Complex Formation Between The Anionic Polymer (PAA) and a Cationic Drug (Procaine HCl): Characterization by Microcalorimetric Studies

Thirumala Govender,[1] Touraj Ehtezazi,[1,2] Snjezana Stolnik,[1] Lisbeth Illum,[1] and Stanley S. Davis[1]

Received November 10, 1998; accepted March 19, 1999

Purpose. Due to the importance of drug-polymer interactions in, inter alia, drug loading/release, supramolecular assemblies and DNA delivery for gene therapy, the aim of this study was therefore to establish the mechanism of interaction between a model polymer (Polyacrylic acid, PAA) and a model drug (procaine HCl).

Methods. This was performed by studying the effect of salt (KCl) concentration on their heat released values using Isothermal Titration Microcalorimetry (ITM). The integrated released heat data were computer fitted to a one class binding model and the thermodynamic parameters (K_{obs}, ΔH, and N) were determined.

Results. As the KCl concentration was increased, K_{obs} decreased thus establishing the salt dependence of the interaction. The linear variation of ΔG_{obs} with ΔS_{obs} indicated that their interaction was entropically driven. The stoichiometry of the interaction was calculated to be one procaine molecule per monomer of PAA. Dissection of the total observed free energy at each KCl concentration indicated that the contribution of the non-electrostatic attractions to the interaction of PAA with procaine HCl was greater than those of the electrostatic attractions.

Conclusions. We have shown that the interaction between PAA and procaine HCl is dependent upon the presence of counterions (monovalent ions) and is mainly entropically driven. The calculated stoichiometry indicated that one procaine HCl molecule neutralised one carboxylic acid group on PAA. Although electrostatic interactions were necessary for initiating complex formation, the non-electrostatic forces were dominant in stabilising the PAA-procaine HCl complex.

KEY WORDS: drug-polymer interactions; salt dependence; isothermal titration microcalorimetry; electrostatic and non-electrostatic interactions.

INTRODUCTION

Drug-polymer interactions can have considerable importance in optimising drug delivery. For example, the drug release profiles of a polymeric matrix system for controlled drug release have been directly correlated to the interactions of drugs with polymers (Eudragit[R] RL and RS) (1). Also a newly reported concept of micelle formation, which involves the spontaneous assembly of macromolecules, is driven via drug-polymer interactions (2). A potential application of this concept is improving the drug loading capacity of nanoparticulates which can be employed for drug targeting, gene therapy and sustained drug release (3–5). In addition, the interaction of DNA with polymers such as poly(lysine) has been employed in the field of gene therapy (6). Therefore the importance of drug-polymer interactions in drug release and incorporation, supramolecular assemblies and DNA delivery, necessitates preliminary studies to evaluate and determine the mechanisms of interaction between a drug and polymer.

Isothermal titration microcalorimetry (ITM) would be an ideal analytical method for this purpose since it is the only technique which allows simultaneous determination of all binding parameters (K, ΔH, ΔS and N) in a single experiment (7). Being highly accurate and precise it is emerging as the premier tool for characterising the affinity and stoichiometry of binding interactions (8).

In this study poly(acrylic acid) (PAA) has been chosen as a model polyionic polymer to study its interaction with a model water soluble cationic drug, procaine HCl. PAA was selected due to its ready availability and also because of its other widely reported pharmaceutical applications based on interaction/complex formation between drugs or other polymers (9–11). More specifically, the mechanism of their interaction has been determined by investigating the influence of salt (KCl) concentration using ITM. The data were computer fitted to a one class binding model and the thermodynamic parameters determined.

Theory

When a drug and polymer molecule in an aqueous solution carry opposite electrical charges an interaction between the drug and polymer molecule can occur, as was expected in this study. The interaction between a polyelectrolyte and a poly or monovalent counter ion has been studied by many investigators (12–24). The principles of this interaction are outlined in the following sections.

Interaction of Monovalent Counterions with a Polyelectrolyte

In an aqueous solution, when a polyelectrolyte such as PAA is negatively charged (due to the carboxylic acid groups), monovalent counterions (originating from buffer or added salt in solution) interact with the polyelectrolyte. This interaction can be been explained by Manning's theory (13–16). The fraction of a M^+ counterion bound per polyelectrolyte charge (in a thermodynamic sense) ψ, is given by Eq. 1:

$$\psi = 1 - (2\xi)^{-1} \quad (1)$$

where ξ is given by Equation 2

$$\xi = \frac{e^2}{\varepsilon kTb} \quad (2)$$

here ε denotes the dielectric constant of the medium, k is the Boltzmann constant, b denotes the axial distance of the charged groups of the polyelectrolyte, T represents the absolute temperature, and e is the magnitude of the electronic charge.

[1] School of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD UK.

[2] To whom correspondence should be addressed. (e-mail: snjezana.stolnik@nottingham.ac.uk)

0724-8741/99/0700-1125$16.00/0

Interaction of a Charged Ligand with a Counterion Polyelectrolyte

Now, consider further an aqueous solution which contains a negatively charged polyelectrolyte (such as PAA) in the presence of excess monovalent M^+ ions and to which is added an aqueous solution of charged ligand (such as drug molecule or an oligopeptide) which has an opposite charge to the polyelectrolyte. The electrostatic attraction between the opposite charged ions will be a part/or the main driving force for the initiation of complex formation between the drug and polymer molecules. The molecular interaction can be written as

$$\text{Polymer} + \text{Drug} \underset{\leftarrow}{\rightarrow} \text{Complex} + a\text{M}^+ + b\text{X}^- + c\text{H}_2\text{O} \quad (3)$$

where the coefficients a, b, c represent the net preferential interaction coefficients of cations, anions, and water, respectively. The sign of these coefficients can be positive or negative for a net release or uptake, respectively. An experimentally determined equilibrium binding constant (observed equilibrium constant) K_{obs} is defined as

$$K_{obs} = \frac{[Complex]}{[Drug][Polymer]}. \quad (4)$$

Applying Le Chatelier's principle to Eq. (3) shows that by increasing salt concentration, K_{obs} will decrease (21). It has been shown that at constant pressure and temperature, Eq. 5 can be used for the variation of K_{obs} with M^+ concentration for the process shown in Eq. 3 (17)

$$\left(\frac{\partial \ln K_{obs}}{\partial \ln [M^+]}\right)_{T,P} = -(a + b) + \frac{2[MX]}{[H_2O]} c \quad (5)$$

According to Manning's theory, when a drug (or ligand) with z positively charged binding sites binds to a charged polymer with successive negatively charged binding sites, in the presence of excess monovalent cation (M^+), $z\psi$ counterions will be released from the charged polymer (17,22). Therefore in Eq. 5, we will have $a = z\psi$, where the physical meaning of ψ is described above. When a drug molecule binds with a charged polymer in an aqueous solution with negligible uptake/release of anions or water molecules, Equation 5 simplifies to Eq. 6.

$$\left(\frac{\partial \ln K_{obs}}{\partial \ln [M^+]}\right)_{T,P} = -z\psi \quad (6)$$

The release of $z\psi$ M^+ counterions into the solution is followed by the dilution of the released counter ions. Therefore, the entropy of the solution increases due to this dilution and according to the Manning's theory this is the sole driving force for complex formation (17,20–22). However, it is shown in this paper that, when a drug molecule replaces one M^+ cation condensed on the polyelectrolyte (polymer), the formation of non-electrostatic interactions between the drug molecule and polymer stabilise the complex and gives priority to drug molecules to occupy the binding site on the polymer for a longer time than M^+ cations. The free energy of dilution of the released ions (due to the complex formation between the drug and charged polymer), which is also called free energy of the electrostatic interaction ΔG_{es}, is calculated from Eq. 7 (21).

$$\Delta G_{es} = z\psi RT \ln[M^+] \quad (7)$$

It has been shown that in addition to the electrostatic forces between a charged ligand and polyelectrolyte in a complex, non-electrostatic interactions (such as hydrogen bonding and van der Waals forces) can also play a significant role in the complex formation (18). The free energy of contribution of non-electrostatic interactions ΔG_{nes}, is calculated from Eq. 8.

$$\Delta G_{nes} = -RT \ln K_{obs} - \Delta G_{es} \quad (8)$$

Equation 8 is derived from the fact that the total free energy of interaction ΔG_{total} equals to $-RT \ln K_{obs}$ and that the sum of the free energy of electrostatic and non-electrostatic interactions equals to the total free energy of interaction.

Other thermodynamic quantities that are important in a complex formation are the enthalpy ΔH and entropy ΔS of the interaction. The enthalpy of binding is determined from the analysis of the binding isotherms (see below), and the entropy of the interaction is determined using $\Delta S = (\Delta H - \Delta G_{total})/T$.

MATERIALS AND METHODS

Materials

Poly(acrylic acid) (Average Mw = 2000 Da) and procaine HCl were purchased from Sigma, UK. Potassium chloride (reagent grade) was obtained from Aldrich, UK. Water used for all experiments was ultrapure Elgastat^R Option 3 water (Elga Ltd., UK). All other chemicals used were of pharmaceutical grade.

Methods

Isothermal Titration Microcalorimetry

Calorimetric experiments were performed using a Thermal Activity Monitor (TAM 2277, Thermometric AB, Sweden) operated at 298K. Samples of procaine HCl and PAA were prepared in water containing varying concentrations of KCl i.e. 0.25; 1; 10; 25 and 50 mM. The samples were adjusted to pH 8 (±0.05) using dilute KOH. In each experiment, a 3 mL sample of a 1 mg/mL solution of procaine HCl was placed in a sample cell and inserted into the instrument. Once the thermal equilibrium was reached, the titration was performed by consecutive injections (Lund 6100 syringe pump) of a 9.88 mg/mL solution of PAA. Heats of dilution/mixing were determined in blank titrations by injecting aliquots (20 μL) of PAA (9.88 mg/mL) into the appropriate KCl solution (3 mL). Data presented are the mean of a minimum of 2 replicate titrations at each KCl concentration. A typical output of the raw ITM data for the injection of PAA into procaine HCl and a blank experiment are shown in Fig. 1A. The signs of the released heat values were reversed for data analysis since the output from the instrument employed in this study is from the perspective of the equipment and not the system under study.

Analysis of Binding Isotherms

The interaction of procaine and PAA was evaluated using the one class binding model for the binding of non-interacting ligands to a lattice of ligand binding residues (25–26). This model is equivalent to the model that was developed by McGhee and Hippel (27), by having one site exclusion size on the ligand.

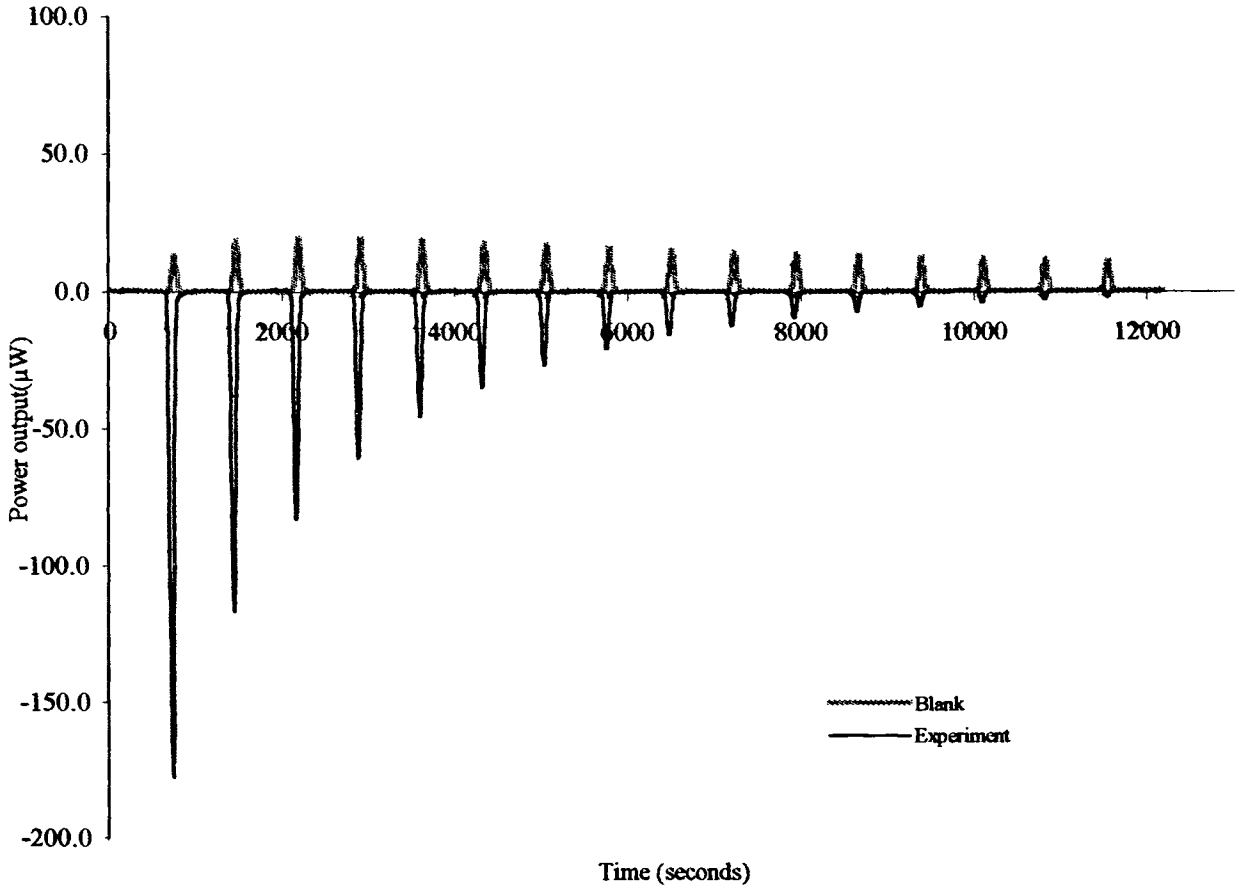

Fig. 1A. Experimental calorimetric data for the isothermal titration at 298K for PAA, into procaine HCl (0.25 mM KCl solution). The blank titration i.e. injection of PAA into 0.25 mM KCl solution is also shown.

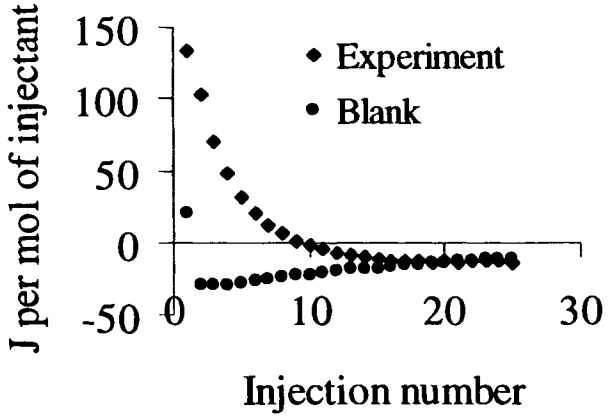

Fig. 1B. Integrated heats of interaction for the injection of PAA into procaine HCl (1 mM KCl solution) as well as heats of dilution for the injection of PAA into 1 mM KCl solution.

In the model used, the reaction heat content Q_i after i injections is given by Eq. 9.

$$Q_i = 0.5V_i\Delta H(A - \sqrt{A^2 - 4P_{t,i}ND_{t,i}} \quad (9)$$

where

$$A = \frac{1}{K_{obs}} + NP_{t,i} + D_{t,i}. \quad (10)$$

and V_i denotes the volume of the solution in the cell after i injections, ΔH represents the enthalpy of interaction between the drug molecule (procaine HCl) and a binding site on the polymer (PAA), $P_{t,i}$ and $D_{t,i}$ denote the total concentration of the polymer and drug in the cell after i injections, respectively, N represents the number of interacting sites on each polymer molecule and K_{obs} is the observed association constant.

In practice the heat released at the ith injection, $\Delta Q_{i,obs}$, was determined by ITM. The observed experimental released heat data also includes the heat of dilution for the drug and polymer. The heat of dilution at each injection can be determined either by; 1) performing a blank titration i.e. injection of drug or polymer solution in the buffer into the buffer solution without drug or polymer (Fig. 1B), or 2) from the end part of the released heat titration profile, which represents just the heat of dilution of drug and polymer, which can be seen in Fig. 1A. In the latter case, an average of the dilution heat from the end part of the observed released heat profile is calculated and subtracted from the observed released heat values at each data point. In this study the latter method was applied for the data analysis of the raw released heat profiles. Since before and after each injection, the solution in the cell of the calorimeter was in thermal equilibrium, it was therefore assumed that the released heat at the ith injection can be related to the heat reaction contents before and after the ith injection by Eq. 11.

$$\Delta Q_i = Q_i - Q_{i-1} \quad (11)$$

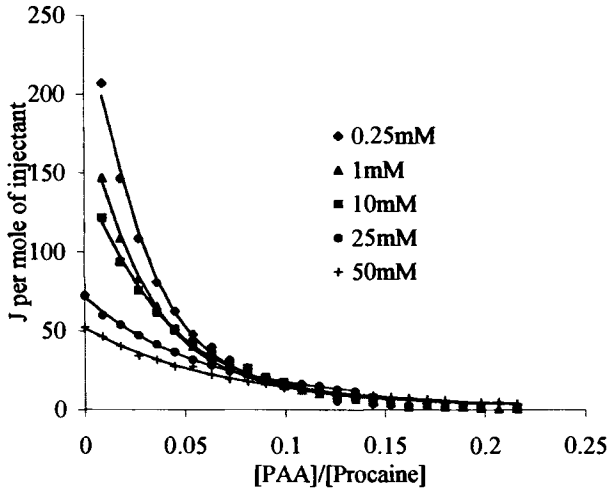

Fig. 2. Integrated heats of interaction corrected for heats of dilution from titrations of PAA into procaine HCl in varying concentrations of KCl solution. Solid lines represent the best fit binding isotherms to the experimental data.

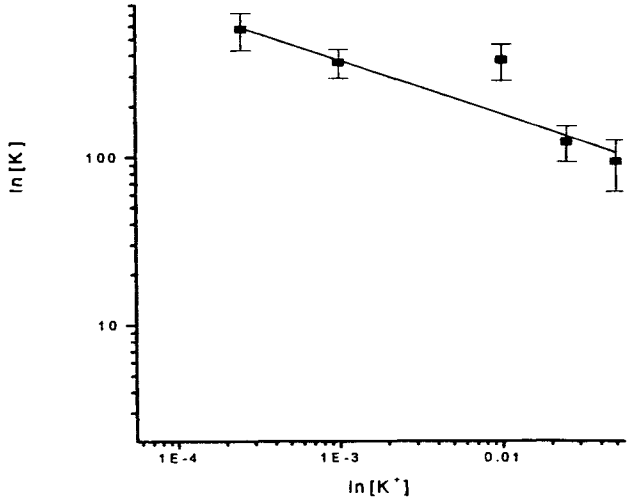

Fig. 3. Variation of the association constant (K_{obs}) for the interaction of PAA with procaine HCl as a function of salt concentration.

The equilibrium parameters, K_{obs}, N, and ΔH were determined by using the non-linear least squares fits of equations 11 and 9 to the corrected (for the heat of dilution) observed heat released data. The non-linear curve fittings were performed using the routines available within the Origin 5.0 program package (Microcal Software, Inc).

RESULTS AND DISCUSSION

Effect of Salt Concentration

Figure 2 illustrates the results of calorimetric titrations of PAA into procaine HCl and the fitted curves to the experimental data. It can be seen from Table I, that by increasing the salt concentration from 0.25 to 50 mM, K_{obs} decreased from 571 M^{-1} to 94 M^{-1}. The values of K_{obs} as a function of KCl concentration are plotted in Fig. 3 and a linear trend can be observed for the ln-ln plot of K_{obs} vs [KCl]. Linear regression analysis of the natural logarithm of salt concentration and K_{obs} yielded a slope value of -0.32 ± 0.07. These results therefore confirm the dependence of the interaction of procaine HCl with PAA on salt concentration. The interactions of a polyelectrolyte such as DNA with a counterion such as protein have also been reported to be strongly salt dependent (12,13–18,20,22).

Dependency of ΔG_{obs} on ΔS_{obs}

The three calculated thermodynamic parameters, ΔG_{obs}, ΔH_{obs} and $T\Delta S_{obs}$ of the corresponding titration curves (Fig. 2) are illustrated in Table I. When the KCl concentration was increased, ΔG_{obs} increased (the absolute value decreased) whereas $T\Delta S_{obs}$ and ΔH_{obs} both decreased. However, the values of ΔH_{obs} are negligible in comparison with $T\Delta S_{obs}$ values. Therefore, the total free energy change ΔG_{obs} was determined mainly by the variation of the total entropy change ΔS_{obs}. It can also be seen that ΔG_{obs} varies linearly with ΔS_{obs} (Fig. 4). These results therefore, indicate that entropic effects mainly determined the driving force for the complex formation between procaine HCl and PAA. As explained in the theory section, dilution of the PAA associated monovalent counterions by interaction of PAA with the charged ligand represented partly/or mainly the sole driving force for their interaction.

Effect of Electrostatic Charges of PAA and Procaine HCl on the Complex Formation

In order to show that complex formation between PAA and procaine HCl was due at least partly to electrostatic attractions and then followed by the release of bound monovalent counterions, the interaction of the drug and polymer was performed at two additional pH values, i.e., 2.8 and 11. An appreciable released heat was not observed by introducing the PAA solution to the drug solution in the cell, at pH 2.8 and 11 (Fig. 5). Therefore, we can infer that at these two pH values, an appreciable interaction between procaine and PAA did not occur. At pH 2.8 procaine HCl (pKa = 9) (28) will be virtually 100% ionised and PAA (pKa = 4.5) (11) only 1.96% ionised. At pH 11, procaine HCl will be only 0.99% ionised while PAA

Table I. Effect of Salt (KCl) Concentration on the Thermodynamic Parameters for the Injection of PAA into Procaine HCl

[K+] (mM)	K_{obs} (M^{-1})	ΔG_{obs} (kJ mol^{-1})	ΔH_{obs} (kJmol^{-1})	$-T\Delta S_{obs}$ (kJmol^{-1})	N_{obs}
0.25	571 ± 143	−15.63 ± 0.62	3.51 ± 0.12	19.14 ± 0.84	36 ± 5
1	361 ± 70	−14.55 ± 0.5	2.84 ± 0.04	17.38 ± 0.54	40 ± 4
10	373 ± 90	−14.63 ± 0.58	2.67 ± 0.08	17.26 ± 0.67	32 ± 4
25	122 ± 29	−11.83 ± 0.58	2.42 ± 0.04	14.21 ± 0.67	33 ± 5
50	94 ± 31	−11.11 ± 0.84	2.38 ± 0.08	13.38 ± 0.92	31 ± 7

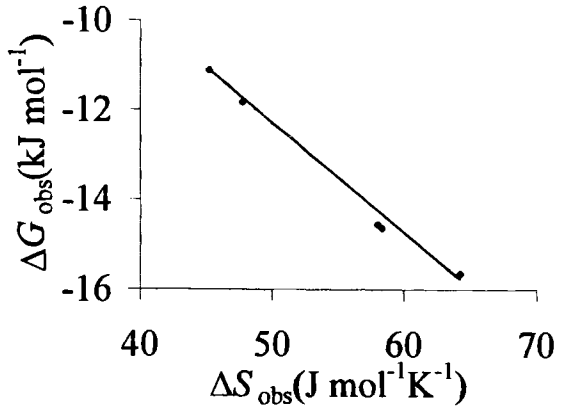

Fig. 4. Dependence of ΔG_{obs} on ΔS_{obs} for the injection of PAA into procaine HCl in varying concentrations of KCl solution.

will be almost 100% ionised. Hence at these two pH values, one of the 2 interacting species exists mainly in its unionised form which may explain the absence of appreciable released heat values (Fig. 5) at these pH conditions. The appreciable released heat values observed at pH 8 (Fig. 5), where both species are >90% ionised, indicate that electrostatic forces are indeed a prerequisite for an interaction between PAA and procaine HCl to occur i.e. a simultaneous electrical charge for the drug and polymer is essential for complex formation.

Dissection of the Free Energy of Procaine HCl Binding to PAA

The total observed free energy can be partitioned into two contributions: the non-electrostatic (hydrogen bonding and van der Waals interactions) and electrostatic (polyelectrolyte) contributions (18). As shown in the theory section, the electrostatic contribution is calculated from Eq. 7, and the non-electrostatic contribution from Eq. 8. Interestingly it is seen that ΔG_{nes} is larger than ΔG_{es} (Table II), which indicates that non-electrostatic forces played a dominant role in stabilising the drug-polymer complex. Table II also shows that the magnitude of ΔG_{nes} is nearly salt independent; which is consistent with the expectations from non-electrostatic interactions. Also, the ratio of ΔG_{nes} to ΔG_{es} was increased by increasing the salt concentration (Table II), since the magnitude of ΔG_{nes} remained constant,

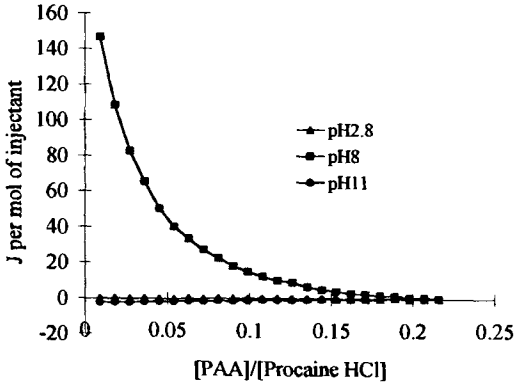

Fig. 5. Integrated heats of interaction corrected for heats of dilution from titrations of PAA into procaine HCl in 1 mM KCl solution adjusted to various pH values.

Table II. Dissection of the Binding Free Energy into Its Electrostatic and Non Electrostatic Contributions for the Injection of PAA into Procaine HCl

[K+] (mM)	ΔG_{obs} (kJ mol^{-1})	*ΔG_{es} (kJ mol^{-1})	**ΔG_{nes} (kJ mol^{-1})	$\Delta G_{nes}/\Delta G_{es}$
0.25	−15.63	−6.56	− 9.07	1.38
1	−14.55	−5.47	− 9.07	1.65
10	−14.63	−3.64	−10.91	3
25	−11.83	−2.93	− 8.9	3.1
50	−11.12	−2.38	− 8.78	3.7

* Calculated from Equation 7.
** Calculated from Equation 8.

whereas ΔG_{es} decreased by increasing the salt concentration. This trend is illustrated in Fig. 6. It has been previously shown that the protonated amine group substantially facilitates hydrogen bond formation (29). Therefore, the existence of non-electrostatic interactions in this study can be explained by the formation of hydrogen bonds between the amine group of procaine HCl and the carboxylic acid group of PAA.

The Stoichiometry of Binding

As mentioned earlier, the calculated value of $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ for the interaction of procaine HCl and PAA is equal to 0.32 ± 0.07. The counterion condensation theory (13–15) predicts that $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ is equal to $z\psi$ (ca. Eq. 6). Therefore by calculating ψ, one can determine the number of monovalent cations released by complex formation between the drug and polymer. It is known that the charge density of PAA varies with pH, and reaches a minimum when the polymer is fully ionised (30). Since our measurements were carried out at pH 8 and at this pH most of the carboxylic acid groups were ionised and have their maximum distance from each other, then ξ will take its maximum value of 1 (13,16). Therefore, from Eq. 2 it is calculated that $\psi = 0.5$. Then, from Eq. 6 and from the experimental results where $-\partial(\ln K_{obs})/\partial(\ln[M^+]) = 0.32 \pm 0.07$, it is calculated that $z = 0.64 \pm 0.14$. Therefore, we can conclude that one monovalent cation (K^+) releases by interaction of a procaine molecule to each binding site on PAA. Since a released K^+ cation is associated with a carboxylic group on PAA, it can be concluded that each procaine HCl molecule neutralised one carboxylic acid group on PAA. This finding can also be confirmed from the number of binding site, N, on

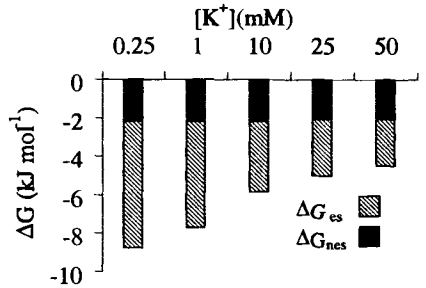

Fig. 6. Dissection of the binding free energy into its electrostatic and nonelectrostatic contributions for the injection of PAA into procaine HCl at varying KCl concentrations.

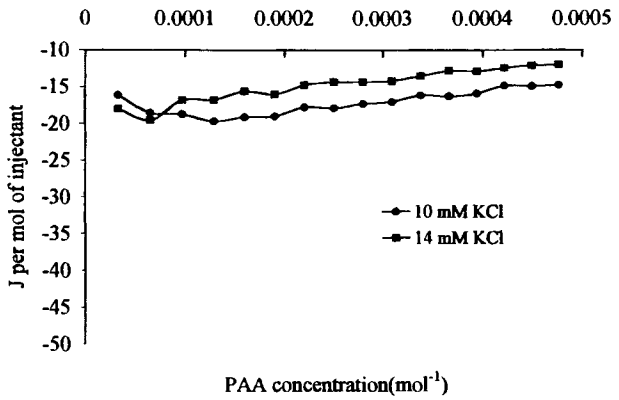

Fig. 7. Integrated released heat values for the injection of PAA (dissolved in 10 mM KCL solution) into 10 mM KCl solution as well those for into 14 mM KCl solution.

each PAA molecule, which was calculated from the analysis of the binding isotherms. These values are listed in Table I and it can be seen that N varies around 30. Also, the mean number of monomers (carboxylic acid groups) on each PAA molecule was calculated theoretically to be 28. Therefore, analysis of the binding isotherms also confirmed that one procaine molecule neutralised one carboxylic acid group on the PAA macromolecule.

Ion Condensation

So far we have shown that one procaine molecule replaced one M^+ cation, which was in a thermodynamical sense bound to one carboxylic acid group of PAA and that also the procaine molecule interacted both electrostatically and non-electrostatically with PAA. In order to confirm that the heat outputs measured by ITM were not just simply the condensation of procaine HCl molecules on PAA and that its manner of interaction was different from K^+ ions, the following experiment was conducted. The released heat profile for the injection of PAA dissolved in 10 mM KCl into 10 mM KCl solution was compared to the injection of the same solution into 14 mM KCl. The difference of 4 mM KCl in the second cell solution was a replacement for the concentration of procaine HCl in our previous experiments. It is seen that both profiles for released heat are almost superimposable (Fig. 7). This confirms that the net released heats measured previously in the interaction of procaine HCl and PAA were due to the dilution of M^+ cations that were condensed on PAA and which was followed mainly by non-electrostatic interactions between procaine HCl molecules and PAA macromolecules. In other words, procaine molecules did not perform simply as monovalent cations and non-electrostatic interactions between drug and polymer molecules played a significant role in complex formation.

CONCLUSIONS

In this study ITM has been employed to elucidate the mechanism of interaction between a model polymer (PAA) and a model cationic drug (procaine HCl). The heat released data for interaction in KCl solutions of varying concentrations were determined and the binding isotherms fitted to a one class binding model to establish the relevant thermodynamic parameters.

The interaction of PAA with procaine HCl was shown to be salt dependent, with K_{obs} decreasing with increasing salt concentrations. The linear variation of ΔG_{obs} with ΔS_{obs} indicated that their interaction was entropically driven. At pH 2.8 and pH 11, where only one of the interacting species was ionised, no appreciable interaction was observed, while the heat released outputs at pH 8, where both species are ionised, indicated an appreciable interaction. It was, therefore, necessary for both species to be ionised and hence electrostatic forces were essential for complex formation. However, dissection of the total observed free energy indicated that the contribution of non-electrostatic attractions to the interaction of PAA with procaine HCl was greater than those for electrostatic attractions. Hence, we conclude in this study that, although electrostatic attractions are necessary for initiating complex formation between PAA and procaine HCl, it is the non-electrostatic forces which played a dominant role in stabilising the complex between this drug and polymer. Analysis of binding isotherms and the slope of $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ revealed that one procaine HCl molecule bound to one monomer (or carboxylic group) of PAA. This was further confirmed by the experimentally calculated N values which varied around 30, while the calculated theoretical value was 28. Finally, superimposable heat released profiles for PAA dissolved in 10 mM KCl and injected into 10 mM and that injected into 14 mM KCl aqueous solution confirmed that the heat outputs measured were not due to the condensation of monovalent ions on PAA but rather to the dilution of M+ cations condensed on PAA as well as mainly to non-electrostatic interactions between this polymer and procaine HCl.

ITM therefore proved a powerful technique in providing comprehensive information on the interaction mechanism between a polymer and drug.

ACKNOWLEDGMENTS

Ms Thirumala Govender is grateful to the Association of Commonwealth Universities for financial support.

REFERENCES

1. M. R. Jenquin and J. W. McGinity. Characterisation of acrylic resin matrix films and mechanisms of drug-polymer interactions. *Int. J. Pharm.* **101**:23–34 (1994).
2. A. Harada and K. Kataoka. Novel polyion complex micelles entrapping enzyme molecules in the core: Preparation of narrowly distributed micelles from lysozyme and poly (ethylene glycol)-poly(aspartic acid) block copolymer in aqueous medium. *Macromolecules* **31**:288–294 (1998).
3. E. Allémann, R. Gurny, and E. Doelker. Drug loaded nanoparticles: Preparation methods and drug targeting issues. *Eur. J. Pharm. Biopharm.* **39**:173–191 (1993).
4. S. S Davis. Biomedical applications of nanotechnology-implications for drug targeting and gene therapy. *Trends Biotechnol.* **15**:217–224 (1997).
5. T. Govender, S. Stolnik, M. C. Garnett, L. Illum, and S. S. Davis. PLGA nanoparticles prepared by nanoprecipitation: Drug loading and release studies of a water soluble drug. *J. Contr. Rel.* **57**:171–185 (1999).
6. P. R. Dash, V. Toncheva, E. Schacht, and L. W. Seymour. Synthetic polymers for vectorial delivery of DNA: characterisation of polymer-DNA complexes by photon correlation spectroscopy and stability to nuclease degradation and disruption by polyanions in vitro. *J. Contr. Rel.* **48**:269–276 (1997).
7. T. Wiseman, S. Williston, J. F. Brandts, and L. N. Lin. Rapid measurement of binding constants and heats of binding using a

new titration calorimeter. *Anal. Biochem.* **179**:131–137 (1989).
8. M. L. Doyle. Characterisation of binding interactions by isothermal titration calorimetry. *Curr. Opin. Biotechnol.* **8**:31–35 (1997).
9. M. F. Lu, S. Borodkin, L. Woodward, P. Li, C. Diesner, L. Hernandez, and M. Vadnere. A polymer carrier system for taste masking of macrolide antibiotics. *Pharm. Res.* **8**:706–712 (1991).
10. B. O. Haglund, R. Joshi, and K. J. Himmelstein. An in situ gelling system for parenteral delivery. *J. Contr. Rel.* **41**:229–235 (1996).
11. J. R. Robinson and W. J. Bologna. Vaginal and reproductive system treatments using a bioadhesive polymer. *J. Contr. Rel.* **28**:87–94 (1994).
12. T. Lundback and T. Hard. Salt dependence of free energy, enthalpy, and entropy of nonsequence specific DNA binding. *J. Phys. Chem.* **100**:17690–17695 (1996).
13. G. S. Manning. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.* **2**:179–246 (1978).
14. G. S. Manning. Limiting laws and counterion condensation in polyelectrolyte solutions. I. Colligative properties. *J. Phys. Chem.* **51**:924–933 (1969).
15. G. S. Manning. On the application of polyelectrolyte limiting laws to the helix-coil transition of DNA. I. Excess univalent cations. *BIPMA.* **11**:937–949 (1972).
16. M. T. Jr. Record, C. F. Anderson, and T. M. Lohman. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Quart. Rev. Biophys.* **2**:103–178 (1978).
17. M. T. Jr. Record, M. T. Lohman, and P. de Haseth. Ion effects on ligand-nucleic acid interactions. *J. Mol. Bio.* **107**:145–158 (1976).
18. I. Haq, P. Lincoln, D. Suh, B. Norden, B. Z. Chowdhry, and J. B. Chairs. Interaction of Δ- and Λ-$[Ru(Phen)_2DPPZ]^{2+}$ with DNA: A calorimetric and equilibrium binding study. *J. Am. Chem. Soc.* **117**:4788–4796 (1995).
19. T. Lundback, H. Hansson, S. Knapp, R. Ladenstein, and T. Hard. Thermodynamic characterization of non-sequence-specific DNA-binding by the sso7d protein from sulfolobus solfataricus. *J. Mol. Biol.* **276**:775–786 (1998).
20. T. M. Lohman, P. L. de Haseth, and M. T. Jr. Record. Pentalysine-deoxyribonucleic acid interactions: A model for the general effects of ion concentrations on the interactions of proteins with nucleic acids. *Biochemistry* **19**:3522–3530 (1980).
21. T. M. Lohman and D. P. Mascotti. Thermodynamics of ligand-nucleic acid interactions. *Methods Enzymol.* **221**:400–424 (1992).
22. L. B. Overman, W. Bujalowski, and T. M. Lohman. Equilibrium binding of Escherichia coli single-strand binding protein to single-strand nucleic acids in the $(SSB)_{65}$ binding mode. Cation and anion effects and polynucleotide specificity. *Biochemistry* **27**:456–471 (1988).
23. L. B. Overman and T. M. Lohman. Linkage of pH, anion and cation effects in protein-nucleic acid equilibria. *J. Mol. Biol.* **236**:165–178. (1994).
24. D. P. Mascotti and T. M. Lohman. Thermodynamics of single-strand RNA binding to oligosines containing tryptophan. *Biochemistry* **31**:8932–8946 (1992).
25. H. Aki and M. Yamamoto. Biothermodynamic characterization of monocarboxylic and dicarboxylic aliphatic acids binding to human serum albumin: A flow microcalorimetric study. *Biophys. Chem.* **46**:91–99 (1993).
26. H. Aki, M. Goto, and M. Yamamoto. Thermodynamic aspects of the molecular recognition of drugs by human serum albumin. *Thermochimica Acta* **251**:379–388 (1995).
27. J. D. McGhee and P. J. Hippel. Theoretical aspects of DNA-protein interactions: Co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. *J. Mol. Biol.* **86**:469–489 (1974).
28. A. C. Moffat, J. V. Jackson, M. S. Moss, and B. Widdop *Clarke's isolation and identification of drugs*, The Pharmaceutical Press, London, 1986.
29. C. B. Black and J. A. Cowan. Quantitative evaluation of electrostatic and hydrogen-binding contributions to metal cofactor binding to nucleic acids. *J. Am. Chem. Soc.* **116**:1174–1178 (1994).
30. J. J. Kiefer, P. Somasundaran, and K. P. Ananthapadmanabhan. Interaction of tetradecyltrimethylammonium bromide with poly (acrylic acid) and poly (methacrylic acid). Effect of charge density. *Langmuir* **9**:1187–1192 (1993).