Poly(vinyldiaminotriazine) nanoparticle adsorption of small drug molecules in aqueous phase and the role of synergistic interaction between hydrogen bonding and hydrophobic affinity

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Abstract
Poly(vinyldiaminotriazine) nanoparticles adsorbed 5-fluorouracil, thymine, theobromine, and xanthine in aqueous phase. Each drug molecule formed triple hydrogen bonds with a diaminotriazine moiety at the nanoparticle surface. Adsorption isotherm studies suggested monolayer adsorption while the adsorption capacity was strongly dependent on the drug compositions/structures (0.053 mmol/g 5-fluorouracil, 0.094 mmol/g thymine, 0.0078 mmol/g theobromine, and 0.0065 mmol/g xanthine). The drug compositional/structural effects were also reflected in the adsorption kinetics. In addition to neutral pH adsorption, studies were performed at pH below the polymer’s pKa and above the drugs’ pKa, separately. Reducing hydrophobicity of the hydrogen bonding sites by protonating the diaminotriazine moieties at pH 4.5 resulted in more than 3 times decrease in adsorption capacities whereas deprotonating the drug molecules at pH 9 increased the adsorption capacity due to electrostatic attraction between the negatively charged drugs and the positively charged nanoparticles, which was supported by strong dependency of the adsorption capacity on ionic strength. The adsorption was reversible for all the four drugs. While increasing temperature caused faster desorption, reducing hydrophobicity of the diaminotriazine moieties by protonation at pH 4.5 had a stronger effect on both cumulative desorption and burst release. The results of this study demonstrated an important role of synergistic interaction between hydrogen bonding and hydrophobic affinity in poly(vinyldiaminotriazine) nanoparticle adsorption of the drugs in the aqueous phase.

Keywords 2-Vinyl-4,6-diamo-1,3,5-triazine polymer nanoparticles · Drug molecules · Hydrogen bonding · Hydrophobic affinity · Physical adsorption in aqueous phase · pH and ionic strength

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Introduction
Liquid-phase adsorption refers to a phenomenon of accumulation of dissolved adsorbate molecules by the solid surface of an adsorbent, either physical or chemical in nature. Physical adsorption occurs under physical interactions and has found applications in a variety of fields, e.g., from separation, purification, and chromatography [1, 2] to drug delivery, selective recognition, and novel sensing technologies owing to its reversibility, versatility, and diversity [3]. The physical interactions include hydrogen bonding (H-bonding), electrostatic attraction, hydrophobic affinity, and Van der Waal’s forces. Van der Waal’s forces are normally weak while the electrostatic attraction is susceptible to variations in pH and/or ionic strength. In contrast, H-bonding is a strong physical interaction with high directionality and hence has attracted increasing interests for both academia research and industrial development as an effective driving force for physical adsorptions...
However, challenges exist to achieve strong and stable H-bonding in aqueous phase, particularly for water-soluble adsorbates because water molecules are an effective competitor for H-bonding and can weaken or even destroy the H-bonding between an adsorbate and adsorbent.

Nevertheless, nature provides inspirations in this respect as H-bonding plays a critical role in DNA, RNA, and proteins. It is believed that base pairing in nucleic acids is the result of more than just H-bonding as base pairing stability is dependent on nearest-neighbor interactions, such as solvophobic effects [7]. The formation of stable H-bonding between proteins and their target ligands in aqueous phase stems from the unique chemical composition and spatial structure of the amino acid units. In addition to having multiple functional groups capable of forming hydrogen bonds, other atoms/groups in the amino acid unit also play an important role. They provide the freedom of rotation of the polypeptide chain to allow the functional groups to enter the optimal binding sites by chain folding. Furthermore, the adjacent parts of the polypeptide chain can restrict the entry of water molecules into the binding site through certain interactions, resulting in a significant increase in the strength of hydrogen bonds between the protein and the ligand [8]. In other words, the synergetic effect of H-bonding groups and hydrophobic affinity is the key for the H-bonding in aqueous phase. In the literature, attempts have been made by copolymerization of lipophilic monomers with “H-bonding” monomers which were highly water-soluble, such as acrylamide and its derivatives [9, 10]. However, due to the secondary-structure complexity of the hydrophilic monomer and the lipophilic monomer copolymers, it is difficult to achieve an ideal synergy of the hydrophobic affinity and H-bonding.

2-Vinyl-4,6-diamino-1,3,5-triazine (VDAT) is a monomer with multiple sites of both donor and receptor for H-bonding as depicted in Scheme 1. Interestingly, VDAT has very low solubility in not only water but also the majority of the organic solvents. This is attributed to its unique physiochemical characteristics which enable multiple intermolecular hydrogen bands that are too strong to be broken in neither water nor the organic solvents. By taking advantage of the VDAT functionalities, our research group has recently prepared a novel high-strength dual physically crosslinked hydrogel of the copolymer of VDAT, acrylamide, and acrylic acid [11]. The hydrogel was synthesized by free radical polymerization in dimethyl sulfoxide (DMSO), followed by replacing DMSO with Fe(NO₃)₃·9H₂O aqueous solution, leading to the formation of carboxylic-Fe³⁺ coordination and H-bonding between the pendent dianamotriazine (DAT) moieties. Publications from other research groups also suggested that VDAT units incorporated in a copolymer chain could form multiple H-bonding [12].

Apart from the self-intermolecular H-bonding, the DAT moieties incorporated in polymers hold potentials to form H-bonding with target molecules in water [13–16]. For physical adsorption, the preferred morphology of functional polymers is micro- or nanoparticles with spherical shape and well-controlled size and polydispersity due to their large specific surface area and flexibility to meet various application needs. Because of the poor solubility of solid VDAT, however, the traditional synthetic methods are not suitable for preparing PVDAT nanoparticles. In their pioneer work on VDAT functional monomer, Asanuma et al. demonstrated that in water, VDAT homopolymer (PVDAT) and its copolymer with acrylamide selectively adsorbed nucleic acid bases, nucleotides, and nucleosides through H-bonding [14]. The binding strength was positively correlated with the number of hydrogen bonds between the DAT moieties and the target molecules since stable adsorption occurred only for the target molecules, such as uric acid and thymine, capable of forming three hydrogen bonds with the DAT moiety, whereas little adsorption was detected for pyrimidine, cytosine, adenine and guanine molecules bearing one or two binding sites for the H-bonding. However, the authors prepared their VDAT polymers by free radical polymerization in DMSO using azobisisobutyronitrile as the initiator, and the synthesized polymers were in the form of particles with poor morphology and size distribution. Recently, our research group has successfully synthesized PVDAT nanoparticles via semi-continuous precipitation polymerization in water [17, 18]. By continuously feeding VDAT solution in DMSO to hot water, the concentration of dissolved VDAT was carefully kept below its saturation and the PVDAT nanoparticles with a uniform size about 136 nm were formed by a mechanism of homogeneous initiation, oligomers/polymers nucleation and particle growth. By using 2,2′-azobis(2-methylpropionamidine)dihydrochloride (V50) as a cationic free radical initiator, positive charges were introduced to the particle surface to protect the particles from agglomeration without the need of using any surface-active substances which could complicate the physical adsorption studies. This article reports our studies on physical adsorption of the PVDAT nanoparticles for a series of target drug molecules, including 5-fluouracil (5-FU), thymine, theobromine, and xanthine. Each of the drug molecules has potentials of forming three hydrogen bonds with one DAT moiety at the nanoparticle surface as depicted in Scheme 2. 5-FU is a derivative of pyrimidine with the C5 methyl group of thymine substituted by fluorine, while theobromine and xanthine are...
purine alkaloids with and without two methyl substituents at N3 and N7, respectively. These compositional/structural differences provide opportunities for studying hydrophobic affinity effects on drugs’ H-bonding with the DAT moiety. In addition, the differences between the drugs pKa (from 7.53 to 9.9) and PVDAT pKa (5.3) allows manipulation of hydrophobic affinity by adjusting the electrostatic charge state of the drug molecules and DAT moieties separately with pH variations and therefore provides insights into the synergistic effects of the H-bonding and hydrophobic affinity on the adsorption capacity, thermodynamics, and kinetics. A preliminary study on desorption of the adsorbed drug molecules was also performed under varied pH and temperature. Understanding of the synergistic role of hydrophobic affinity in aqueous phase H-bonding is important, not only for the design of new functional polymers which are potentially useful in the physical adsorption related areas but also from the fundamental standpoint as it is a basic part of biological function that its significance is almost implicit.

Materials and methods

Materials

5-FU, thymine, theobromine, and xanthine were purchased from Aladdin. All chemicals were analytical grade and used without further purification. All aqueous solutions and subsequent dilutions were prepared using distilled deionized (DDI) water. VDAT was synthesized in our laboratory by following the method described in reference [19]. Briefly, to a 100-mL three-necked flask equipped with a condenser, nitrogen inlet and outlet, 0.1 mol 2MA, 0.1 mol epichlorohydrin, and 2 wt% (weight percent of 2MA) inhibitor Na2S·9H2O were added. Then, 50 mL DDI water was added to dissolve the chemicals under mechanical agitation at about 200 rpm. The flask sat in a thermostatically controlled heating mantle (IKA RCT B S025) and the solution was heated under a slow nitrogen flow, followed by reflux at 100 °C for 30 min. After removing the heating mantle, the reaction solution was left to stand to cool to room temperature. Finally, the crude product was filtered out, dried, and re-crystallized from water to give the final product which was then dried in a vacuum oven at 50 °C until constant weight.

PVDAT nanoparticle preparation

A typical synthesis was performed by following the method described in our previous publication [18]. Prior to the polymerization, 1.6 g VDAT and 90% of 0.016 g V50 were dissolved separately in 15 mL DMSO and 15 mL DDI water in two reservoirs, while the other 10% V50 was added to 50 mL DDI water in the reactor under a mechanical agitation of around 200 rpm at 80 °C. After applying slow nitrogen flow to the reactor and the two reservoirs for about 1 h, the peristaltic pump was switched on to start feeding the solutions of VDAT and V50 to the reactor at about 43.65 μL/min. The reaction continued for a further 16 h after the feedings were completed. The prepared dispersion was purified by three times of centrifugation and re-dispersing in DDI water, then dried PVDAT nanoparticles were stored at room temperature for further use.

Adsorption evaluations

The PVDAT nanoparticle dispersion and the drug solutions were thoroughly mixed by using a mechanical shaker and all the experiments were conducted at an ambient temperature of 299 ± 5 K to minimize temperature interference in the adsorption process. Then, the solid phases were separated by centrifugation by using a desktop high-speed centrifuge (TLL-C) at 10000 rpm for 10 min, and then the supernatant was filtered off using a 0.45-μm PES Syringe Filter. The absorbances of residual drug in the aqueous solutions were determined by using a UV-visible spectrophotometer (Hitachi U3900) and the residual drug concentrations were calculated by using the corresponding calibration equations for each drug.

For adsorption isotherm studies, a series of experiments were carried out in 3-mL centrifuge tubes containing 1 mg dried PVDAT nanoparticles and 2 mL target drug solution under the same pH 7.4 and ionic strength 200 mmol/L; the initial concentrations were chosen as 1–40 μg/mL for 5-FU, 15–120 μg/mL for thymine, 1.5–8 μg/mL for xanthine, and 0.5–8 μg/mL for theobromine. A small volume of 200 mmol/L NaH2PO4 and 200 mmol/L Na2HPO4 aqueous solution was added to adjust pH and ionic strength.

The adsorption capacity was determined by Eq. (1):

$$q = \frac{(C_0 - C)}{V} \cdot \frac{1}{M}$$

where $q$ is the adsorption capacity (mg/g); $C_0$ is the initial drug concentration (mg/mL); $C$ is the residual drug concentration at
equilibrium (mg/mL); \( V \) is the solution volume (mL); \( M \) is mass of the PVDAT nanoparticles (g).

Four isotherm models of Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich (D-R), expressed in Eqs. (2) to (5), were employed to fit the equilibrium data and obtain the key adsorption constants.

\[
C_e = \frac{1}{q_m \cdot K_L} + \frac{C_e}{q_m}
\]  

\[
\ln q_e = \ln K_F + \frac{1}{n} \cdot \ln C_e
\]  

\[
q_e = B \cdot \ln A + B \cdot \ln C_e
\]  

\[
\ln q_e = \ln Q_0 - K_D \cdot \varepsilon^2
\]

where \( C_e (\mu g/mL) \) is the equilibrium concentration, \( q_e (mg/g) \) is the adsorption capacity at equilibrium, \( q_m (mg/g) \) is the maximum monolayer adsorption capacity calculated by the Langmuir model, \( K_L \) is the Langmuir adsorption constant.

The characteristics of the Langmuir isotherm can also be expressed in a term of equilibrium parameter \((R_L)\), \( R_L = 1/ (1 + K_L \cdot C_0) \), \( C_0 \) is the initial concentration. \( K_F \) and \( n \) are Freundlich constants, \( K_F \) represents the relative adsorption capacity of adsorbent, and \( n \) is related with adsorption intensity. \( B \) is the Temkin isotherm constant and \( A \) is the Temkin isotherm energy constant. \( \varepsilon \) is the Polanyi potential, \( \varepsilon = RT \ln (1 + 1/C_e) \). \( Q_0 (mg/g) \) is the theoretical adsorption capacity, and \( K_D \) is the activity coefficient.

The Langmuir isotherm assumes that the adsorption cannot proceed beyond monolayer coverage of adsorbate over a homogeneous adsorbent surface [20]. The Freundlich model is an empirical expression used to describe both the heterogeneous surfaces and multilayer adsorption [21]. The Temkin isotherm assumes that the binding energy is distributed uniformly for the adsorption [22]. The D-R isotherm can describe the adsorption process at low concentration and be suitable for both homogeneous and heterogeneous surfaces [23].

Under the same conditions, adsorption kinetic studies were conducted at the equilibrium concentration of each target drug, and the adsorption time varied between 5 and 90 min for 5-FU, 2 and 20 min for thymine, 3 and 30 min for xanthine, 10 and 160 min for theobromine. Adsorption quantity at a given time \( (q_t) \) was calculated by Eq. (6):

\[
q_t = (C_0 - C_t) \cdot V / M
\]

where \( C_0 \) and \( C_t (\mu g/L) \) are the drug concentrations at start and time \( t \), respectively; \( V \) is the solution volume (mL); \( M \) is the mass of the PVDAT nanoparticles (mg).

The adsorption at different times was fitted by pseudo-first-order model, pseudo-second-order model, Elovich equation, and intra-particle diffusion [24–27]. The four corresponding equations are expressed in Eqs. (7) to (10):

\[
\ln(q_e - q_t) = \ln q_e - K_1 \cdot t
\]

\[
\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e}
\]

\[
q_t = \frac{1}{\beta} \cdot \ln(\alpha \beta) + \frac{1}{\beta} \cdot \ln t
\]

\[
q_t = K_i \cdot t^\frac{1}{2} + C
\]

where \( q_e (mg/g) \) and \( q_t (mg/g) \) are respectively the quantity of drug adsorbed at equilibrium and time \( t \); \( k_1 (min^{-1}) \) and \( k_2 (g \cdot mg^{-1} \cdot min^{-1}) \) are respectively the rate constants of pseudo-first-order model and pseudo-second-order model; \( \alpha \) is the initial adsorption rate constant (mg/(g min)) while \( \beta \) is the desorption rate constant (g/mg); \( k_i (g \cdot mg^{-1} \cdot min^{-0.5}) \) is the intra-particle diffusion rate constant, and \( C \) is the thickness of boundary layer.

**pH and ionic strength studies**

Experiments were performed with varied pH in a range of 4.4 to 10 at ionic strength 200 mmol/L. Studies were also performed to investigate the effects of ionic strength which was varied in a range of 20 to 200 mmol/L at pH 7.4.

**Desorption evaluations**

The drug-loaded PVDAT nanoparticles (6 mg) were collected by centrifugation, then re-dispersed in 2 mL of DDI water, and finally poured into dialysis tubes with a molecular weight cutoff of 8000–14,000 (Spectra/Por, Spectrum Laboratories, Inc., USA). The dialysis tubes were placed in reservoirs with 10 mL of phosphate buffer under different temperature and pH conditions. The solution (1.0 mL) was periodically sampled to determine concentrations of the desorbed drug by UV-vis analysis, and then the test solution was returned to the reservoir with minimal delay.

**Results and discussion**

**Adsorption of the drug molecules by PVDAT nanoparticles**

Scanning electrical microscopy on the prepared PVDAT nanoparticles revealed uniform size with an average diameter of about 136 nm. A transmission electron microscope image of the synthesized PVDAT nanoparticles is shown in Fig. 1.

As depicted in Scheme 2, 5-FU, thymine, theobromine, and xanthine comprise an identical composition unit to form simultaneous triple hydrogen bonds with each DAT moiety and hence, according to Asanuma and coworkers’ findings [15, 16], are all potentially capable of achieving stable adsorption
on the PVDAT nanoparticles in aqueous phase. After mixing with the PVDAT nanoparticles in pH 7.4 phosphate buffer at room temperature, adsorption was evident for the four drugs as indicated by clear decreases in their residual concentrations.

Adsorption isotherm studies were conducted to not only evaluate the adsorption capacity but also define how the adsorbate interacts with adsorbents. Figure 2 shows the adsorption isotherms of the four drugs on the PVDAT nanoparticles at pH 7.4 and ionic strength of 200 mmol/L. The equilibrium adsorption increased rapidly with increasing drug initial concentration in the low concentration range, then gradually slowed down and eventually reached their maxima. Linear fitting of $C_e/q_e$ versus $C_e$, $\ln q_e$ versus $\ln C_e$, $q_e$ versus $\ln C_e$, and $\ln q_e$ versus $\varepsilon^2$ was applied to determine the corresponding isotherm constants of the four isotherm models (see Supplementary Information, Figs. S1–S4), and the obtained results are listed in Table 1. Langmuir model is the best fit for describing adsorption for all the drugs as indicated by the highest correlation coefficient values ($R^2 > 0.99$). This suggests that the drug molecules probably formed monolayer coverage at the PVDAT nanoparticle surfaces, and all the nanoparticle adsorption sites could have the same adsorption energy. Langmuir equilibration parameter $R_L$ provides information about the adsorption nature: irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$), or unfavorable ($R_L > 1$) [28, 29]. The $R_L$ values over the given initial concentration range were calculated to be in a range of 0.009–0.155 for 5-FU, 0.465–0.874 for thymine, 0.019–0.092 for xanthine, and 0.073–0.559 for theobromine. These $R_L$ values indicate that the adsorptions are favorable for all the four drugs.

As shown in Table 1, the adsorption capacities are 0.053 mmol/g for 5-FU, 0.094 mmol/g for thymine, 0.0078 mmol/g for theobromine, and 0.0065 mmol/g for xanthine. It is interesting that the adsorption capacity of thymine is nearly twice that of 5-FU. This could be attributed to the different effects of fluorine and methyl substituents of the two drugs on their H-bonding. Due to fluorine’s strong electronegativity, 5-FU is less hydrophobic than thymine. Moreover, fluorine is a poor acceptor of H-bonding due to the low polarizability of its electron pairs which contribute only weakly to the electron transfer $n \rightarrow \sigma^*$. On the other hand, the electron-withdrawing effect of the 5-FU fluorine atom weakens its neighboring C=O as H-bonding acceptor. On the contrary, the electron-donating effect of the methyl substituent in thymine probably makes its triple hydrogen bonds with the DAT moiety stronger than that of 5-FU. Xanthine and theobromine have greater steric hindrance effects than 5-FU and thymine, which probably accounts for the large difference (about one magnitude) in the adsorption capacity. The steric hindrance effects were also reflected by Asanuma and coworkers’ results: thymine adsorbed on the VDAT polymer more than ten times than its nucleotide derivatives [16]. It is noteworthy that theobromine has larger steric hindrance, but slightly higher adsorption capacity than xanthine. This agrees with

![TEM image of PVDAT nanoparticles](image)

**Figure 1** TEM image of PVDAT nanoparticles

| Isotherm models         | Constant  | 5-FU       | Thymine    | Theobromine | Xanthine |
|------------------------|-----------|------------|------------|-------------|----------|
| **Langmuir**           | $q_m$ (mg/g) | 6.897      | 11.846     | 1.398       | 0.987    |
|                        | $q_m$ (mmol/g) | 0.053      | 0.094      | 0.0078      | 0.0065   |
|                        | $R^2$      | 0.9997     | 0.9986     | 0.9908      | 0.9988   |
|                        | $R_L$      | 0.009–0.155 | 0.465–0.874 | 0.073–0.559 | 0.019–0.092 |
| **Freundlich**         | $n$        | 6.481      | 1.430      | 1.704       | 19.763   |
|                        | $K_F$      | 4.430      | 0.456      | 0.480       | 0.879    |
|                        | $R^2$      | 0.7993     | 0.9945     | 0.8838      | 0.8725   |
| **Temkin**             | $A$        | 377.947    | 0.121      | 5.738       | $1.325 \times 10^8$ |
|                        | $B$        | 0.774      | 4.232      | 0.357       | 0.047    |
|                        | $R^2$      | 0.8643     | 0.9777     | 0.7939      | 0.8686   |
| **Dubinin-Radaushkevich** | $Q_0$ | 6.496      | 9.509      | 0.921       | 0.962    |
|                        | $E$        | 0.501      | 306.748    | 0.478       | 0.231    |
|                        | $R^2$      | 0.9513     | 0.8333     | 0.921       | 0.8415   |
the hypothesis about the important role of synergy between the H-bonding and hydrophobic affinity because the two methyl groups in theobromine probably are of help in repelling water molecules from vicinity of the H-bonding sites.

Our PVDAT nanoparticles (around 136 nm, Fig. 1) are much smaller than the irregular particulates (average diameter 1 μm) used in Asanuma and coworkers’ studies and hence could provide much larger specific surface area for adsorption. However, our measured equilibrium adsorption capacity for thymine is significantly lower than Asanuma and coworkers’ results reported in reference 16 (0.094 mmol/g vs 0.38 mmol/g). This could be attributed to the differences between the two particle surfaces. Our PVDAT nanoparticle surfaces were highly positive charged (zeta potential 35.15 mV) due to the use of V50 initiator, whilst Asanuma and coworkers’ particulates were synthesized by using azobisisobutyronitrile as an initiator and had no surface charge. The electrostatically neutral surfaces could be beneficial to the synergy between the H-bonding and hydrophobic affinity, therefore facilitating thymine adsorption.

Apart from Langmuir isotherm, some key constants of the other three models were also listed in Table 1. The Freundlich model \( n \) values for the four drugs are in the range of 1.43–19.763, suggesting the adsorption was a physical process [30, 31]. For the D-R isotherm, the magnitude of \( E \) is used to estimate whether an adsorption process is chemical (8 kJ/mol \( < E < 16 \) kJ/mol) or physical (\( E < 8 \) kJ/mol) [32], where \( E \) is the mean adsorption energy and can be determined by the equation: \( E = 1/(2K_D)^{1/2} \). The values of \( E \) in Table 1 are under 8 kJ/mol and hence suggest a physical adsorption process for the four drugs.

Figure 3 shows the drug adsorption capacity variations with time. All the drugs reached the adsorption equilibrium within 40 min. Linear fitting of the experimental data with the four kinetic models are presented in Supplementary Information (Figs. S5–S8) for the four drugs. The obtained parameters of the kinetic models are listed in Table 2. The pseudo-second-order model supposes that the number of binding sites relies on the equilibrium adsorption capacity for thymine and hence the differences between the two particle surfaces. Our PVDAT nanoparticle surfaces were highly positive charged (zeta potential 35.15 mV) due to the use of V50 initiator, whilst Asanuma and coworkers’ particulates were synthesized by using azobisisobutyronitrile as an initiator and had no surface charge. The electrostatically neutral surfaces could be beneficial to the synergy between the H-bonding and hydrophobic affinity, therefore facilitating thymine adsorption.

To gain further understanding about the synergistic interaction between H-bonding and hydrophobic, experiments were performed with varied pH in a range of 4.5 to 9 for 5-FU, 4.5 to 10 for thymine and theobromine, and 4.5 to 8 for xanthine across of both PVDAT pKa (around 5.15) and pKa of the four drugs (5-FU 8.0, thymine 9.9, theobromine 9.9, xanthine 9.9).
and xanthine 7.53) [33–35]. Variations of the adsorption capacity with pH are shown in Fig. 4. The same trend was observed for all the four drugs: the adsorption capacities showed a step decrease as pH dropped below PVDAT pKa while a step increase was detected at pH above the drug’s pKa.

As an example, Scheme 3 depicts H-bonding and electrostatic charge states of the DAT moieties and 5-FU molecules. At pH 6 and pH 7.4, tautomerization of the drugs likely occurred and the equilibrium probably shifted towards the lactam at the lower pH. Nevertheless, both the DAT moieties and the drug molecules were primarily in their electrostatic neutral state, in favor of forming the triple H-bonding. Adjusting pH to 4.5 was deliberate to protonate the DAT moieties. It is interesting that at pH 4.5, the four drugs achieved about a quarter to one-third of the pH 7.4 adsorption capacities (26.42% for 5-FU, 26.06% for thymine, 32.93% for theobromine, and 30.77% for xanthine) although the protonation should dramatically facilitate H-bonding competition from the water molecules. Given that all the receptors and donors for the triple H-bonding should be intact at pH 4.5, the

### Table 2 Adsorption kinetic parameters and correlation coefficients ($R^2$) of the four models

| Kinetics models | Parameters | 5-FU | Thymine | Theobromine | Xanthine |
|-----------------|------------|-----|---------|-------------|----------|
| Pseudo-first-order model | $q_e, exp$ | 6.853 | 11.914 | 1.237 | 1.137 |
| | $q_e, calc$ | 6.844 | 11.932 | 1.234 | 1.128 |
| | $K_1$ | 0.107 | 0.928 | 0.084 | 0.127 |
| | $R^2$ | 0.9995 | 0.9997 | 0.9978 | 0.9987 |
| Pseudo-second-order model | $q_e, exp$ | 6.853 | 11.914 | 1.237 | 1.137 |
| | $q_e, calc$ | 7.257 | 12.225 | 1.278 | 1.182 |
| | $K_2$ | 0.033 | 0.212 | 0.341 | 0.937 |
| | $R^2$ | 0.9987 | 0.9989 | 0.9962 | 0.9969 |
| Elovich equation | $\alpha$ | 7.998 | 5154.837 | 1.949 | 112.347 |
| | $\beta$ | 0.864 | 0.9133 | 7.616 | 8.666 |
| | $R^2$ | 0.8859 | 0.6054 | 0.8765 | 0.8249 |
| Intra-particle diffusion model | $C$ | 0.411 | 0.714 | 0.0431 | 0.066 |
| | $K_i$ | 3.699 | 9.358 | 0.913 | 0.838 |
| | $R^2$ | 0.7211 | 0.4616 | 0.6457 | 0.7101 |

Fig. 3 PVDAT nanoparticles adsorption kinetics. Experimental and model predictions for a 5-FU; b thymine; c theobromine; d xanthine
significant decrease in the drug adsorption capacity was very likely due to the undermined H-bonding as the DAT protonation caused an increase in hydrophilicity of the H-bonding sites and hence, weakened the synergistic interaction between the H-bonding and hydrophobic affinity. This probably also accounts for the slightly lower 5-FU adsorption capacity at pH 6 (0.049 mmol/L) than that at pH 7.4 (0.053 mmol/L) because the protonated and non-protonated DAT moieties always co-exist even at the neutral pH and the lower pH is, the higher percentage of the protonated form [15]. As pH increased from 7.4 to 9, 5-FU molecules underwent deprotonation and became negatively charged. Furthermore, the increased pH could also induce lactam-to-lactim tautomerism through an intramolecular proton transfer. A 5-FU molecule in the lactim form can only form two hydrogen bonds with a DAT moiety (see Scheme 3) and hence compromise the adsorption. This obviously contradicted the experimental observations and suggested two possible scenarios: first, the electrostatic attraction between the negatively charged drug molecules and the positively charged PVDAT nanoparticle surfaces could be strong and play a dominant role in the adsorption under this condition, and secondly, a significant proportion of 5-FU molecules were in the lactam form capable of forming triple H-bonding while the electrostatic attraction could complement the adsorption.

Studies were also performed to investigate the effects of ionic strength which was varied in a range of 20 to 200 mmol/L at pH 7.4. As shown in Fig. 5, a gradual decrease in the adsorption capacity was detected with increasing the ionic strength. These results agreed with the above-discussed adsorption increases as pH increased higher than the drugs’ pKa1, likely due to an increased shielding effect on the electrostatic attraction between the drug molecules and the oppositely charged PVDAT nanoparticles.

**Desorption of the four drugs**

Owing to the nature of physical interactions, drug adsorptions are expected to be reversible. Drug desorption experiments

![Scheme 3](image-url)

*Scheme 3* pH effects on H-bonding and hydrophobic affinity
were conducted at varied pH and temperature and the cumulative desorption results are presented in Fig. 6. In these desorption experiments, all the desorbed drug molecules were kept in the system by immediately returning the supernatant samples after the UV-vis analysis. Therefore, it is expected that a new equilibrium between the adsorbed and desorbed drug molecules would be eventually achieved for the four drugs, and the equilibration time would vary with the drugs and depend on the desorption conditions.

At room temperature and pH 7.4, xanthine and theobromine reached equilibrium within 6 h whereas 5-FU and thymine demonstrated much longer equilibration time. In terms of cumulative desorption at equilibrium, thymine had the lowest percentage (4.66%) while xanthine and thymine reached around 25%. These desorption results are probably not too difficult to understand from the perspective of adsorption dependency on the drug molecular composition and/or structure as discussed in
the preceding section. The stronger a drug adsorbed by the nanoparticles, the slower and less it desorbed.

The room temperature desorption was dramatically accelerated for all the drugs as the pH dropped to 4.5, reflected by the faster desorption rate and the higher cumulative desorption at equilibrium. For instance, thymine reached equilibrium with 39.4% desorption within 16 h (versus about 4.7% over 100 h at pH 7.4). The pH drops also resulted in a “burst desorption” with about 19.5% thymine, 26.6% 5-FU, 65.88% theobromine, and 57.6% xanthine desorbed within the first 30 min. These are probably ascribed to the compromise of H-bonding resulting from the pronation-induced hydrophobicity diminution at the bonding site. Once again, the drug desorption differences at pH 4.5 are in alignment with the effects of drug composition and/or structure on the physical interactions between the drug and nanoparticles.

While keeping pH at 7.4, the temperature increases facilitated desorption of all the drugs. As the temperature increased from 25 °C, through 50 °C to 70 °C, stepwise increases were apparent not only in the desorption rate but also in the cumulative desorption at equilibrium. The observed temperature effects on the drug desorption is a typical phenomenon for physically adsorbed molecules. In the present study, it is particularly interesting that compared with the temperature effects, the pH drops from 7.4 to 4.5 resulted in more profound increases in the drug desorption, including the burst desorption within 30 min and the cumulative desorption maxima, therefore providing further evidence for the strong dependency of the H-bonding stability in aqueous phase on its synergy with hydrophobic affinity.

Conclusions

PVDAT nanoparticles successfully adsorbed 5-FU, thymine, theobromine, and xanthine in aqueous phase. The results of this study suggested that the adsorptions could be primarily driven by the simultaneous formation of triple hydrogen bonds between a diaminotriazine moiety at the nanoparticle surface and each of the drug molecules while hydrophobic affinity probably played an important role due to its synergistic interaction with the H-bonding, not only to facilitate the formation but also to enhance the stability of the hydrogen bonds by restricting water molecules entering the bonding sites. The exocyclic amino protonation of the diaminotriazine moieties at pH 4.5 could compromise the synergistic interaction and weaken the H-bonding, hence reduce the adsorption capacities. On the other hand, both the adsorption capacities and kinetics were dependent on the drug compositional/structural differences. Thymine had the largest adsorption capacity with the shortest time to reach adsorption equilibrium. The lower adsorption capacities and slower adsorption kinetics of the other drugs could be attributed to the presence of electron-withdrawing fluorine in 5-FU and greater steric hindrance effects of theobromine and xanthine molecules. Deprotonating the drug molecules at pH higher than the drugs’ pKa resulted in higher adsorption capacities than that at the lower pH, probably due to electrostatic attraction between the negatively charged drugs and the positively charged nanoparticle surface although one of the triple hydrogen bonds could be compromised. The adsorptions were reversible and the desorption was accelerated with increased temperature while adjusting pH to below PVDAT pKa had a more profound effect on the desorption as the synergistic interactions were probably weakened.

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Conflict of interest The authors declare that they have no conflict of interest.

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