desorption of melamine only by temperature change. The TSA process through the adsorbents described here will provide an energy-saving and environment-friendly process for the concentration and purification of melamine by intermolecular hydrogen bonding interaction. Additionally, nonlinear isotherms models, including Langmuir and Freundlich models were tested to fit the experimental data.

Experimental

Materials

N-isopropylacrylamide (NIPAAm) purchased from Tokyo Kasei Kogyo (Tokyo, Japan) was purified by recrystallization from a mixture consisting of benzene and *n*-hexane(3:7, v/v) and dried at room temperature under vacuum prior to use. *N,N*′-methylenebisacrylamide (BIS) was produced by Tianjin Kermel Chemical Reagent Development Center (Tianjin, China). Ammonium persulfate (APS) and *N,N,N*′*,N*′-tetramethylethylenediamine (TEMED) were purchased from Shanghai Chemical Plant (Shanghai, China). PEG-20,000 was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Melamine was kindly provided by the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Acetonitrile and ammonium acetate are of HPLC grade and were obtained from Yili Co., Ltd. (Beijing, China). Ultrapure and deionised water was prepared from a Millipore-Q water-purification system (Taiwan, China).

A series of melamine standard aqueous solutions were prepared in appropriate ultrapure water and were stored in a refrigerator at 4 °C. Liquid milk was purchased from a local supermarket and spiked with different concentrations of melamine.

Instrumentation and chromatography conditions

Scanning electron microscopy (SEM) measurements were performed by the Hitachi S-4300 SEM (Hitachi, Tokyo, Japan). Chromatography was performed on an Agilent 1100 series HPLC system (Agilent, America) at ambient temperature. The HPLC system consisted of a quaternary pump with an online vacuum degasser, an autosampler and a UV detector. Agilent liquid chromatography system chemical software was used and operated under Windows XP for data acquisition and integration. Chromatographic separations were carried out on a C18 Agela column (250 × 4.6 mm i.d., 5 μm, Agela Technologies Inc., USA). UV spectroscopy was set at the wavelength of 210 nm and the mobile phase was acetonitrile–water (2:98, v/v) containing 0.01 mol/L ammonium acetate at the flow rate of 1.0 mL/min. All solutions for determination were filtered through a 0.45 μm membrane throughout the HPLC studies.

Synthesis protocols

For calculation of the polymerization reactant component, the % *C* (crosslinker concentration/100 mL) and % *T* (total monomer concentration/100 mL) nomenclature suggested by Hjertén [13] was adopted. The polymerization mixtures for the PNIPAAm cryogels adsorbents were prepared as follows: a mixture consisting of BIS (% $C = 40\%$), NIPAAm (% $T = 26\%$) and PEG-20,000 (50 mg/mL) was dissolved in 1.4 mL ultrapure water, vigorously mixed, sonicated for 20 min and bubbled with nitrogen for another 5 min to remove oxygen. Ten microliters of a 10% w/v APS solution was added and the mixture was briefly mixed, before 10 μL of a 10% v/v TEMED solution was finally added. Then, the stainless-steel columns (100 mm × 4.6 mm i.d.) sealed at the bottom were filled with the polymerization mixture and then sealed at the top. After the polymerization was allowed to proceed at −12 °C for 24 h, the seals were removed from the chromatographic tubes,

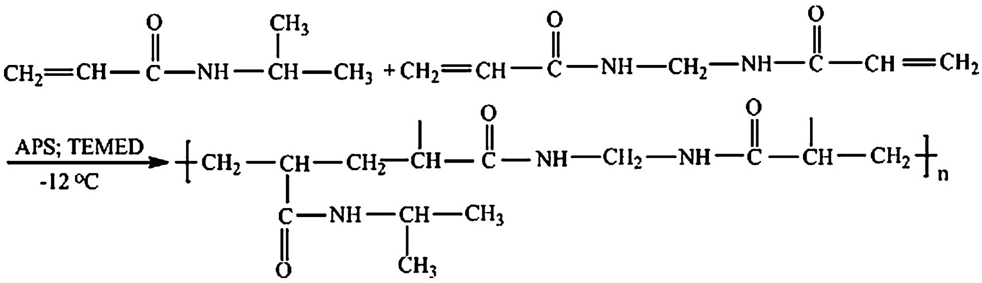

Fig. 2 Synthesis scheme of the PNIPAAm cryogels adsorbents

the columns were attached to the HPLC system, and washed with water at a flow rate of 1 mL/min for 60 min to remove PEG-20,000 and other unreacted compounds in the PNIPAAm cryogels adsorbents at room temperature. The scheme of polymerization is shown in Fig. 2.

Experiments of adsorption and desorption

The adsorption experiments of melamine using PNIPAAm cryogels were carried out in a typical batch fashion at 25 °C. Before starting the melamine adsorption experiment, approximately 0.05 g of the dry PNIPAAm cryogels were weighed accurately and introduced into 25 mL distilled water at 25 °C for 12 h to attain swollen PNIPAAm cryogels. The swollen PNIPAAm cryogels were taken out and then immersed in 20 mL of melamine aqueous solutions and spiked liquid milk at various initial concentrations, C_0 (mg/L) (viz. 8.85, 15.95, 23.25, 34.90, 48.35, 56.30, 66.35, 77.75, 107.70, 121.15; 4.15, 11.25, 21.60, 27.20, 35.45, 42.15, 48.45, 56.25, 60.30, 63.60, respectively). The airproof flasks containing the samples were shaken at 150 rpm in a thermostatic oscillator at 25 °C for 4 h during the adsorption experiments. This period of time was chosen based on preliminary adsorption studies that showed that the required time to reach equilibrium was approximately 4 h. The equilibrium concentrations of adsorbate in the outside melamine aqueous solutions and spiked liquid milk, C_e (mg/L), were determined chromatographically using the HPLC system as stated in "Instrumentation and chromatography conditions" section. The UV absorbency of the melamine standard aqueous solutions with different known concentrations was measured and produced a calibration curve where the melamine concentration C, was defined as $A = 71.56C - 2.51$ with the correlative factors $r = 0.99994$. The equilibrium concentrations, C_e (mg/L), were calculated based on the calibration curves. In addition, the equilibrium adsorption capacity of melamine adsorbed onto the PNIPAAm cryogels adsorbents, q (mg/g), was calculated from the integral mass balance equation of melamine inside and outside the adsorbent: $q = V(C_0 - C_e)/W$, where W is the mass of the dry adsorbent, and V is the volume of adsorption solution. Note that, before the chromatographic analysis for spiked liquid milk samples, pre-treatment procedures were conducted as follows: five milliliters of the spiked liquid milk samples which had been adsorbed were transferred to a 25 mL centrifuge tubes. Then, 10 mL of acetonitrile was added, and these tubes were thoroughly shaken for 1 min and then sonicated for 10 min at room temperature to achieve proteins precipitation. After centrifugation at 10,000 rpm for 5 min, the supernatant was collected and filtered for HPLC determination.

In desorption experiments, melamine adsorbed PNIPAAm cryogels adsorbents were transferred into 20 mL desorption medium, where 5% (v/v) acetic acid aqueous solution was employed. These experiments were performed in order to evaluate the possibility of concentrating melamine. The desorption experiments were carried out by the same procedure as above except that the experimental condition was at 50 °C for 3 h which were the appropriate temperature and time to reach desorption equilibrium based on preliminary studies. The effluent concentrations of melamine in the outer acetic acid aqueous solution, C_d (mg/L), were measured by the HPLC method as previously described. The desorption recovery, D, was calculated as the percentum of desorbed amount of melamine (mg) to the amount adsorbed onto the PNIPAAm cryogels adsorbents (mg).

Results and discussion

Internal morphology studies by SEM

In order to observe the porous property of the PNIPAAm cryogel adsorbent prepared in our laboratory, scanning electron microscopy (SEM) was utilized. Based on the scanning electron micrograph shown in Fig. 3, internal morphology of PNIPAAm cryogel adsorbent was composed of a regular three dimensional bicontinuous skeleton structure with an excellent balance between micropores (which give specific surface area for the required interactions) and macropores (which allow efficient mass transfer). Two main aspects could contribute to this remarkable appearance: (1) the ice crystals formed after freezing could act as a porogen and (2) the dissolved monomers and

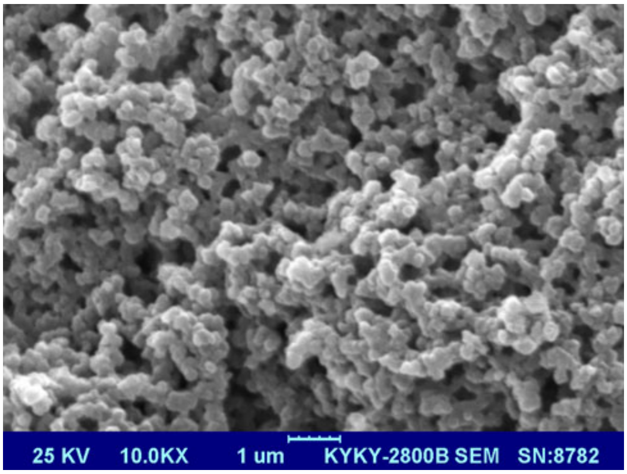

Fig. 3 Internal morphology of PNIPAAm cryogel adsorbent

initiator were concentrated in a small fraction of a non-frozen fluid in which polymerization proceeded efficiently. After melting, a continuous porous monolithic cryogel was formed via this cryopolymerization system [14]. On the other hand, the introduction of PEG-20,000 induces lateral aggregation of polymer chains, thus contributing to the formation of a more porous structure [15].

Temperature swing adsorption

The possibility of TSA, i.e., the control of adsorption and desorption by changing the temperature, was investigated using 30 mg/L melamine aqueous solutions. The temperature dependence for the adsorption capacity q of melamine from aqueous solution onto the PNIPAAm cryogel adsorbent was shown in Fig. 4. The amount of melamine adsorbed on cryogels decreases sharply in a stepwise manner with an increase in temperature in the range 30–35 °C. The reason might be that, below the LCST, the melamine was bound to the hydrophilic moieties (amide groups) of PNIPAAm cryogels mainly by the hydrogen bonds, and the adsorbents existed in an extended coil conformation; in turn, above the LCST, the hydrogen bonds were disrupted, and the adsorbents transferred to be hydrophobic resulting from the exposed hydrophobic moieties (isopropyl side groups) and shriveled into a compact globule conformation [16]. Therefore, in this temperature range, NH···O and NH···N intermolecular hydrogen bonding between melamine and PNIPAAm cryogels adsorbents was broken and the releasing melamine was then squeezed out from adsorbents along with the occurrence of phase transition behavior. It can be said that the change in the swollen/shrunken and hydrophilic/hydrophobic transition have a significant effect on adsorption performance. Therefore, this temperature dependence indicates the feasibility of the TSA.

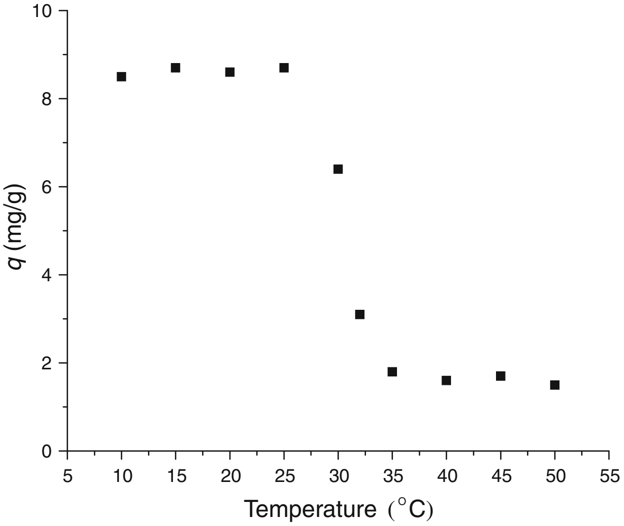

Fig. 4 Adsorption capacity of melamine adsorbed onto the PNIPAAm cryogels adsorbents as a function of temperature

Modeling of the adsorption isotherms

The type of the adsorption can be deduced from data fitting to model equations for the adsorption isotherm. In the present study, Langmuir and Freundlich isotherms are adopted to describe the adsorption process at 25 °C [17, 18]. The Langmuir model for q is given by $q = q_m bC_e/(1 + bC_e)$, where q_m and b denote the maximum adsorption capacity of the adsorbent of unit mass and Langmuir constant, respectively. The Freundlich model is as follows: $q = kC_e^{1/n}$, where k and n are the characteristic constants to be determined for each adsorption system. The fitted curves to the Langmuir and Freundlich models were displayed in Figs. 5 and 6.

The equilibrium constants and the corresponding R^2 adjustment coefficients were listed in Table 1. Apparently, the experimental equilibrium data were found to be well correlated to both models. According to the Freundlich theory (Slejko, 1985), the parameter k was taken as a relative indicator of adsorption capacity and $n > 1$ indicated the favorable adsorption. From Table 1, both the exponents (n) in these two cases were larger than 1, which demonstrated the adsorption of melamine onto the PNIPAAm cryogel adsorbent was favorable. Moreover, it was quite remarkable that Langmuir isotherms characterized the experimental results better than Freundlich isotherm due to $R^2 > 0.97$, which indicated that the adsorption may be a monolayer adsorption process and the surface energy of the adsorbent may be homogeneous. Additionally, maximum adsorption capacity value in single-component solutions was greater than the corresponding one in spiked liquid milk at the same temperature as a result of the complexity of dairy products.

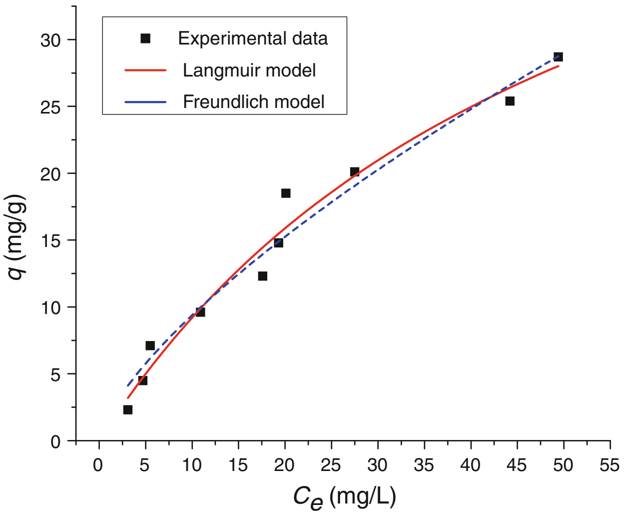

Fig. 5 Adsorption isotherms in melamine aqueous solutions

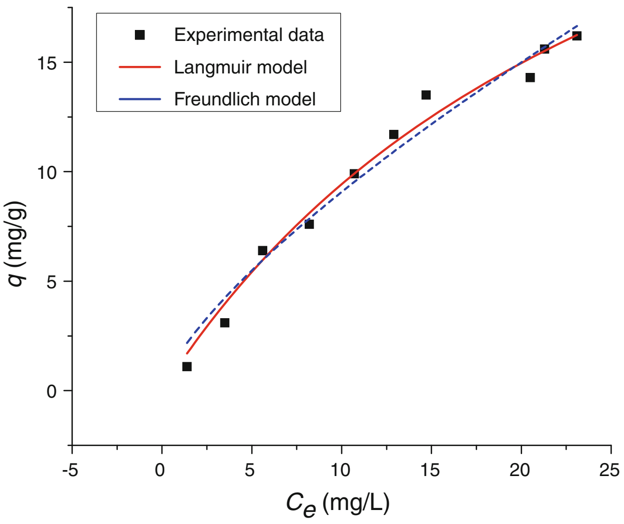

Fig. 6 Adsorption isotherms in spiked liquid milk

Desorption and adsorption/desorption cycles

In the current study, 5% (v/v) acetic acid aqueous solution was applied as desorption medium in order to promote the desorption progress and maximize the desorption amount by disrupting intermolecular hydrogen bonding interaction. The desorbed concentrations of melamine aqueous solutions and spiked liquid milk, C_d (mg/L), was determined chromatographically as follows: 4.77, 9.79, 15.27, 21.12, 26.75, 32.56, 39.78, 44.22, 54.61, 65.29; 1.93, 5.27, 10.72, 14.06, 17.08, 21.65, 26.33, 28.96, 30.81, 32.40, respectively). The above-mentioned desorption recovery, D, was calculated and presented in Table 1. In the same manner, desorption recovery values of single-component solutions were higher than that of complex spiked liquid milk matrixes. In addition, adsorption/desorption cycles were evaluated in spiked liquid milk and was shown in Fig. 7. The experimental procedure of adsorption/desorption was described in "Experiments of adsorption and desorption" section for successive cycles. As shown in Fig. 7, the adsorbed amount decreased somewhat from one cycle to another, presumably due to the occupancy or deactivation of active sites. However, the PNIPAAm cryogels adsorbents retained the approving adsorption/desorption capacity for successive cycles. Therefore, this confirmed the reliability and efficacy of the proposed adsorbent for long term use for the enrichment melamine residues in real samples.

Conclusions

In the present study, the viability of using thermosensitive PNIPAAm cryogel as sorbent for extraction and enrichment of melamine from aqueous solutions and spiked liquid milk by TSA was confirmed. The proposed mechanism associated with the TSA of melamine onto the PNIPAAm cryogel adsorbent is as follows: in the case of the swelling gel at temperatures lower than the LCST, melamine might be adsorbed dominantly by multiple sites NH···O and NH···N intermolecular hydrogen bonding between melamine and PNIPAAm cryogel adsorbent, while at temperatures higher than the LCST, melamine is desorbed and ejected along with free water in the shrinking process. The adsorbent effectively played the dual role of a separator and a mediator to enrich melamine from aqueous solutions and spiked liquid milk. The TSA technique is simple, environmentally friendly, and potentially applicable to a variety of melamine purification processes in different dairy products matrixes.

Table 1 Langmuir and Freundlich parameters adjusted for adsorption experimental data and desorption recovery

	Langmuir model			Freundlich model			D (%)
	q_m	b	R^2	k	n	R^2	
Melamine aqueous solutions	58.3	0.019	0.9729	1.867	1.426	0.9666	87 ± 4
Spiked liquid milk	36.2	0.035	0.9827	1.707	1.379	0.9689	74 ± 7

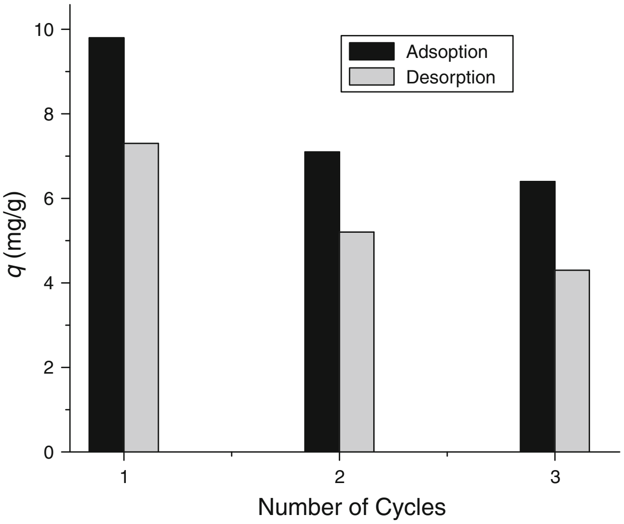

Fig. 7 Adsorption/desorption cycles on PNIPAAm cryogels adsorbents in spiked liquid milk

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