

Titration Microcalorimetry Study: Interaction of Drug and Ionic Microgel System

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Abstract— Doxorubicin (DOX) and Pluronic-PAA interaction was investigated using isothermal titration calorimetry (ITC). DOX/polymer interaction is governed primarily by electrostatic interaction. The uptake of DOX results in the formation of insoluble polymer/DOX complex. Addition of salt weakens the interaction of drug and polymer by charge shielding effect between positive ionized amino group on DOX and oppositely charged polymer chains. However high drug-loading capacity in high salt condition implied that self-association property of DOX also play a role in the drug loading process.

Index Terms—Anticancer drug, ionic microgel, electrostatic interaction, hydrophobic interaction

I. INTRODUCTION

THE Pluronic-PAA copolymers are obtained via a one-step polymerization of acrylic acid in the presence of Pluronic, with a chain transfer to the Pluronic.^{1,20,21,22,26} In aqueous solutions, these copolymers exist as temperature- and pH-sensitive microgel, containing fixed ionic groups bound to their backbone that give them ion-exchange properties. Hydrophobic domain of Pluronic chains also affords a hydrophobic environment to stabilize hydrophobic drugs such as Taxol. Hence, ionic microgel of Pluronic-PAA can be used as drug carriers for both hydrophobic and ionic anticancer drugs

Building upon previous research work on the Pluronic-PAA systems, the interaction of Doxorubicin with Pluronic-PAA was examined, by making use of the high sensitivity of isothermal titration calorimetry (ITC) to detect the effect of charge density, hydrophobicity and salt (NaCl) concentration. Doxorubicin is a weak monocationic base and is currently in clinical use as anticancer drug. The *n*-octanol-*to*-water partition coefficient (logP) is

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1.85.⁶ Figure 1 shows the molecular structure of Doxorubicin HCl. ITC is one of the most sensitive techniques for measuring the thermodynamic changes during the course of binding. The results obtained provide information that would facilitate the use of ionic microgel as drug carriers.

II. EXPERIMENTAL

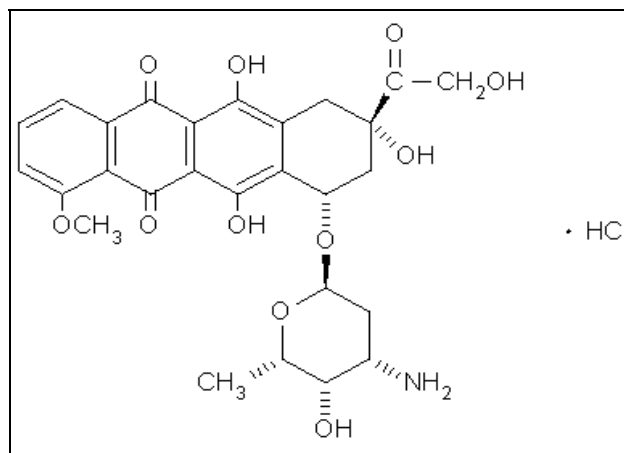


Figure 1. Molecular structure of Doxorubicin HCl

A. Materials

Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-Poly(acrylic acid) copolymer (Pluronic-PAA) synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto the polyether backbone of the Pluronic copolymer. Such Pluronic-PAA copolymers typically consist of about 50wt% of PAA.^{1,20,21,22} Nonionic copolymer Pluronic F127 NF was obtained from BASF Corp. Doxorubicin hydrochloride (99%) was purchased from Hande Tech USA (Houston, TX), a subsidiary of Yunnan Hande Technological Development Co. (Kunming, CHINA). Polyacrylic acid (PAA) with

molecular weight 5700Da was purchased from Sigma Chemical Company. All chemicals were used without further treatment. 0.1wt% stock solutions of F127-PAA and L92-PAA-EGDMA were prepared by dispersing polymer in distilled water and magnetically stirred at 4°C for 48h. Doxorubicin hydrochloride was prepared at concentration 10mg/g solution (17mM) using deionized water and stored at 4°C prior to use.

B. Potentiometric Titration

Ion-exchange capacity of F127-PAA and L92-PAA-EGDMA was determined from potentiometric titrations at 25°C using ABU93 Triburette Titration System. The electrodes are Radiometer pHG201 pH glass and Radiometer REF201 reference electrodes. A 1M NaOH standard solution was used as titrant for 0.1wt% F127-PAA and L92-PAA-EGDMA stock solutions. A back-titration was then performed with 1M HCl standard solution to confirm the end-point of the titration. Forty seconds of lag time was allowed between two injections to ensure that the reaction had reached equilibrium. The content of carboxylic acid on polymer was calculated from moles of NaOH at the end-point.

C. Isothermal Titration Calorimetry

Calorimetric experiments were performed using Microcal Isothermal Titration calorimeter (ITC). The principles and limitation of this instrument were previously described in several publications.^{2,3} The sample cell has a volume of 1.346 mL filled with 0.01wt% polymer solution. Sample of 0.01wt% F127-PAA or L92-PAA-EGDMA and doxorubicin HCl (17mM solution) were prepared in deionized water using stock solutions. The polymer solutions were adjusted to different pHs using 0.1M NaOH. In each experiment, doxorubicin solution was placed in a 250µL continuously stirred (400rpm) syringe and injected into sample cell. Measurements were carried out at 25.0°C. To determine the dilute heat of DOX, blank titrations were made by injecting the drug solution into the sample cell filled with deionized water. Data analysis was performed using the Microcal ORIGIN software.

D. Drug loading

The uptake of doxorubicin for the polymer was assayed using UV-Vis spectrophotometer. Measurements of free drug amounts were made on the supernatant solutions of the drug/polymer suspensions at wavelength of 481nm, which were obtained directly from ITC experiments. The total drug concentration was determined from blank titrations. Appropriate dilution (~100 times) was made to obtain absorbance readings in the linear range of Beer's law. The extinction coefficient of doxorubicin ($\lambda=481\text{nm}$) was $10410 \text{ M}^{-1}\text{cm}^{-1}$ at 25°C. It corresponded well with published data.^{1,4}

III. RESULTS AND DISCUSSION

A. Potentiometric Titration

Figure 2 shows the comparison of pH curves obtained from titrating 1M NaOH into 0.1 wt% F127-PAA and L92-PAA-EGDMA solution. The maximum carboxyl group concentration of F127-PAA and L92-PAA-EGDMA is 6.5 mmol/g and 7.02 mmol/g dry polymers respectively. This result corresponds well with the known value of polyether-modified poly (acrylic acid).¹

The ionization degree is calculated by the equation

$$\alpha = \frac{([BASE] + [H^+] - [OH^-])}{[COOH]_t}$$

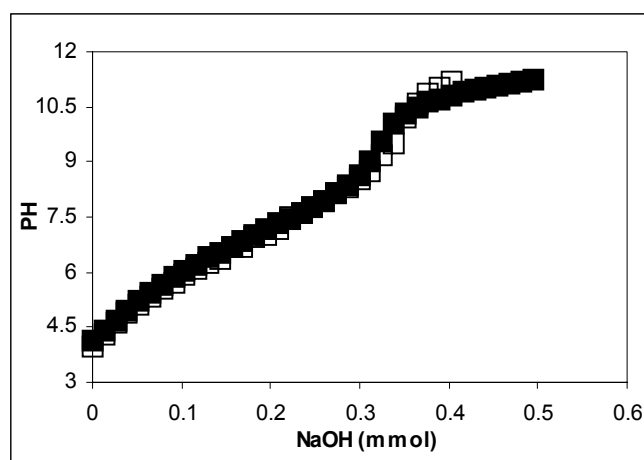


Figure 2. Potentiometric titration (25°C) of F127-PAA and L92-PAA-EGDMA: (□) 0.1wt% L92-PAA-EGDMA; (■) 0.1wt% F127-PAA

(1)

In these calculations the activity was assumed to be equaled to the concentration, and $[BASE]$, $[H^+]$, and $[OH^-]$ are the molarities of added NaOH, free hydrogen ion and hydroxide ion, which are calculated from pH measurements.

B. Isothermal Titration Calorimetry (ITC)

Effect of Charge Density. The effect of polymer charge density on the interaction of DOX with polymer was investigated by varying the ionization degree (α) of L92-PAA-EGDMA using NaOH. The enthalpy binding curves obtained from titrating 17mM Doxorubicin to 0.01wt % L92-PAA-EGDMA ($[COO^-] = 0.7\text{mM}$) were plotted in Figure 3, where the dilute heat of DOX into water has been subtracted.

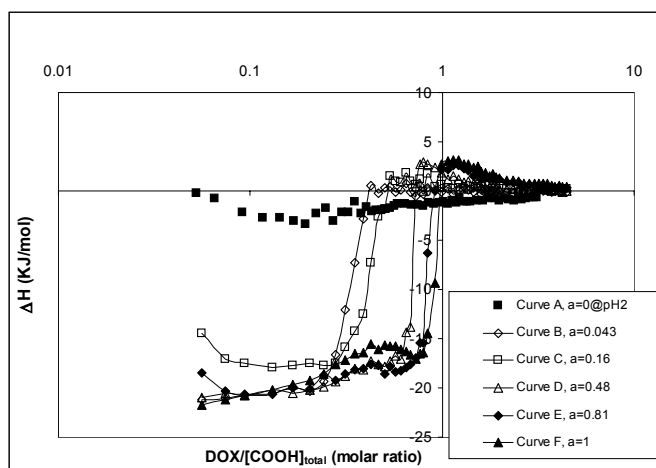


Figure 3. Differential enthalpy curves of titrating 17mM Doxorubicin into 0.01 wt% L92-PAA-EGDMA solutions at 25°C.

These differential enthalpy curves exhibit the favorable binding process. Experimental results indicate that DOX binds on L92-PAA-EGDMA immediately due to electrostatic force between negatively charged carboxylic group of PAA and positively charge amine group of DOX. All curves shows a plateau region followed by a sharp enthalpy increase corresponding to a distinct transition point. Only curve A exhibits little or no interaction between DOX and L92-PAA-EGDMA at pH=2, which corresponds to a zero ionization degree.

For fully neutralized L92-PAA-EGDMA, the enthalpy curve levels off at $\text{DOX}/[\text{COOH}]_{\text{total}} \approx 1$, suggesting that each doxorubicin binds to one positive charge site on the polymer. The comparison from PAA, F127-PAA and L92-PAA-EGDMA suggests the same phenomena at $\alpha=1$, as shown in Figure 4. This result implies that, at high charge density, the hydrophobic modification does not influence the loading behavior of Pluronic-PAA system. The binding

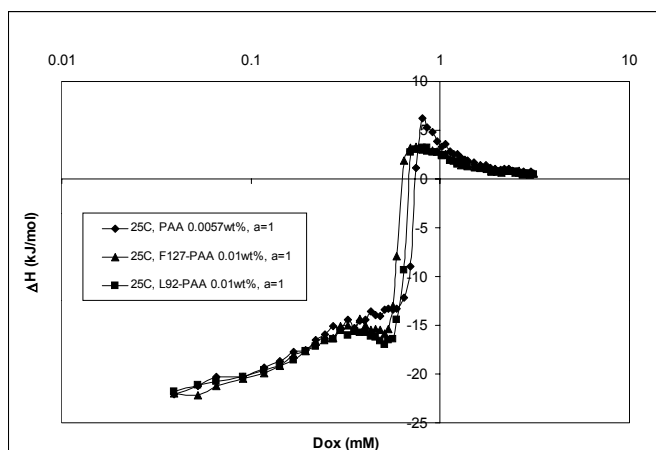


Figure 4. Calorimetric titration of 17mM DOX into different hydrophobic modified poly(acrylic acid) at 25C, absence from NaOH.

of DOX molecules to polyether modified PAA is governed by electrostatic interactions and does not rely on hydrophilic-hydrophobic balance of the polymer. A small

difference between PAA, F127-PAA and L92-PAA-EGDMA arises from the conformation difference.

At low degree of ionization (e.g. $\alpha=0.04$), the experimental results (Figure 3) show that a small negative charge on the polymer is required to attract DOX ions to the vicinity of the polymer coil.¹⁰ But without electrostatic interaction, no drug loading was observed, as shown in curve A of Figure 3. However the plateau region of the binding enthalpy curve is not proportional to the ionization degree. At low ionization degree, the binding fraction is greater than α , which means that the ratio of DOX to ionized carboxyl groups is not 1:1. This suggests that other interactions besides the electrostatic interaction may have contributed to the drug loading process at low ionization degree. Ho *et al* reported the presence of chemical-cross linked hydrophobic domains in Pluronic-modified poly(acrylic acid).¹⁸ Considering the amphiphilic property of DOX, hydrophobic interaction between dihydroxyanthraquinone of DOX and hydrophobic domain of Pluronic-PAA is possible as observed for polymer/surfactant system. The higher hydrophobicity of the polymer at lower charge density may contribute to the observed binding at lower ionization degree. More DOX can be incorporated into existing polymer-DOX aggregates. To identify hydrophobic effect on the Pluronic-PAA/DOX complex formation, it is interesting to investigate different hydrophobic Pluronic-PAA system at low ionization degree; including PAA, F127-PAA and L92-PAA-EGDMA. The difference between these three polymers is the hydrophobic nature and conformation. PAA is a relatively hydrophilic polymer, adopting a random coil conformation at low charge density.¹⁰ In contrast, Pluronic modified PAA possesses a compact form, which is thought to result from the chemical-cross linked hydrophobic PPO segments. But L92-PAA-EGDMA showed more hydrophobic character due to its high amounts of PPO chains (80% PPO in L92 backbone) compared to F127-PAA (20% PPO in F127 backbone).¹

Figure 5 depicts the comparison of the differential enthalpy curves obtained from titrating 17mM DOX into these three polymer aqueous solutions in which concentration of carboxylic group is 0.7mM, in the absence NaOH solution. The carboxylic group of F127-PAA and L92-PAA-EGDMA is 4.5% and 4.3% ionized, respectively. In the presence of a few COO^- groups on poly(acrylic acid) chain (without the addition of NaOH) the interaction between DOX and polymer can be induced. The presence of hydrophobic domain in F127 and L92 modified PAA doesn't influence the shape of the binding curves, which exhibit the similar sigmoidal shape associated with identical transition point. The hydrophobicity of polymer may not be the main reason for the high bind fraction at low charge density. One possible explanation is that DOX can displace proton from the unionized carboxyl group on polyacrylic acid chain and increase the number of available binding sites beyond the

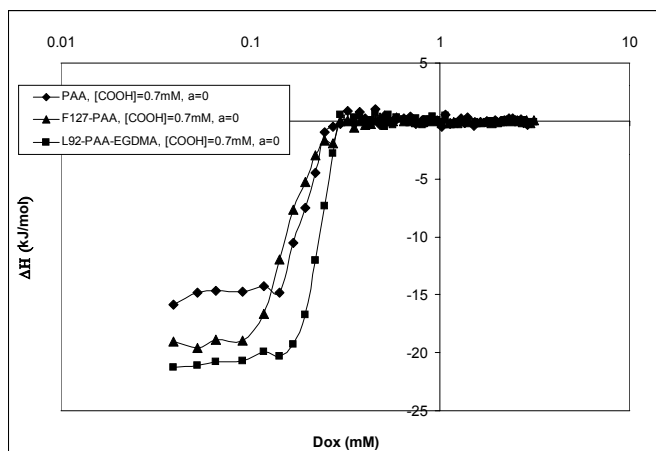


Figure 5. Calorimetric titration of 17mM DOX into different hydrophobic modified poly(acrylic acid) at 25°C, absence from NaOH.

calculated ionization degree.¹⁹ The hydrophobic nature of polymer influence only the deprotonation process during drug loading. Study to confirm the actual mechanism is underway.

Effect of Salt Concentration. Figure 6 shows the differential enthalpy curves at 25°C, which were measured from the gradual injection of 4mM DOX solutions into fully neutralized F127-PAA solution in different salt concentrations. The experimental results indicate that the interaction of polymer with DOX is strongly salt dependent. In salt free solution, the curve increases sharply at the equivalent point, which indicates the complete polymer-DOX complex conformation and higher equilibrium constant.¹¹ Negative enthalpy values decrease gradually and exhibit a less distinct equivalence point by increasing the salt concentration from 0mM to 0.3mM. This indicates that the binding is weakened by the addition of salt due to the charge shielding effect on the carboxylic group and ionized amino group, and counter-ions (Na^+) cannot be fully released because of relatively stronger condensation on polymer chain in high-salt concentration.

We expected to observe lower DOX uptake in higher salt concentration condition because electrostatic

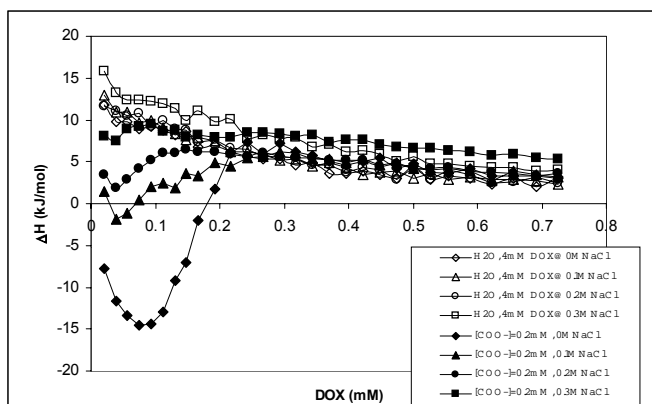


Figure 6. Calorimetric titration of 4mM DOX into fully neutralized F127-PAA solution at 25°C.

interaction is weakened by the addition of salt. However UV measurement (Figure 7) shows the enhancement of drug loading with increasing salt concentrations. Drug uptake suggests that other non-electrostatic interaction may also play a role in the polymer-DOX complex formation. The aggregation behavior of doxorubicin could explain this unexpected DOX uptake. Many research groups^{12,13,14,23} had reported the self-association behavior of doxorubicin in aqueous solution, whether the sugar portion of the drug forms a helical arrangement on the periphery of the stacked anthracycline rings; and there are no steric reasons for limited aggregation. The physical state of DOX in aqueous solution can be influenced by counterions. It can form a dark and transparent Cl-gel in concentrated sodium chloride solution. In our study, although 4mM DOX cannot form gel in 0.1~0.3 M NaCl solutions, partition of doxorubicin in hydrated polymer

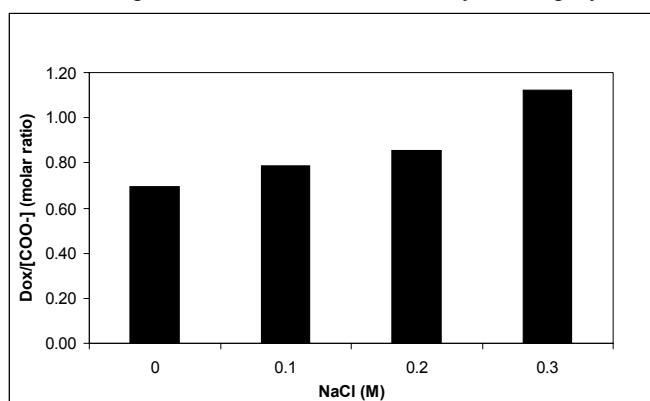


Figure 7. Equilibrium DOX loading as a function of the salt concentration at 25°C

chain and bulk water could be varied with the aggregation of the drug by the addition of salt. Thus, this may explain the higher DOX uptake in higher salt concentration. Hence, in the presence of salt, drug loading can be made highly efficient by taking advantage of DOX's accumulation inside the polymer matrix.

IV. CONCLUSION

The effect of charge density on DOX loading to F127-PAA and L92-PAA-EGDMA was studied using ITC. Experimental results suggest that at ionization degree α of between 0 and 1.0, the interaction of drug and Pluronic-PAA copolymer is governed by electrostatic interaction with a counter-ion exchange process. At low charge density, drug loading is induced by small amounts of COO^- and possibly followed by partial deprotonation of uncharged carboxylic group on polyacrylic acid chain, where the binding fraction exceeds the expected ionized degree. At high charge density, Pluronic-PAA loaded with DOX via ion exchange and sodium ion is released. Addition of salt weakens the interaction of drug and polymer by charge shielding effect on the carboxyl group and ionized amino group. And counterions (Na^+) cannot be

fully released because of relatively stronger condensation on polymer chain in high-salt concentration. However at higher salt conditions, the aggregation of DOX is enhanced^{12,13}, while the affinity of the drug for polymer is decreased. Self-association property of DOX cannot be ignored in assessing the drug uptake. Its effect on the interaction of DOX and polymer is currently being investigated.

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