Cationic Polyelectrolytes as Drug Delivery Vectors: Calorimetric and Fluorescence Study of Rutin Partitioning[†]

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The interaction between hydrophobically modified cationic polysaccharides based on dextran and a flavonoid drug (Rutin) was studied by isothermal titration calorimetry (ITC) and fluorescence spectroscopy, in order to assess the factors responsible for the interaction and characterize its energetics, as well as for evaluating their encapsulation capacity, for possible applicability of these polymers as drug delivery vectors. To address the importance of the hydrophobic pendant groups in the solution behavior of these polymer/drug systems, we also studied the interaction of Rutin with a cationic surfactant, cetyltrimethylammonium chloride (CTAC). The interaction enthalpies and drug binding constants for D40R30/Rutin systems were derived from ITC through a simple binding model. The binding constants were independently derived from fluorescence results, with fair agreement between the parameters obtained from both methods. By changing the Rutin concentration, we were able to get evidence for a solubility enhancement induced by the presence of the polymers, a promising effect regarding its use to improve bioavailability.

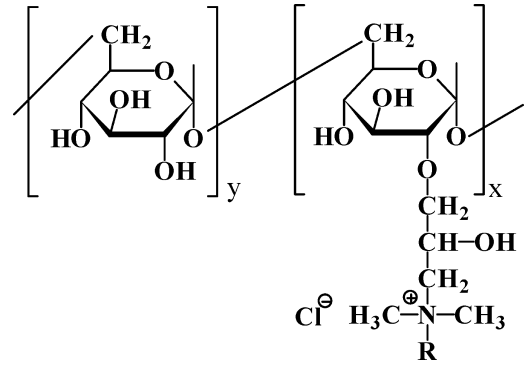
Introduction

Studies of water-soluble associative colloids (micelles, liposomes,and self-assembling polymers) are prone to have an importance both for the understanding of natural processes and for the development of new vectors for drug delivery.[1−3] The design of colloidal gallenic vectors allows the modulation of the biodistribution of drugs by variation of size, morphology,and charge. Their applications in drug delivery and controlled release rely upon the ability of the associative polymer species to encapsulate the drug and later release it to the external medium or target organs in a controlled manner. The encapsulation usually reduces systemic toxicity, prevents premature degradation, and increases bioavailability.

Amphiphilic polymers can self-assemble in aqueous solution into nanoaggregates or polymeric micelles above a certain concentration.[4,5] During the last two decades, they have attracted substantial interest as drug delivery systems, particularly for anticancer drugs, because of their high diversity, biocompatibility, biodegradability, and the multiple functional groups.[6−13] The macromolecular architecture plays a key role in obtaining the desired properties in different polymer-based drug delivery systems.[14−17] The main purpose of these studies is to exploit the solubilizing potential of polymeric micelles toward poorly water-soluble pharmaceutical active ingredients in order to improve their bioavailability.

Nowadays, hydrophobically modified biocompatible polymers (HMP) are recognized as an important and attractive class of drug carriers, especially for intravenous administration of hydrophobic drugs.[17−22] They are prepared by the introduction of hydrophobic ionic moieties on environmentally friendly nonionic polymers or polyelectrolyte precursors, leading to a strong tendency of their hydrophobic groups to aggregate so as to minimize their contact with the solvent water, forming nanostructures that are stable in aqueous solution.[23] The interaction of HMP with drugs often generates a variety of unusual properties, with a pattern different from their unmodified counterparts. Available studies also reported that aggregates formed of hydrophobized polysaccharides are able to solubilize poorly water-soluble drugs, as they become entrapped into their hydrophobic microdomains.[24−27]

SCHEME 1: Chemical Structure of Cationic Amphiphilic Polymer Based on Dextran (D40R30)[a]



[a] R (alkyl) = octyl, dodecyl, and cetyl. Degree of substitution with ammonium groups, DS = $x100/(x + y)$ = 30 mol %.

In our previous work, we reported the aggregation behavior of a series of hydrophobically modified cationic polysaccharides, as studied by fluorescent and viscosity techniques,[28] and the thermodynamics of their self-assembling in aqueous solution.[29,30] Our HMPs derived from dextran by grafting of *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-alkylammonium chloride pendant groups (D40R30, Scheme 1). The critical aggregation concentrations (cac's) of these polymers are several orders of magnitude lower than the ones for alkyltrimethylammonium chloride surfactants (C_nTAC) of alkyl chain length (C_n) equal to that of the pendent group in the polymer.[30] It is expected that these polymers are

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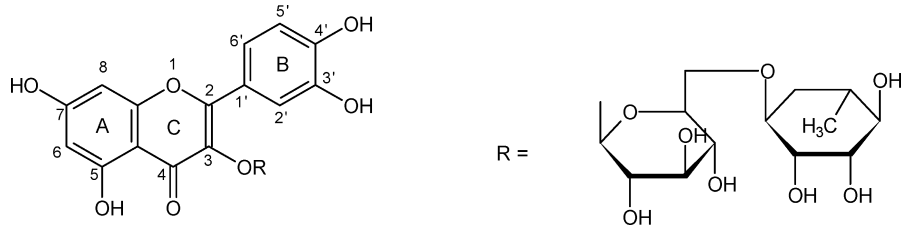
SCHEME 2: Chemical Structure of Rutin, ((3-(6-Deoxy-α-L-mannopyranosyl)-β-glucopyranosyl)oxy)-2-(3′,4′,5,7-tetrahydroxyflavone)



able to solubilize poorly water-soluble drugs as they can be entrapped into their hydrophobic microdomains.

The present work aims at studying the interaction between a series of D40R30 polymers (R: octyl, dodecyl, and cetyl) and Rutin, taken as a model drug. There are several spectroscopic studies of the interaction of Rutin with a variety of entities,[31−42] such as cyclodextrins[31−34] and cetyltrimehtylammonium bromide,[35] but to the best of our knowledge, this is the first report about the interaction of Rutin with a synthetic hydrophobically modified polyelectrolyte. Due to its polyphenolic structure, with several ionizable OH groups, Rutin might interact with cationic amphiphiles by both hydrophobic and electrostatic interactions.

Rutin ($C_{27}H_{30}O_{16}$) (Scheme 2), a glycoside of the flavonol quercetin, is one of the major polyphenols available. This compound has been extensively studied and is known to exhibit antitumor,[43] anti-inflammatory,[44] antiplatelet,[45] and vasodilation[46] properties. Nevertheless, due to its poor solubility (the solubility at $T = 298.15$ K is $(0.66 \pm 0.03) \times 10^{-5}$ (mole fraction value)[48]), it shows a slow dissolution rate in solid oral forms, and the drug absorption from the gastrointestinal tract is generally slow and irregular, thus restricting its use in therapy.[47] Therefore, it is important to improve its solubility and bioavailability.

Isothermal titration calorimetry (ITC) is nowadays well established as an excellent tool for providing a thorough characterization of the energetics of the interactions involved.[29,30,49] Therefore, we see it as very important to provide new microcalorimetric studies of the interactions present in self-associated polymer/drug interactions, to permit the development of new promising delivery vectors.

We also studied the interaction between a surfactant having the same alkyl chain as one of the cationic polymer's pendant groups, namely, cetyltrimethylammonium chloride (CTAC), aiming at ascertaining the role of the hydrophobic effect on the drug/polymer association. In fact, it is to be expected that the interaction between the studied polymers and Rutin shares some characteristics with the ones observed with this cationic surfactant. Guo et al[35] have studied the interaction between CTAB and Rutin using UV−vis and fluorescence emission spectroscopy, cyclic voltammetry, and electron spin resonance methods, but so far no information on the energetics of the interaction is available. The thermodynamic information obtained here will help the characterization of the interactions present in these systems of hydrophobically modified polyelectrolytes (D40R30) and Rutin, as well as to ascertain the importance of the change in alkyl chain length of the polymer pendant groups on the interactions, and thus will be a contribution to the unravelling of the complex patterns displayed by these mixed systems.

Experimental Section

Materials. The studied polyelectrolytes (D40Oct30, D40Dod30, and D40Cet30 (Scheme 1)) were synthesized according to previously described methods[28] by chemical modification of one dextran sample, D40 (Sicomed S.A. Bucharest) with M_w = 40 000 g/mol, and had 30 mol % quaternary ammonium groups. We studied three polyelectrolytes with R having 8, 12, and 16 CH_2 groups, which will therefore be denoted as D40Oct30, D40Dod30, and D40Cet30 (Scheme 1).

Rutin (quercetin-3-*O*-rhamnoglucoside) (Scheme 2), cetyltrimethylammonium chloride (CTAC) (Fluka, >98%), and methanol (Sigma-Aldrich, 99.8%) were used as received.

For ITC experiments, D40R30 polymer solutions (10 $g \cdot L^{-1}$), CTAC (0.02 $mol \cdot L^{-1}$), Rutin solution (1×10^{-4} $mol \cdot L^{-1}$), and its suspension (5×10^{-4} $mol \cdot L^{-1}$) were prepared using ultrapure water (Milli Q Gradient, Millipore, Billerica, MA). All solutions contained 1% (v/v) methanol, as that was needed for preparation of Rutin solutions, and was therefore also added in polymer solutions to avoid high dilution heats.

For fluorescence experiments, stock solutions of D40R30 polymers (20 $g \cdot L^{-1}$ in Millipore water) and Rutin (1×10^{-2} $mol \cdot L^{-1}$ in methanol) were prepared and aliquots of these solutions were added to vials so as to obtain the desired final concentrations: 1×10^{-4} $mol \cdot L^{-1}$ for Rutin and 0.001−20 $g \cdot L^{-1}$ for polymers. All solutions contained 1% (v/v) of methanol.

Isothermal Titration Microcalorimetry (ITC). The microcalorimeter unit used in this work consists of a twin heat conduction calorimeter equipped with a 3 mL titration cell (ThermoMetric AB, Järfalla, Sweden), a water bath and its controller, built at Lund University, Sweden, and a71/2 digit HP nanovoltmeter connected to the calorimetric channel and to the computer. This unit as well as the instrumental procedure have been described in detail in our previous work.[29] Briefly, the volume of pure solvent or Rutin suspension in the calorimetric vessel was 2.6 mL. The calorimetric titration experiments consisted of a series of consecutive additions of D40R30 polymer solution or concentrated CTAC surfactant solution into pure solvent or Rutin suspension contained in the calorimetric vessel. The titrating solution was added to the vessel automatically, in aliquots of 8−12 μL, from a modified gastight Hamilton syringe, through a thin stainless-steel capillary, until the desired range of concentrations had been covered. A special Kel-F turbine, made at Lund University's workshop (Sweden), was used throughout, as we have shown that it promotes very good mixing.[29] All experiments were carried out at 308.15 ± 0.01 K.

Fluorescence Measurements. Steady-state fluorescence emission spectra were obtained with a LS 55 Perkin-Elmer fluorescence spectrometer, using an excitation wavelength (λ_{ex}) of 440 nm, and the slits set at 3 and 10 nm for excitation and emission, respectively. The prepared solutions (see above) were allowed to equilibrate for 24 h before the fluorescence measurements were performed. Emission spectra were recorded in the range 450−650 nm.

Results and Discussion

Thermodynamic Description of the Amphiphile−Rutin Interaction in the Studied Systems. In the present work, two kinds of amphiphiles were used: a series of polymers D40R30 and a cationic surfactant CTAC, along with a drug taken as a model of poorly soluble polyphenols, Rutin. CTAC closely resembles the cationic pendant group of one of the studied polymers, namely, D40Cet30, and was therefore used to carry out a comparison between the interactions of the surfactant and the amphiphilic polyelectrolyte with Rutin, in an attempt to discriminate between hydrophobic interactions with a pendant group and the importance of the presence of the hydrophilic polymer backbone. In a previous study, we found the information retrieved from the study of surfactant association very useful for a deeper understanding of the self-association of HMP.[30] We believe it is also important for clarification of drug encapsulation driving forces. ITC measurements were performed as follows: (i) Rutin solution (or suspension) contained in the calorimetric vessel was titrated with concentrated amphiphile solution; (ii) dilution experiments were taken care of separately, by having solvent media in the calorimetric vessel titrated with concentrated amphiphile solution (heats of dilution of the amphiphiles) and Rutin solution (or suspension) titrated with solvent media (heats of dilution of Rutin). The heat of dilution of Rutin was found to be negligible, within the instrument resolution, and therefore, the results obtained in (i) were only corrected for dilution of the concentrated amphiphile solutions.

Consider the polymer/Rutin interaction process (i) to take place in two steps: (1) the concentrated polymer solution (aggregates) are diluted; (2) Rutin associates with the polymer molecules. The equation representing the processes can be written as

$$\mathrm{P(aq}, c_1) \rightarrow \mathrm{P(aq}, c_2) \quad (1)$$

$$\mathrm{P(aq}, c_1) + \mathrm{Rutin(aq)} \rightarrow \mathrm{[P\text{-}Rutin](aq)} \quad (2)$$

And by subtracting eq 1 from eq 2 we get

$$\mathrm{P(aq}, c_2) + \mathrm{Rutin(aq)} \rightarrow \mathrm{[P\text{-}Rutin](aq)} \quad (3)$$

where P stands for the polymer. Reaction 1 represents the dilution of the polymer into the solvent media, leading to the enthalpy of dilution of concentrated polymer solution from initial concentration c_1 to final concentration in the vessel c_2 ($\Delta H_{obs}(1)$), and it can be obtained from experiment type (ii). Reaction 2 represents the global process ($\Delta H_{obs}(2)$), i.e., dilution of the polymer into Rutin solution, accompanied by Rutin partitioning to the aggregated polymer, with possible formation of a complex, here represented by [P-Rutin] (aq), and finally, reaction 3 represents the process corrected for dilution, i.e., interaction of the polymer already at concentration c_2 with Rutin leading to the formation of Rutin−polymer aggregates. Thus, the corrected enthalpies (ΔH_{corr}) can be calculated from the difference in observed enthalpy values with and without Rutin as

$$\Delta H_{corr} = \Delta H_{obs}(2) - \Delta H_{obs}(1) \quad (4)$$

ΔH_{corr} therefore reflects a balance of various contributions: possible partial polymer dehydration and Rutin partitioning, mediated by electrostatic and hydrophobic interactions.

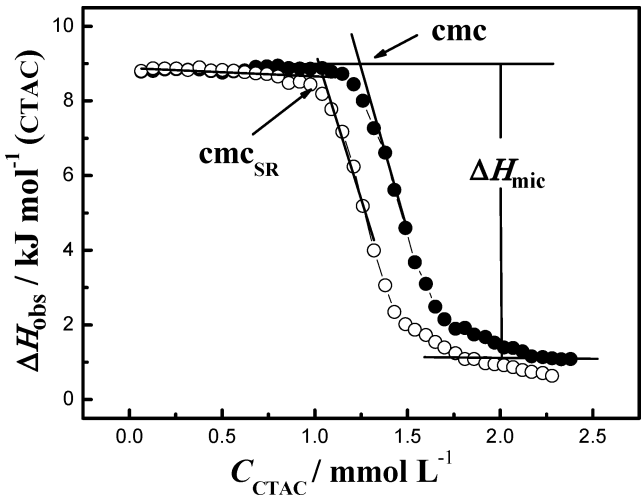


Figure 1. The observed enthalpy curve for the titration of 0.02 mol·L^{-1} CTAC to Rutin solution (○) ($C_R = 1 \times 10^{-4}$ mol·L^{-1}). The solid symbol (●) represents the dilution of 0.02 mol·L^{-1} CTAC into the solvent media (1% methanol (v/v)).

The same type of experiments (i) and (ii) were performed with CTAC, but in this case, the heat evolved in the dilution experiments (titration of CTAC into solvent media) reflects initially both the breaking up of the micelles and monomer dilution, until the surfactant cmc is reached, and thereafter dilution of the micelles.[30]

Part I. Interaction between Rutin and Cationic Surfactant CTAC. Calorimetric titration measurements with CTAC were performed at 308.15 K, at a Rutin concentration of $C_R = 1.0 \times 10^{-4}$ mol·L^{-1}. The calorimetric curves representing the variation of the observed enthalpies (ΔH_{obs}) with CTAC concentration ($C_{surfactant}$) are shown in Figure 1, and the corresponding dilution enthalpy curve of CTAC into the solvent media (water/methanol 1% (v/v)) is also included in the figure for comparison. The calculated enthalpy of micelle information ($\Delta H_{mic} = -8.0$ kJ·mol^{-1} of CTAC) and the critical micelle concentration (cmc) (1.24 mmol·L^{-1}) (Figure 1) agree with the values we obtained previously using pure water as a solvent,[30] showing that methanol does not significantly alter the thermodynamic parameters of micellization. The observed enthalpy pattern in the presence of Rutin is similar to the one observed in its absence, but the presence of Rutin causes a small decrease in cmc. Further, a break is observed in each curve, where the corresponding critical concentration represents the onset of CTAC aggregation in the presence of Rutin, maybe accompanied by Rutin encapsulation, i.e., the formation of CTAC/Rutin mixed aggregates, and should therefore be considered as a critical micellar concentration of the surfactant in the presence of Rutin (cmc_{SR}). The cmc_{SR} values (about 1.0 mmol·L^{-1}) are only slightly lower than the cmc of CTAC in pure solvent, showing that the presence of Rutin has no significant effect on the CTAC aggregation parameters.

The observed behavior is attributed to (i) hydrophobic interaction, that leads Rutin to partition into the micelles, due to its aromatic rings that cause poor water solubility; (ii) the interaction of Rutin with CTAC micelles. The most acidic phenolic OH group of Rutin is at position 7 in the molecule, and its dissociation results in a mixture of neutral and anionic species.[35] Upon Rutin partitioning to CTAC micelles, the rigidity of the B−C ring is reinforced and the $\pi-\pi$ conjugation increased. CTAC micelles, being positively charged, can also promote the adsorption of Rutin anions. As a result of these

events, the cmc of CTAC will decrease. After cmc_{SR}, the observed enthalpies start to decrease, leading to deviation of the curves representing the titration containing Rutin from the dilution curve in its absence. It should be observed that, for the same CTAC concentration, the observed enthalpy after cmc_{SR} is always less positive (Figure 1), indicating that the interaction of Rutin with CTAC is an exothermic event.

Part II. Interaction between Rutin and D40R30. The hydrophobic pendant group of the polymer D40Cet30 closely resembles the cationic surfactant CTAC. As seen above, Rutin can interact with the cationic surfactant CTAC. It is thus tempting to infer that it will also do so with the cationic hydrophobic pendant groups of the polymer D40Cet30. A comparison between the interactions of the two types of amphiphiles (surfactant CTAC and D40Cet30) with Rutin should provide the basis for a better understanding of the interaction characteristics of the studied series of polymers D40R30.

The Interaction Enthalpy Patterns. According to the above thermodynamic description, the difference between the observed enthalpies with and without Rutin can be ascribed to the interaction between Rutin and polymer. Initially, we studied the interaction of the three polymers D40R30 (R = Oct, Dod, and Cet) with Rutin solution at a concentration of $C_R = 1.0 \times 10^{-4}$ mol·L^{-1}, as that was the concentration used in the fluorescence experiments (see below). Nevertheless, due to the sensitivity limit of our calorimeter, we could not observe resolvable peaks for D40Oct30 at this Rutin concentration. Therefore, we decided to perform experiments also at a higher Rutin concentration, $C_R = 5.0 \times 10^{-4}$ mol·L^{-1}, which is slightly above the solubility limit in water [$(0.66 \pm 0.03) \times 10^{-5}$ (mole fraction value) at $T = 298.15$ K].[48] Further, as we had observed visually that addition of polymer to a saturated Rutin solution induced drug solubilization, we also aimed at studying the effect of polymer-induced Rutin solubilization.

The calculated corrected enthalpy curves (ΔH_{corr} vs $C_{side\ group}$) for the D40Cet30/Rutin and D40Dod30/Rutin systems are presented in Figure 2 for $C_R = 1.0 \times 10^{-4}$ mol·L^{-1} and $C_R = 5.0 \times 10^{-4}$ mol·L^{-1}, where the polymer concentration ($C_{side\ group}$) is the final concentration of the polymer side groups in the calorimetric vessel after each injection.

For the CTAC/Rutin system, it is clearly seen that no interaction was found when the CTAC concentration was below cmc_{SR}. However, in the case of D40Cet30 and for the same Rutin concentration, ΔH_{obs} deviates quite significantly from the dilution curve of the polymer (not shown) from the beginning of the titration. We should remember that, in the case of D40Cet30, its concentration in the vessel is already above its own cac, since the first titration point. Therefore, the observed dependence of ΔH_{corr} on polymer side group concentration, indicates that Rutin partitions to the polyelectrolyte aggregates. Finally, we can also see that the ΔH_{corr} vs $C_{side\ group}$ curves depend on Rutin concentration for both D40Dod30 and D40Cet30 (Figure 2).

Although we observe in all cases that partitioning of Rutin only occurs after amphiphile self-aggregation, the details of the interaction depend on the aggregates' characteristics, both among the studied polymers (D40R30) and as compared to the micelles formed by CTAC. D40R30's first cac is much lower than the cmc of C_nTAC,[28−30] with the aggregation of hydrophobic moieties of D40R30 considered to be micelle-like. The microdomains are formed mainly intramolecularly, except for the polymer with octyl groups, which also form intermolecular aggregates. As said above, in the studied systems, the final concentrations of the polymer side group in the calorimetric

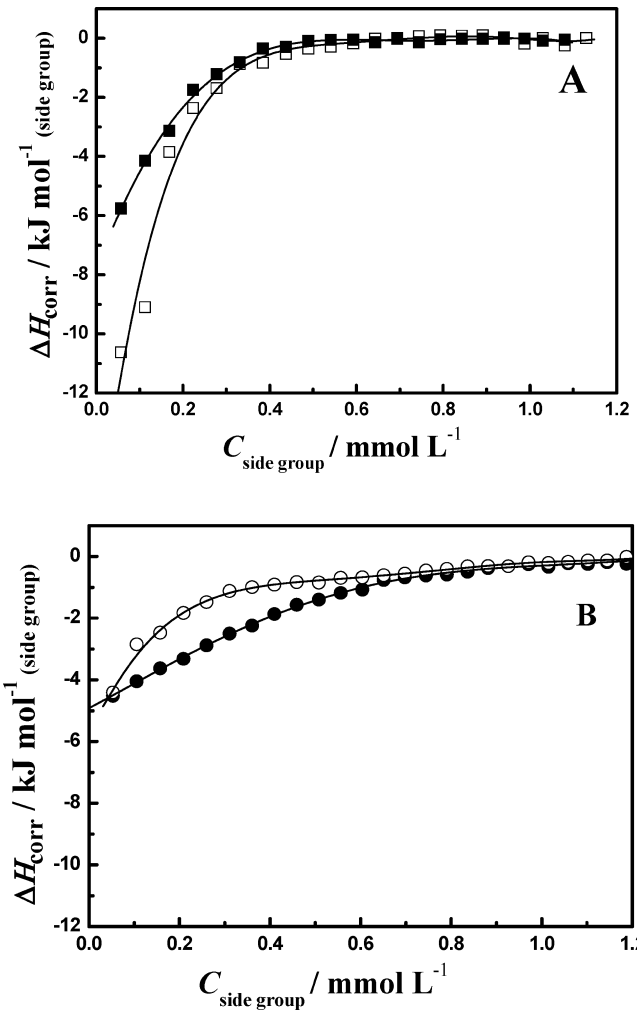


Figure 2. Calculated interaction enthalpy curves (ΔH_{int} vs $C_{side\ group}$) at different Rutin concentrations. (A) D40Dod30 (squares); (B) D40Cet30 (circles). The Rutin concentrations (C_R) are as follows: (■, ●) 1.0×10^{-4} mol·L^{-1}; (□, ○) 5.0×10^{-4} mol·L^{-1}. The lines plotted are just a "guide for the eye".

vessel are always above their first critical aggregation concentrations (cac_1),[28] indicating that the partitioning/binding of Rutin occurs to micelle-like aggregates of polymer. As such, the addition of polymer results in Rutin "solubilizing" in already formed micelle-like aggregates of the polymer, due mainly to the hydrophobic effect. Upon partitioning, interaction between Rutin molecules and the cationic side group of polymer can determine a partial ionization of Rutin's OH groups, and thus be partly responsible for the observed exothermic effect, as due to electrostatic interaction between Rutin anions and the cationic pendant group.

The change in interaction enthalpies with polymer concentration reflects the progressive partitioning/solubilization of Rutin in polymer microdomains. A decrease in ΔH_{corr} (in absolute values) with polymer addition was found, typical for a partition/binding isotherm. The continuing addition of polymer leads to a leveling off, as less Rutin is available in the calorimetric vessel.

In Figure 3, we show the calculated corrected enthalpy curves obtained for the three polymers at the higher Rutin concentration, for comparison of the effect of pendant group on the interaction. ITC measurements show a strong interaction between Rutin and D40Dod30 and D40Cetyl30, whereas for D40Oct30 we could only detect a small exothermic effect when $C_R = 5.0 \times 10^{-4}$ mol·L^{-1}, with no significant polymer

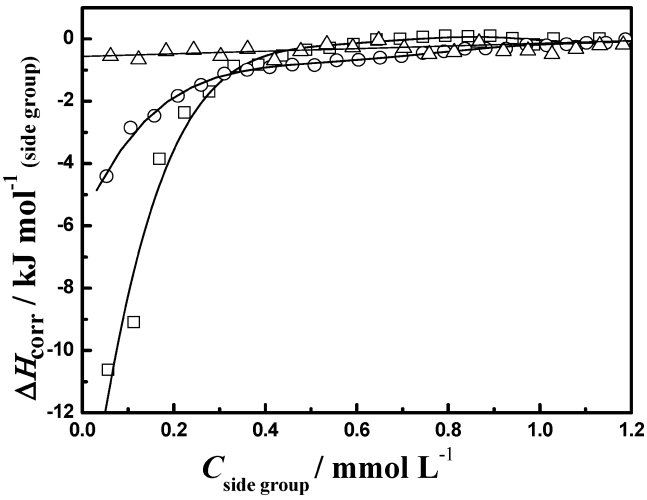


Figure 3. Effect of the polymer alkyl side chain length on the interaction of D40R30 with Rutin ($C_R = 5 \times 10^{-4}$ mol L^{-1}). D40Oct30 (△); D40Dod30 (□); D40DCet30 (○).The lines plotted are just a "guide for the eye".

concentration dependence. This indicates that the interaction takes place, but it is small and not well resolved within the sensitivity of our instrument. The titration curves for D40Dod30 and D40Cet30 present a similar pattern for the enthalpy change as a function of polymer side group concentration. One would expect the interaction of Rutin with the series of HMPs to be enhanced by the increase in polymer hydrophobicity, thereby showing a somewhat lower performance of the dodecyl polymer (12 CH_2) as compared to cetyl (16 CH_2), which is not observed. This effect can be ascribed to the cetyl polymer having more compact hydrophobic microdomains than dodecyl,[28] implying that the former microdomains have a lower ability to accommodate the bulky Rutin molecules. The difference in interactions for the three cases suggests a similar electrostatic interaction contribution (all three polymers have the same charge density) and a major importance of hydrophobic interactions, which depends not only on the hydrophobic alkyl chain length but also on the compactness of their self-aggregation microdomains.

Rutin Partitioning into D40R30 Aggregates. Rutin's behavior in the presence of HMPs can be accounted for by a simple partition/binding equilibrium, driven mainly by the hydrophobic effect. In the following, we shall discuss the analysis of the binding equilibrium between Rutin and D40R30 on the basis of calorimetric and fluorescence measurements.

(A) Partitioning of Rutin to Polymer Aggregates as Measured by ITC. Titration calorimetry allows a model-independent measurement of binding isotherms.[50] This technique has been applied with success to the binding of drugs to proteins[51] and to partitioning of drugs or surfactants to lipid membranes.[50,52,53] The results obtained from the titration of D40Dod30 and D40Cet30 into Rutin can be treated in this way, to provide both the interaction enthalpy, ΔH_{int}, and the binding/partition constant.

In brief, the predicted heat evolved in the titration step i is calculated by eq 5

$$q_i = \Delta H_{int}(n^i_{R,b} - n^{i-1}_{R,b}) + q_{dil} \quad (5)$$

where ΔH_{int} is the molar binding enthalpy, $n^i_{R,b}$ is the number of moles of Rutin bound to the polymer after injection i, and q_{dil} is the dilution heat. The concentration of Rutin bound after each injection can be calculated from

$$C^i_{R,b} = \frac{q_i}{\Delta H_{int} \times V(cell)} \quad (6)$$

and the fraction of Rutin bound in each step according to eq 7 where $n_{R,Total}$ is the total number of moles of Rutin in the

$$\frac{n_{R,b}}{n_{R,Total}} = \frac{K \times [Pol]_i}{1 + K \times [Pol]_i} \quad (7)$$

calorimetric cell, K is the binding constant, defined as the ratio between Rutin concentration in polymer aggregates and in water, and $[Pol]_i$ is the concentration of polymer side groups in the calorimetric cell after injection i. From the obtained heat per injection a differential titration curve was generated, and the best fit of the model to the experimental values was performed through the minimization of the square deviations between the experimental and predicted heat *per* injection for all the titration steps, by use of the appropriate equations using Microsoft Excel and Solver, in a worksheet recently designed to treat surfactant partitioning to bilayers.[53] The original equations were changed to the present system, specially as regarding Rutin and polymer concentrations, as in our calorimeter the total volume in the cell increases after each injection.[29]

We started by fitting the experimental data obtained for D40Dod30 and D40Cet30 for a Rutin concentration of $C_R = 1.0 \times 10^{-4}$ mol·L^{-1}. The experimental results along with the fitted lines are shown in Figure 4A, and the retrieved K and ΔH_{int} values in Table 1. We can see in Figure 4A that the curves are quite similar and eventually superimpose, after the fifth injection. Nevertheless, their initial parts are somewhat different, leading to enthalpy values that are similar (within uncertainty) but to K values that are larger for D40Dod30. As mentioned above, the more compact hydrophobic microdomains of D40Cet30[28] imply that the former polymer microdomains will have a lower ability to accommodate the bulky Rutin molecules, which can justify the difference in K values retrieved.

As referred to above, ITC experiments were also performed for a higher Rutin concentration, $C_R = 5.0 \times 10^{-4}$ mol·L^{-1}, where we have initially a Rutin suspension, and not a solution. This system is indeed more complicated, as it can be anticipated that partitioning of Rutin to the polymer aggregates can induce further drug dissolution, and in fact we had observed this outside the calorimeter (by eye inspection). Thus, to try to evaluate this effect, we started by treating the obtained data by a similar model to the one described above. The experimental values and respective fitted lines are shown in Figure 4B, and the obtained thermodynamic parameters were $K = (5.0 \pm 0.3) \times 10^3$ mol^{-1}·L, $\Delta H = -6 \pm 1$ kJ·mol^{-1} for D40Dod30 and $K = (4.2 \pm 0.3) \times 10^3$ mol^{-1}·L, $\Delta H = -3 \pm 1$ kJ·mol^{-1} for D40Cet30. Comparing with the experiments performed with $C_R = 1 \times 10^{-4}$ mol·L^{-1}, there is an increase in K values and a large decrease (in absolute value) in the obtained ΔH for both polymers. If partitioning/binding was the only process taking place also here, the thermodynamic parameters should not change with Rutin concentration. Therefore, another process must also be occurring, namely, further drug dissolution upon partitioning, as we referred to above. It is well-known that the enthalpy of dissolution of partly hydrophobic drugs in water is endothermic, and can be quite large; e.g., available enthalpies of dissolution (ΔH_{diss}) for diclophenac and paracetamol are 46 ± 1 and 21.0 ± 0.3 kJ·mol^{-1}. Therefore, we

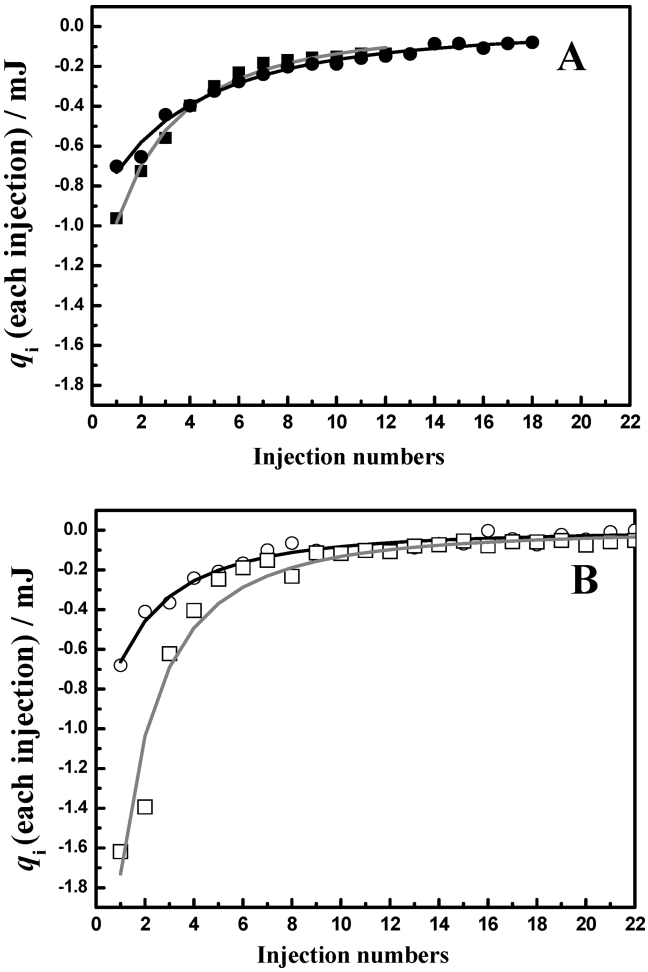


Figure 4. Experimental heat per injection, q_i, from ITC experiments and fitted lines according to eq 7. D40Dod30 (squares); D40Cet30 (circles). Gray line, D40Dod30; black line, D40Cet30. The Rutin concentration is $C_R = 1.0 \times 10^{-4}$ mol·L^{-1} in A and $C_R = 5.0 \times 10^{-4}$ mol·L^{-1} in B.

TABLE 1: Thermodynamic Parameters for Rutin/D40R30 Interaction, as Obtained from ITC and Fluorescence Measurements for a Rutin Concentration of $C_R = 1.0 \times 10^{-4}$ mol·L^{-1}

	$10^{-3} \times$ K/mol^{-1}·L		
polymer (D40R30)	ITC	fluorescence	ΔH_{int}/kJ·mol^{-1}
D40Oct30		0.19 ± 0.07	
D40Dod30	3.6 ± 0.3	2.9 ± 0.3	-22 ± 1
D40Cet30	2.4 ± 0.2	4.8 ± 0.4	-25 ± 1

suggest that the apparent decrease in exothermicity arises from the concomitant drug dissolution, making the simple model above unsuitable to reproduce the observed titration curves in this case.

In order to validate this hypothesis, we did a very rough estimate of the enthalpy of dissolution of Rutin in water from the temperature dependence of available solubility data,[48] using a van't Hoff treatment,[54] obtaining the value +21 kJ·mol^{-1}. Next, we did reevaluate our data by considering that, for the initial titration points, until the amount of Rutin in solution was close to the solubility limit, the total observed enthalpy would be a sum of the interaction (ΔH_{int}) and dissolution (ΔH_{diss}) components of the total observed ΔH. Further, in a very crude approach, we considered that the same number of moles that partition would consequently dissolve, and that the partition

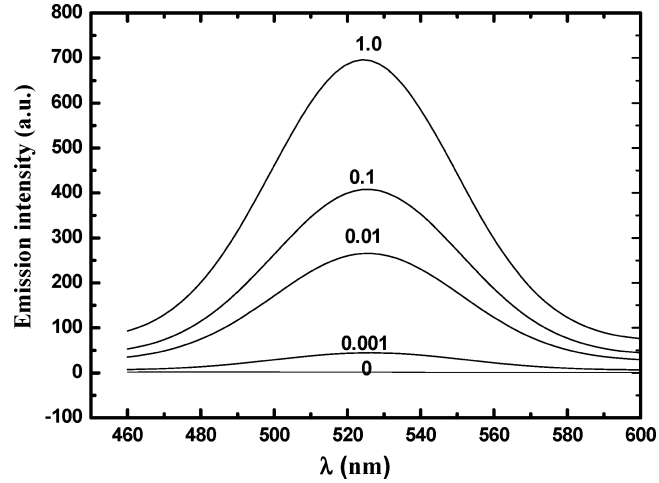


Figure 5. Emission fluorescence spectra of Rutin ($C_R = 10^{-4}$ mol·L^{-1}) in aqueous solution (1% (v/v) methanol), in the presence or absence of polymer D40Dod30. The numbers in the curves indicate the polymer concentration, in g·L^{-1}.

would still be characterized by the same K and ΔH_{int}, as found previously for $C_R = 1.0 \times 10^{-4}$ mol·L^{-1}. This calculation led us to estimate ΔH_{diss} values of +15 and +17 kJ·mol^{-1} for D40Dod30 and D40Cet30, respectively. Therefore, despite the simplicity of the approaches, we believe that the decrease in enthalpy can probably be accounted for by the presence of both partitioning and dissolution taking place for this Rutin concentration. Nevertheless, other possible effects cannot indeed be ruled out based on the present data.

(B) Partitioning of Rutin to Polymer Aggregates as Measured by Fluorescence. Flavonoids with OH groups in position 5 (as Rutin) have a very low fluorescence quantum yield due to the internal hydrogen bonding between C(5)—OH and C(4)═O, which facilitates the nonradiative deactivation of excited flavonoid.[55] Additionally, the substitution of hydroxyl groups at C(3) with a bulky rutoside group was considered to reduce more its intrinsic fluorescence as compared with its aglycon (quercetin, R = H in Scheme 1).[38] However, in the presence of the amphiphilic polymers, clear, broad emission peaks with the maximum at about 522 nm (for excitation at 440 nm) were obtained, and the maximum intensity of these peaks (F values) increased with polymer concentration (Figure 5). This fluorescence enhancement suggests a strong interaction between Rutin and amphiphilic cationic polymers, with formation of a yellow colored complex.

Due to the sensitivity of Rutin emission fluorescence intensity to polymer concentration, we could estimate a binding constant (K) from the fluorescence data, using the Benesi—Hildebrand equation[56] modified to a fluorescence binding isotherm[57] as

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \left(\frac{1}{K|\mathrm{Pol}|_i}\right)\left(\frac{1}{\Delta F_{max}}\right) \qquad (8)$$

where $\Delta F = F - F_0$, $\Delta F_{max} = F_{max} - F_0$, K is the binding constant of Rutin to the D40R30 system, and $|\mathrm{Pol}|_i$ is the polymer concentration in each mixture, expressed in moles of side groups/L. F_0, F_{max}, and F are the fluorescence intensities of free Rutin (in the absence of polymer), bound Rutin (corresponding to the upper plateau of the plots of emission fluorescence vs $C_{side\ group}$), and Rutin in the presence of variable polymer concentrations, respectively.

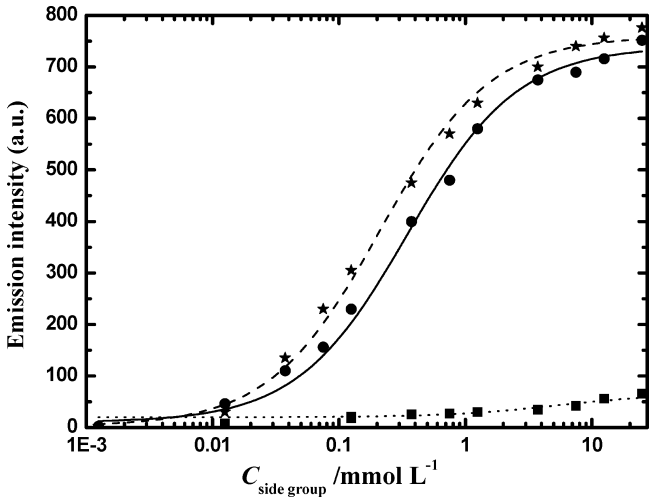


Figure 6. Variation of Rutin fluorescence emission intensity with concentration of cationic amphiphilic polymers. The lines represent the best fits of eq 9 to experimental data. (■) D40Oct30; (●) D40Dod30; (★) D40Cet30.

Equation 8 was rearranged as

$$F = \frac{F_0 + F_{\max} K|\text{Pol}|_i}{1 + K|\text{Pol}|_i} \quad (9)$$

and used to calculate K, by fitting this equation to the experimental data. The K values so obtained are included in Table 1 and the experimental data along with fitted lines presented in Figure 6.

The increase in K values with increasing polymer hydrophobicity indicates a strong contribution of hydrophobic interactions to the partition of Rutin into the hydrophobic microdomains of the amphiphilic polymers. Due to the higher sensitivity of the fluorescence method, a value of K could also be derived for D40Oct30.

The values of the partition constants obtained by microcalorimetric and fluorescence measurements agree fairly in order of magnitude but not on their dependence on the polymer hydrophobicity. Differences in absolute values, mainly the higher K value obtained for the system D40Cet30/Rutin as compared to D40Dod30, at odds with the values retrieved from ITC, can be due to the presence of different fluorescent species of Rutin molecules bound to polymer, as their molar ratio will have a strong influence on the measured fluorescence intensity but probably not on microcalorimetric results.

Conclusions

The hydrophobically modified cationic polyelectrolytes here studied proved to provide a hydrophobic environment favorable to Rutin partitioning/binding. Further, they allowed discrimination of factors responsible for polymer/Rutin interaction, as we could ascertain the effect of alkyl side chain length and microdomain compactness on the interactions.

The calorimetric and fluorescence results were treated as a binding equilibrium, allowing derivation of the relevant thermodynamic parameters, namely, the interaction enthalpy, ΔH_{int}, and the binding constant, K. The K values estimated independently from the two techniques agree in magnitude but not on the dependence on polymer alkyl chain. The use of fluorescence allowed us to also get an estimate of the K value for D40Oct30, which we could not derive from ITC due to sensitivity limits of our instrument. From the studies performed at higher Rutin concentration, we could show that a more complicated process is at stake, and confirm that the polymer induces further Rutin dissolution, from the obtained change in enthalpy values and the estimated enthalpy of dissolution.

The comparison between the results obtained for CTAC/Rutin and D40Cet30/Rutin showed the importance of hydrophobic interactions on the partition process, as it was shown that Rutin only partitions to self-aggregated forms of surfactant and polymer. Finally, it was shown that the hydrophobic interaction in the D40Cet30/Rutin system is stronger than that in CTAC/Rutin (with no "stereo block spacers"), and thus that these polymers can provide better delivery vectors for the drug as compared with the surfactant system.

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