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Inclusion Interactions of Cucurbit[7]uril with Adenine and its Derivatives

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Interactions of cucurbit[7]uril (Q[7] host) with guest adenine (g1), adenosine (g2) and 2′,3′-o-isopropylideneadenosine (g3) were studied in details by ^{1}H NMR, UV absorption spectroscopy, fluorescence spectroscopy and high performance liquid chromatography (HPLC) methods. We found that the suitable pH range for interaction was between 1 and 7, and the optimal pH range was between 2 and 4. The ^{1}H NMR analysis indicated that Q[7] selectively interacted with the adenine moiety of the guests g1 and g2, while Q[7] selectively interacted with the D-ribose sugar ring moiety of the guest g3. Moreover, ^{1}H NMR spectra showed that the exchange between the bound guest and the free guest was fast on the NMR time scale for the Q[7]-g1 and Q[7]-g2 systems. However, an obvious equilibrium between the bound host/guest and the unbound host/guest were observed in the Q[7]-g3 complex. Several methods were used to determine quantitatively the stability of the three host–guest inclusion complexes formed between Q[7] and the guests. The formation constants by UV and fluorescence were $1.90 \times 10^5\,L\,mol^{-1}$ and $1.34 \times 10^5\,L\,mol^{-1}$ for Q[7]-g1, $9.41 \times 10^4\,L\,mol^{-1}$ and $4.24 \times 10^4\,L\,mol^{-1}$ for Q[7]-g2, $4.50 \times 10^4\,L\,mol^{-1}$ and $3.62 \times 10^4\,L\,mol^{-1}$ for Q[7]-g3, respectively. HPLC method was also introduced to explore the interactions between Q[7] and the adenine and its derivatives. The formation constants of the host–guest inclusion complexes, as determined by HPLC, were $6.76 \times 10^4\,L\,mol^{-1}$ for Q[7]-g1, $1.80 \times 10^4\,L\,mol^{-1}$ for Q[7]-g2, $3.01 \times 10^4\,L\,mol^{-1}$ for Q[7]-g3 respectively. Our study suggested that Q[7] could be a suitable host for the delivery of bioactive molecules, such as the adenine and its derivatives.

Keywords: Cucurbiturils; Inclusion complexes; ^{1}H NMR; UV–vis spectra; Fluorescence spectra; HPLC

INTRODUCTION

Synthetic receptors, such as cyclodextrins (CDs), crown ethers and calixarenes, have been extensively explored as drug carriers with the aim to enhance the solubility, stability, bioavailability of drug molecules and to reduce the toxicity of drug molecules [1–5]. Cucurbituril [6] and its homologues [7–9], derivatives [10–17], or analogues [18], as a new family of synthetic receptors, have been widely studied since the structure of cucurbituril(Q[6]) was determined and reported in 1981 [6]. The Q[n] family have common characteristic features, like a hydrophobic cavity and two opening hydrophilic portals. In addition, the varying cavity and portal sizes lead to the formation of inclusion or exclusion complexes with different organic or inorganic species through a combination of dipole-ion, hydrogen bonding and hydrophobic interactions. These studies have been summarized in different reviews in different periods of the development of Q[n]s chemistry [19–25].

However, few works were reported on the study of cucurbit[7 or 8]urils as a drug delivery system [26–28]. Day and Grant have disclosed that the dinuclear platinum complex *trans*-[{Pt(NH3)2Cl}2mdpzm]$^{2+}$ (di-Pt) binds inside cucurbit[7]uril with slow exchange kinetics, which does not significantly affect the cytotoxicity of the dinuclear complex, but reactivity at the platinum centre is reduced [26,27]. Kim and co-workers reported that oxaliplatin can form a stable 1:1 inclusion complex with cucurbit[7]uril. The encapsulation of the drug not only results in a large enhancement in the stability of the drug, but also reduces unwanted side effects caused by protein binding of the platinum drug [28].

Adenine, adenosine and some of their derivatives are natural or synthetic metabolism activators.

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They play an important role in improving substance metabolism, repairing the damaged tissue and accelerating rehabilitation of the abnormal cell and anoxic tissue in organic body. Moreover, purine and pyrimidine base moieties in RNA play an important role in resistance of multiplication of human cancer cells and biosynthesis of protein or RNA [29]. These compounds consist of an aromatic ring connected with a sugar ring. Both of the aromatic and sugar rings could bind with the cucurbit[n]urils, which could protect these drugs from decomposition by enzymes, such as adenosine deaminase, increase their stability and improve the activities of these potential drugs.

Among the cucurbituril and its homologues, the cucurbit[6]uril (Q[6]) and cucurbit[8]uril (Q[8]) are almost insoluble in water. The cucurbit[7]uril (Q[7]) has a moderate solubility (2×10^{-2} mol/L), which is similar to that of β-cyclodextrin (1.6×10^{-2} mol/L). Moreover, the suitable size of cavity and portals of Q[7] make it suitable for binding certain drug molecules, such as adenine and adenosine.

In this work, we have studied the interactions between three nucleoside analogues, adenine (g1), adenosine (g2) and 2′,3′-o-isopropylideneadenosine (g3), with the moderate water soluble Q[7] (Fig. 1) in details. The three guests have a common aromatic adenine moiety and g3 has two more methyl groups than g2. We investigated the selectivity of Q[7] towards the guests, stability of the formed complex of the host and the guest, and the conditions of holding and releasing the guest by the host Q[7].

RESULTS AND DISCUSSION

Effects of PH on the Interactions of Q[7] with Guests

Generally, pH of an interaction system, particularly a biochemical system, could significantly affect the interaction of a host and a guest. Therefore, we first examined the pH effects on the interactions of Q[7] with the guests by UV absorption spectroscopy and fluorescence spectroscopy.

Figure 2 shows the absorbance and fluorescence intensities of three guests recorded in the absence and in the presence of 1.0 equiv. of Q[7] at their respective λ_{max} and λ_{Ifmax} at different pH. Generally, the λ_{max} or λ_{Ifmax} of the guest varied with the pH of the system. The curves of the absorbance (A_{max}) versus pH or the intensity of fluorescence (If_{max}) versus pH of three guests showed similar trend. However, there are differences in absorbance and fluorescence intensity between the free guest and the bound guest in the pH range of 1–6. The curves of the bound guests overlapped with the curves of the free guests in the pH range of pH < 1 or pH > 7, suggesting that the bound guests dissociated from the host Q[7]. The A_{max} of bound g3 increased in the pH range of 2–4 and reached above the curve of free g3 with increasing of the pH. For the same host–guest interaction system, the absorption and fluorescence intensity curves showed similar pH range of interaction. The suitable interaction pH range for the three host–guest systems was between 1–7, particular between 2–4.

^{1}H NMR Spectra Analysis of the Interactions Between Q[7] and Guests

When a guest interacts with a Q[n], it experiences a cavity interaction or a portal interaction or a combined cavity interaction and portal interaction with the host Q[n]. The protons' environment can be changed by the shielding effect of the cavity of the Q[n] or deshielding effect of the portals of the Q[n]. Therefore, ^{1}H NMR technique is a powerful method to investigate the interaction and structure characteristics of the guest and the host [31–33].

Figure 3 shows ^{1}H NMR spectra of g1 in the absence (a) and in the presence of 0.2 equiv (b), 1.0 equiv (c), and 2.0 equiv (d) of Q[7]. Two undeuterated protons Hx and Hy of the guest g1 were detected.

They underwent a gradually upfield shift with increasing equiv of Q[7] (from bottom to top), suggesting that Q[7] can include g1 into its cavity with a fast ingress and egress exchange ratio. Chemical shift changes of certain proton resonances

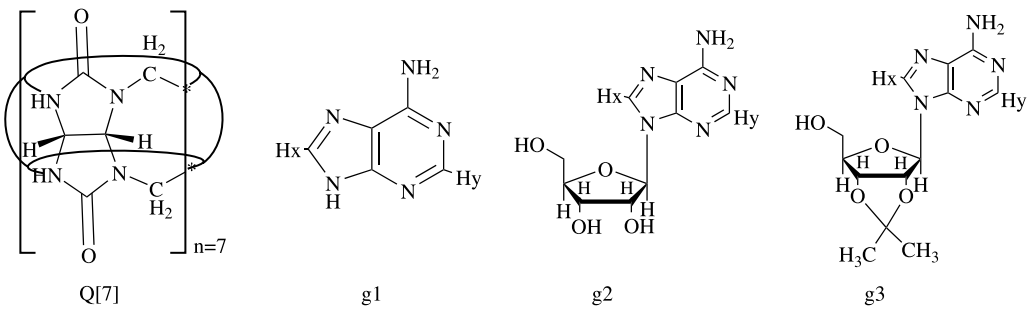

FIGURE 1 Structures of cucurbit[7]uril, adenine and its derivatives.

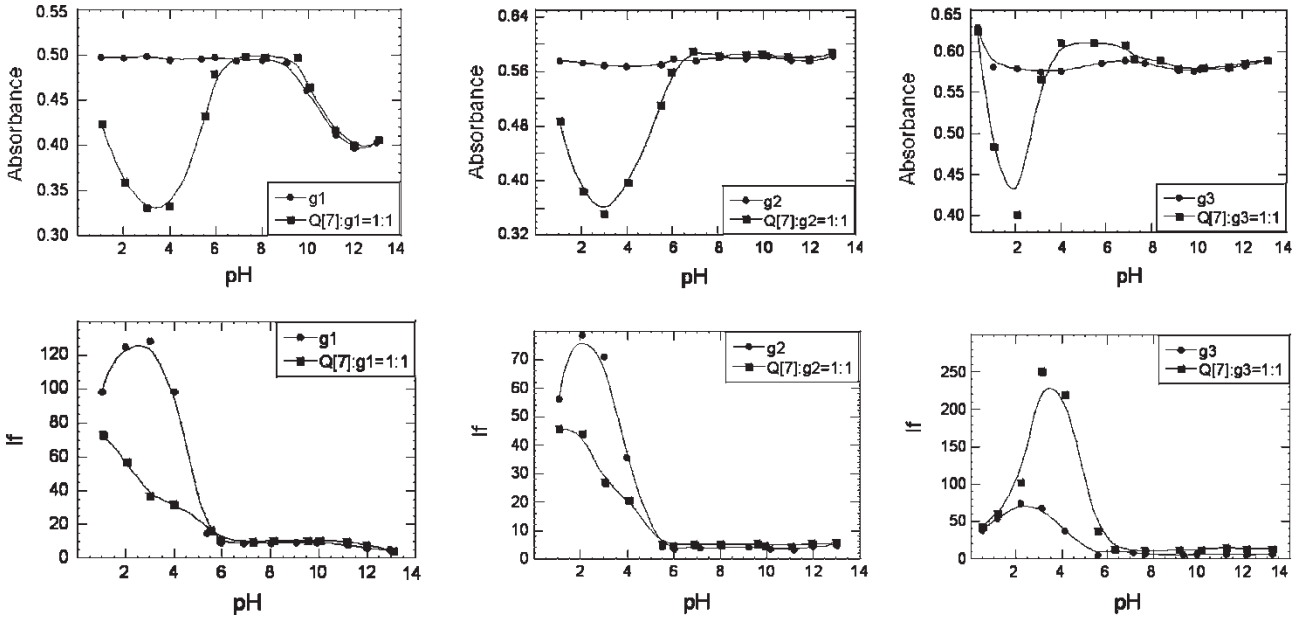

FIGURE 2 A_{max} versus pH and If_{max} versus pH curves of the three guests recorded in the absence and in the presence of 1.0 equiv. of Q[7] at respective λ_{max} and λ_{Ifmax}.

of the guest or host with increasing or decreasing equiv of the guest or host can be used to study host–guest interaction. However, it was difficult to read the accurate chemical shift and the integrity of the guest for the Q[7]-g1 interaction system due to the broad proton resonances of the guest.

In guest g2, there is a D-ribose sugar ring on the adenine. Two moieties in guest g2 could be included in the cavity of Q[7]. ^{1}H NMR spectra of g2 in the absence (a) and in the presence of 0.6 equiv (b), 1.0 equiv (c), and 2.0 equiv (d) of Q[7] are shown in Fig. 4. Similar to guest g1, the protons Hx and Hy on the aromatic moiety of guest g2 showed a gradually upfield shift with increasing equiv of Q[7] (from bottom to top). Moreover, the proton H3 on the D-ribose sugar ring that is close to the adenine moiety is also located in the shielding zone of the cavity of Q[7]. The coupling protons H7/8 showed a downfield shift, suggesting that the most part of D-ribose sugar ring is located at the deshielding portal of the host.

There are also two parts in guest g3, a D-ribose sugar ring with two methyls and an aromatic adenine moiety. ^{1}H NMR spectra of g3 in the absence (a) and in the presence of 1.5 equiv (b) and 0.2 equiv of (c) Q[7] are shown in Fig. 5. Unexpectedly, the two protons Hx and Hy of guest g3 exhibited a downfield shift by ~0.1 ppm and ~0.4 ppm, respectively.

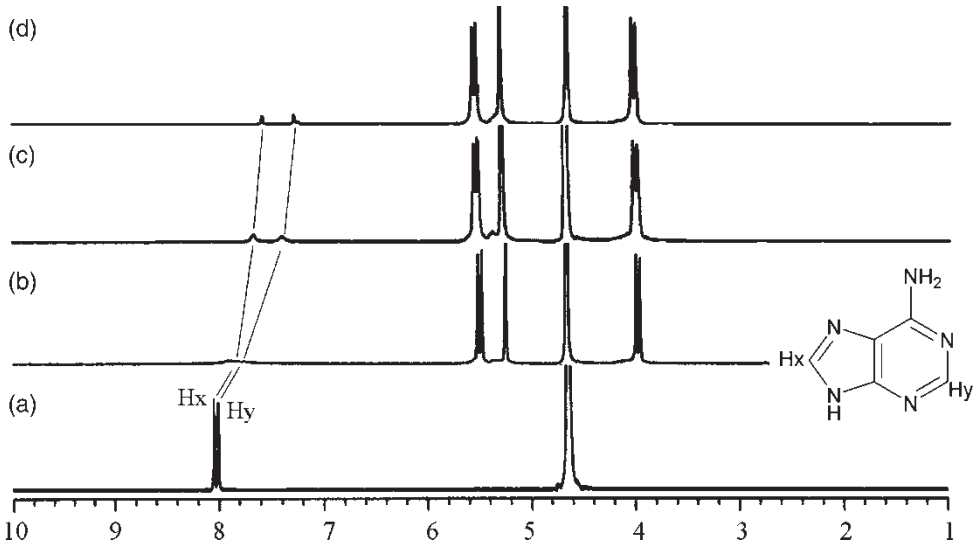

FIGURE 3 ^{1}H NMR spectra (400 MHz, D_2O) of g1 in the absence (a) and in the presence of 0.2 equiv (b), 1.0 equiv (c), and 2.0 equiv (d) of Q[7].

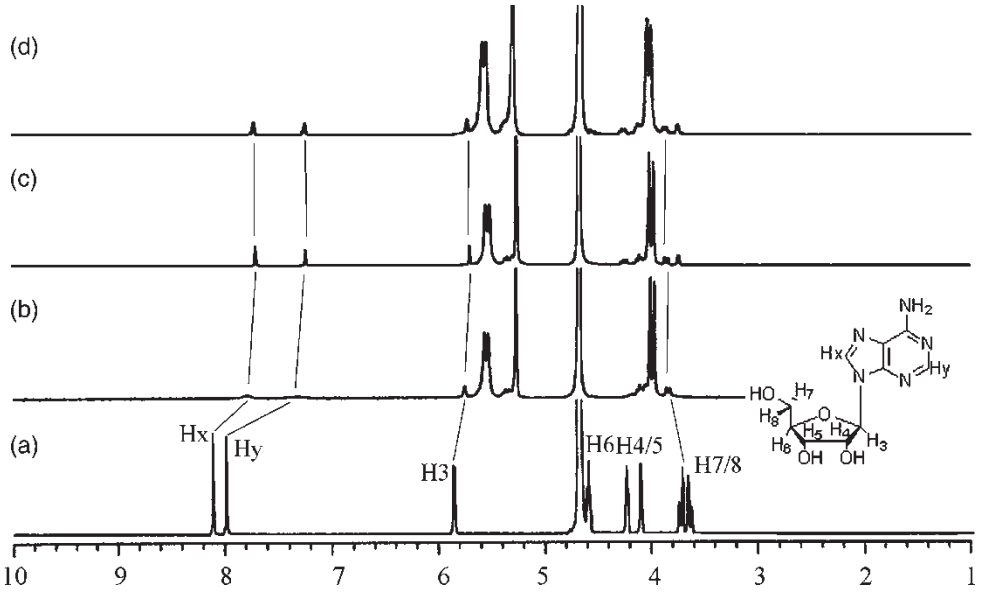

FIGURE 4 ^{1}H NMR spectra (400 MHz, D_2O) of g2 in the absence (a) and in the presence of 0.6 equiv (b), 1.0 equiv (c), and 2.0 equiv (d) of Q[7].

The two methyl protons showed an upfield shift by ~0.7 ppm. Moreover, the proton H3 on the D-ribose sugar ring also showed a downfield shift by ~0.3 ppm and the protons H7/8 showed an upfield shift by ~0.2 ppm. These results suggested that inclusion by Q[7] prefers the methyl substituted D-ribose sugar ring moiety to the aromatic moiety.

The unbound host and guest were observed in the ^{1}H NMR spectra for the Q[7]-g3 system although the host or guest was in excess (Fig. 5b or c), suggesting that the inclusion complex of [7]-g3 has a moderate slow ingress and egress exchange.

UV and Fluorescence Spectroscopy Study of the Interaction Between Q[7] and g1, g2, g3

The ^{1}H NMR spectroscopy revealed that Q[7] bound the three guests and formed the host–guest inclusion complexes. But it was hard to measure the ratio of the host and the guest in these complexes due to the fast ingress/egress of the guest and the broad resonance signals. To determine quantitatively the stability of the host–guest inclusion complexes formed from Q[7] and these guests, UV absorption was recorded at pH = 3.0 for g1 and g2, pH = 2.0 for g3 and fluorescence spectra was recorded at pH = 3.0 for g1, g2 and g3.

The UV spectra obtained with aqueous solutions containing a fixed concentration of the guest (40 mM) and variable concentrations of Q[7] were shown in Fig. 6. As shown, the absorption spectra of the guests, g1, g2, and g3, exhibited a common progressively lower absorbance with a slight red shift as the ratio of $N_{Q[7]}/N_g$ is increased. The hosts show no absorbance in the range of >210 nm. The absorbance (A) *vs* ratio curves can be fitted into a 1:1

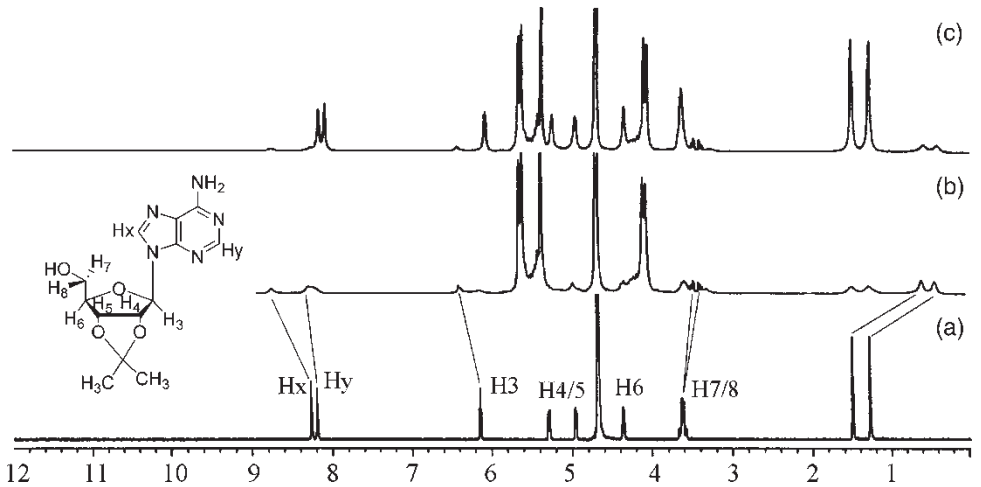

FIGURE 5 ^{1}H NMR spectra (400 MHz, D_2O) of g3 in the absence (a) and in the presence of 1.5 equiv (b) and 0.2 equiv (c) of Q[7].

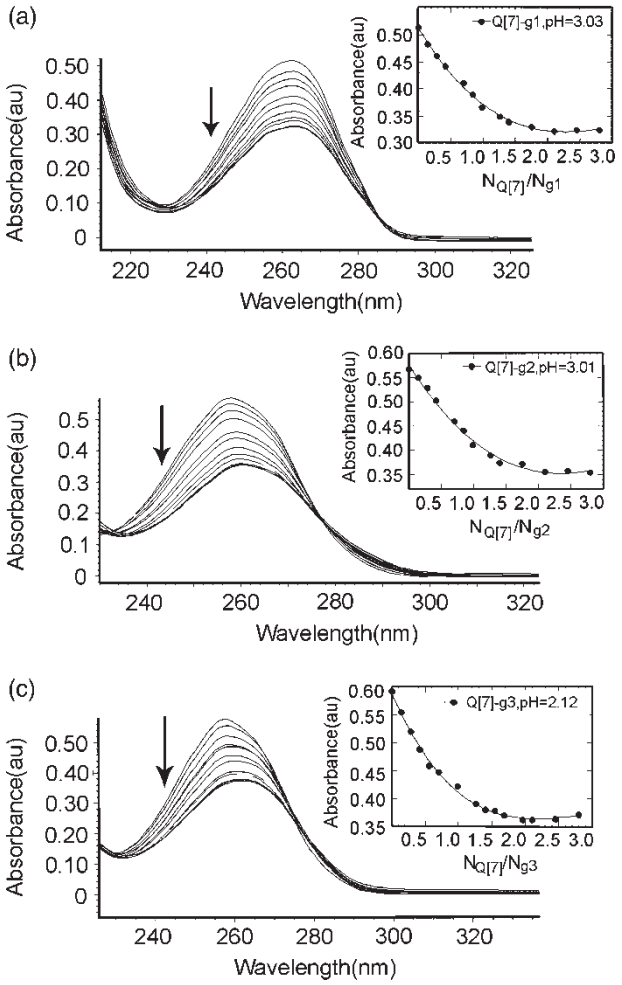

FIGURE 6 UV absorption spectrum of g1–3 in the presence of increasing concentrations of Q[7] and corresponding absorbance versus $N_{Q[n]}/N_g$ curves at $\lambda_{max} = 263$ nm (g1), 264 nm (g2) and 259 nm (g3).

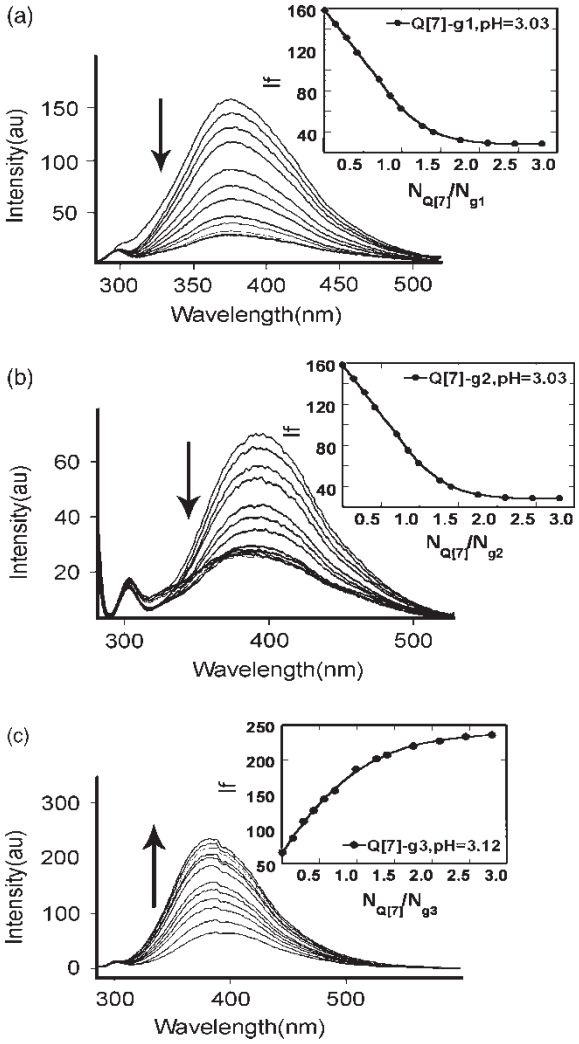

FIGURE 7 Fluorescence emission spectra and corresponding $I_f - N_{Q[n]}/N_g$ curves for the Q[7]-g1-3 systems.

binding model for the Q[7]-g systems. The simple isosbestic points at $\lambda = 284$ nm, 276 nm and 277 nm for the Q[7]-g1-3 systems suggested that these three host–guest inclusion complexes are consistent with a simple interaction between Q[7] and g1-3. The corresponding formation constants (K) are: $1.90 \times 10^5\,L\,mol^{-1}$ for Q[7]-g1, $9.41 \times 10^4\,L\,mol^{-1}$ for Q[7]-g2 and $4.50 \times 10^4\,L\,mol^{-1}$ for Q[7]-g3.

Fluorescence emission spectra of 40 mM guest g1, g2 or g3 in the presence of increasing concentrations of Q[7] are shown in Fig. 7. The inserted plots are corresponding I_f versus $N_{Q[7]}/N_g$ curves of the three systems at $\lambda_{EX}/\lambda_{Em} = 276/376$ nm for g1, 274/390 nm for g2 and 274/388 nm for g3.

Similar to the UV absorption spectra, the fluorescence spectra of g1 and g2 exhibited progressively lower intensity with no shift in wavelength upon addition of increasing amounts of Q[7] to the solution. However, the fluorescence spectra of g3 exhibited progressively higher intensity with no shift in wavelength upon addition of increasing amounts of Q[7]. This difference may correlate with the different interaction models observed in ^{1}H NMR spectra of the corresponding host–guest inclusion complexes. The fluorescence in intensity (If) *vs* ratio curses can be best fitted into a 1:1 binding model for the three Q[7]-guest systems, which were consistent with those from the absorption spectrophotometric analysis. The corresponding formation constants (K) are: $1.34 \times 10^5\,L\,mol^{-1}$ for Q[7]-g1, $4.24 \times 10^4\,L\,mol^{-1}$ for Q[7]-g2 and $3.62 \times 10^4\,L\,mol^{-1}$ for Q[7]-g3, respectively. They are quite close to those obtained by absorption spectrophotometric analysis.

HPLC Studies of the Interactions Between Q[7] and g1, g2, g3

High performance liquid chromatography with β-cyclodextrin modified mobile phase has been extensively used for the study of the interactions of β-cyclodextrin with various guests. It was also used to determine the formation constants of the related inclusion complexes [33–38]. For the relatively new

host family, cucurbituril and its homologues, high performance liquid chromatography with the cucurbit[n]urils modified mobile phase was rarely used to study their interactions with guests due to the poor solubility of cucurbit[n]urils. Although they have a common hydrophobic cavity and two hydrophilic portal rimmed carbonyls, their water solubility is low. However, the discovery of a series of modified cucurbit[n]uril derivatives and analogues that have good water solubility lead to the use of HPLC as a study tool.

Similar to the studies of interaction between cyclodextrin and guest [39], the equilibrium of the Q[7]-guest systems are presented by the following set of equations in which the species concentration in stationary phase and in the mobile phase are denoted by subscripts s and m, respectively.

$$G_m + L_s \overset{K'_G}{\longleftrightarrow} G_m{\cdot}L_s \quad (1)$$

$$Q[7]_m + L_s \overset{K'_Q}{\longleftrightarrow} Q[7]{\cdot}L_s \quad (2)$$

$$G{\cdot}Q[7]_m + L_s \overset{K'_{G\cdot Q}}{\longleftrightarrow} G{\cdot}Q[7]{\cdot}L_s \quad (3)$$

$$G_m + Q[7]_m \overset{K}{\longleftrightarrow} G{\cdot}Q[7]_m \quad (4)$$

The concentrations of all species are defined in mol L^{-1}, and $[Q[7]_m]$ is the concentration of Q[7] in the mobile phase. The formation constants corresponding to Eqs. (1)–(4) are denoted by K'_G, K'_Q, $K'_{G\cdot Q}$ and K, respectively. Equation (1) represents a reversible equilibrium of the guest in the bulk solvent mobile phase (G_m) with the stationary phase sites (L_s) to form an interaction pair. The concentration of the guest ($[G_m]$) should be kept below the bound phase concentration ($[L_s]$). Equation (2) represents the adsorption of $Q[7]_m$ onto the stationary phase of the column to form an unstable interaction pair. Equation (3) also represents the adsorption of $G{\cdot}Q[7]_m$ onto the stationary phase of the column to form an interaction pair. Equation (4) represents a reversible equilibrium of the guest in the bulk solvent mobile phase (G_m) with Q[7] in the mobile phase ($Q[7]_m$) to form an 1:1 inclusion complex, $G{\cdot}Q[7]_m$.

The adsorption of guest, host and host–guest onto the stationary phase can be observed by the change of the peak characteristics, such as retention time, resolution, peak shape, etc.. Generally, the cucurbit[n]urils are treated as polar molecules due to the dipole portals and the hydrophilic nature of the surface of the cucurbit[n]urils. Therefore, the interactions between the cucurbit[n]urils with the nonpolar stationary phase, ODS, are likely limited. A comparison of column characteristics, before and after the column was exposed to the Q[7] mobile phase, showed that the column characteristics were not changed when using Q[7] modified mobile phase. Therefore, the adsorption of Q[7] onto the stationary phase, as expressed in Eq. (2), could not happen. Thus, the retention mechanism could be controlled by Eqs. (1), (3) and (4). The apparent capacity factor of the guest (K′), which represents the ratio of the total concentrations of the species in the stationary phase to the total concentrations of the species in the mobile phase, is given by:

$$K' = ([G{\cdot}L_s] + [G{\cdot}Q[7]{\cdot}L_s])/([G_m] + [G{\cdot}Q[7]_m]) \quad (5)$$

Combination of Eqs. (1), (3)–(5) yields the following expression for the capacity factor in terms of K'_G, $K'_{G\cdot Q}$ and K:

$$K'_G = [G{\cdot}L_s]/[L_s]{\cdot}[G_m] \quad (6)$$

$$K'_{G\cdot Q} = [G{\cdot}Q[7]{\cdot}L_s]/[L_s]{\cdot}[Q[7]_m] \quad (7)$$

$$K = [G{\cdot}Q[7]_m]/[G_m]{\cdot}[Q[7]_m] \quad (8)$$

$$K' = (K'_G + K'_{G\cdot Q}{\cdot}K{\cdot}[Q[7]_m])/(1 + K{\cdot}[Q[7]_m] \quad (9)$$

Equation (9) is transformed to Eq. (10) by rearranging:

$$K' = (K'_G - K')/K{\cdot}[Q[7]_m] + K'_{G\cdot Q} \quad (10)$$

Slope and intercept can be obtained by plotting K′ *vs.* $(K'_G - K')/[Q[7]_m]$ in mol L^{-1}.

$$\text{Slope} = 1/K \quad (11)$$

$$\text{Intercept} = K'_{G\cdot Q} \quad (12)$$

K′ and K'_G are obtained directly from the experiments. Thus, the formation constants of the Q[7]-guest systems can be calculated by Eq. (10).

The chromatograms of three guests with different concentrations of Q[7] added to the mobile phase as a modifier were shown in Fig. 8. The column temperature was kept at 50°C. The retention time of the guests (t_R) decreased as the concentration of Q[7] increased, suggesting that the free guest was retained on the reversed stationary phase longer when no Q[7] was added to the mobile phase. Guest g2 and g3, which have almost the same size, were retained longer on the stationary phase than guest g1. When Q[7] was added to the mobile phase, the interaction of the guest with the stationary phase was changed due to the competitive interaction with host Q[7]. That resulted in the reversal of the elution order of g1, g2 and g3. Moreover, unlike the chromatograms of g1 that showed peak tailing in the presence of Q[7], the chromatograms of g2 or g3 showed peak front tailing.

The correlation of K′ upon $(K'_G - K')/[Q[7]_m]$ is graphically illustrated in Fig. 8d–f. According to Eq. (10), the calculated formation constants for the three host–guest inclusion complexes are 6.76×10^4 L mol^{-1}, 1.80×10^4 L mol^{-1} and 3.01×10^4 L mol^{-1} respectively. They are close to the corresponding formation constants obtained by UV and fluorescence spectroscopy analysis. The function

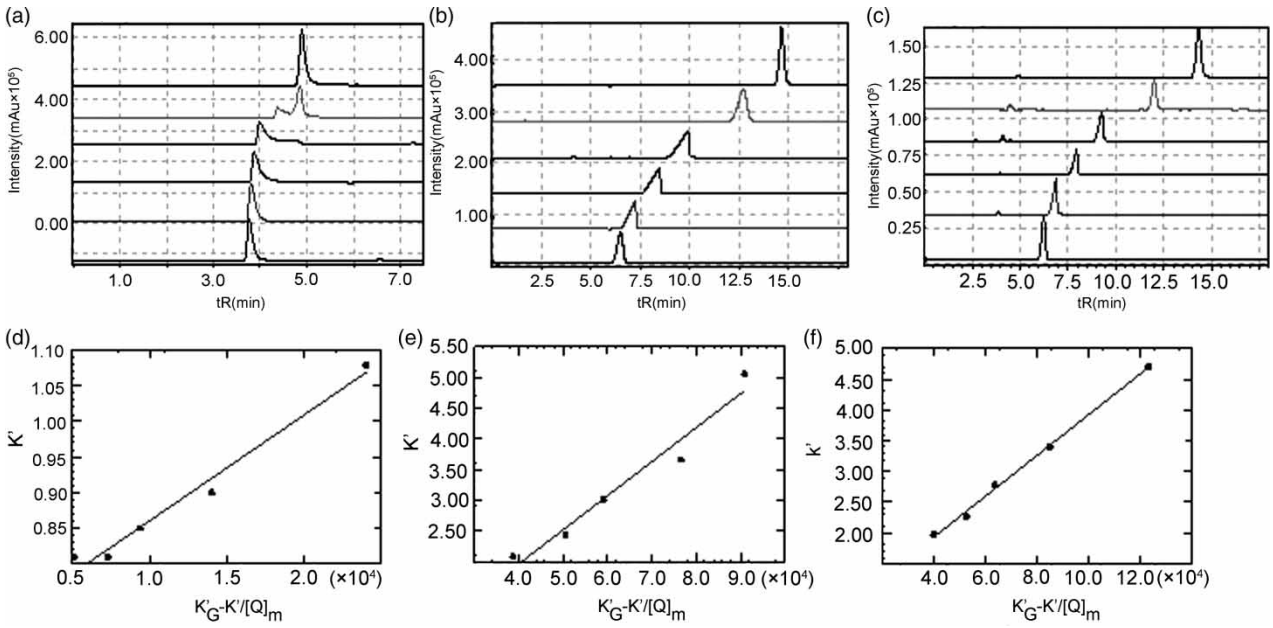

FIGURE 8 Chromatograms of guests with various concentrations of Q[7] in the mobile phase. (a) for g1, (b) for g2, (c) for g3. The concentration of Q[7] were $0, 1 \times 10^{-5}$ mol/L, 3×10^{-5} mol/L, 5×10^{-5} mol/L, 7×10^{-5} mol/L, 1×10^{-4} mol/L from top to bottom; (d), (e) and (f) are the correlation plot of Eq. (10) for g1, g2, and g3, respectively.

constants determined by three methods are listed in Table I.

Based on the experimental results from ^{1}H NMR analysis, the interaction of Q[7] with g2 was different from its interaction with. For g2, Q[7] selectively interacted with the aromatic moiety. For g3, it also interacted with the sugar ring moiety. Although only subtle difference was observed in the chromatograms of Q[7]-g2 and Q[7]-g3, K'_{GQ} data showed that interactions of the host–guest inclusion complex with the stationary phase were quite different. For the inclusion complex Q[7]-g2, a hydrophilic sugar ring tail is left out the portal of Q[7], which could increase the total hydrophilic property of the complex and result in a weaker interaction with the stationary phase.

CONCLUSION

The interactions between cucurbit[7]uril and adenine, adenosine and 2′,3′-o-isopropylideneadenosine have been studied in details by ^{1}H NMR spectroscopy, UV absorption spectroscopy, fluorescence spectroscopy and high performance liquid chromatography (HPLC) methods. The investigation of the pH effects on the interactions between the host and guests revealed that the interaction pH range was between 1 and 7. And the most suitable pH range was between 2 and 4. The ^{1}H NMR spectra analysis indicated that Q[7] selectively interacted with the adenine moiety of guests g1 and g2, but interacted with the D-ribose sugar ring moiety of guest g3. Moreover, ^{1}H NMR also revealed that the H3 on the ring of guest g2 is located in the shielding zone of Q[7], and the H6 on the ring of guest g3 is located in the deshielding zone of the Q[7]. In addition, ^{1}H NMR spectra showed that the exchange between the bound guest and the free guest was fast on the NMR time scale for the Q[7]-g1 and Q[7]-g2 complex. However, an obvious equilibrium between the bound host/guest and the unbound host/guest were observed in the Q[7]-g3 complex.

Three methods have been used to determine quantitatively the stability of the three host–guest inclusion complexes formed from Q[7] and the guests. Measurement by UV spectroscopy and fluorescence spectroscopy gave similar results for the same host–guest system at pH = 3.0. The Formation constants by UV and fluorescence were 1.90×10^5 L mol^{-1} and 1.34×10^5 L mol^{-1} for Q[7]-g1, 9.41×10^4 L mol^{-1} and 4.24×10^4 L mol^{-1} for Q[7]-g2, 4.50×10^4 L mol^{-1}

TABLE I Chromatogram paremeters and formation constants of three host–guest inclusion complexes

Guest	Correlation coefficient (R)	Intercept (K'_{GQ})	Formation constants L.mol^{-1} (K_{HPLC})	Formation constants L.mol^{-1} (K_{UV})	Formation constants L.mol^{-1} (K_{If})
g1	0.9887	0.71	6.76×10^4	1.90×10^5	1.34×10^5
g2	0.9789	0.25	1.80×10^4	9.41×10^4	4.24×10^4
g3	0.9985	0.61	3.01×10^4	4.50×10^4	3.62×10^4

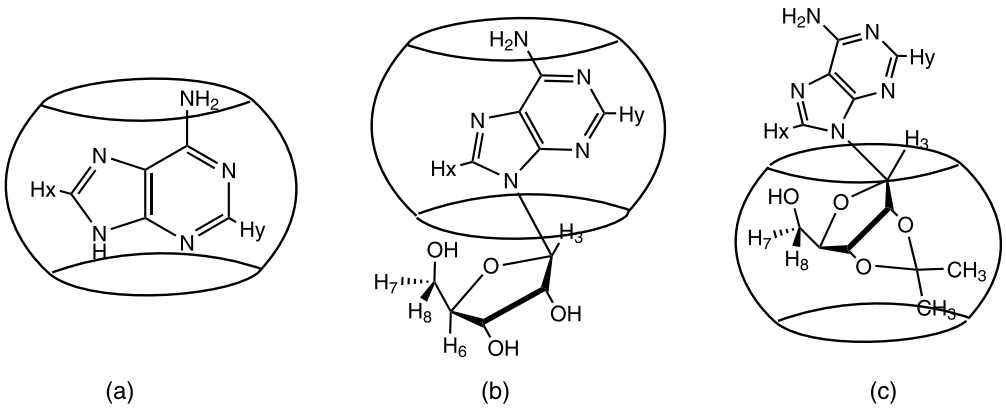

FIGURE 9 The proposed interaction models of (a) Q[7]-g1, (b) Q[7]-g2, (c) Q[7]-g3.

and $3.62 \times 10^4\,\mathrm{L\,mol^{-1}}$ for Q[7]-g3, respectively. HPLC method was also introduced to explore the interactions between Q[7] and the adenine and its derivatives. The formation constants of the host–guest inclusion complexes, as determined by HPLC, were $6.76 \times 10^4\,\mathrm{L\,moL^{-1}}$ for Q[7]-g1, $1.80 \times 10^4\,\mathrm{L\,moL^{-1}}$ for Q[7]-g2, $3.01 \times 10^4\,\mathrm{L\,moL^{-1}}$ for Q[7]-g3 respectively. They are quite close to those obtained from the UV and fluorescence spectroscopy methods.

Moreover, the HPLC method provided information on the interaction of host–guest and showed useful supporting information on the structure characteristics of the complexes. Based on these results, the interaction models for the three host–guest are proposed as shown in Fig. 9.

EXPERIMENTAL

Materials

Cucurbit[7]uril was prepared and purified according to the method developed in our laboratories. Adenine (g1), Adenosine (g2), 2′,3′-o-isopropylideneadenosine (g3) were obtained from Aldrich and used without further purification.

NMR Measurements

To study the host–guest complexation of Q[n] and the guests, $2.0–2.5 \times 10^{-3}$ mmol samples of Q[7] in 0.5–0.7 mL D_2O with an increasing concentrations of guests were prepared, the corresponding ^1H NMR spectra were recorded at 20°C on a VARIAN INOVA-400 spectrometer.

UV and Fluorescence Measurement

UV absorption spectra of the host–guest complexes were recorded on an Agilent 8453 Photospectrameter at room temperature. Fluorescence spectra of the host–guest complexes were recorded on a Varian photofluorescent spectrometer at room temperature. The aqueous solutions of adenine and its derivatives were prepared with a concentration of $1.00 \times 10^{-3}\,\mathrm{mol/L}$. An aqueous solution of Q[7] was prepared with a concentration of $2.00 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$ for both absorption spectra and fluorescence spectra determination. Samples of these solutions were combined to give solutions with a guest:Q[7] ratio of 0, 0.2:1, 0.4:1, 1:1, 1.5:1 and 2:1. pH values of the solutions of the host–guest complexes were monitored with a S-3C pH meter and the pH of the samples was adjusted with HCl and NaOH.

HPLC Analysis

The high performance liquid chromatography (HPLC) system (Agilent 1100 Series) used for the study consisted of an Agilent pump, an Agilent UV–vis detector and an Agilent oven. A Nucleosil 250 mm × 4 mm ODS column (5 μm, particle size) supplied by Macherey–Nagel (Dueren, Germany) was used. The mobile phase (3% formic acid) flow rate was at $1.0\,\mathrm{mL\,min^{-1}}$ and the column temperature was set at 50°C. The appropriate amounts of Q[7] (13.42–134.2 mg) were dissolved in 1L of mobile phase and filtered through a 0.45 μm membrane filter. The wavelength at 263 nm for g1, 264 nm for g2, and 259 nm for g3 were used. The first peak caused by the change in UV absorbance of sample solvent of each injection was used as the dead time (t_0) of the system. The average t_0 of 2.10 min was used for all K′ calculations.

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