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The Capture and Stabilization of Curcumin Using Hydrophobically Modified Polyacrylate Aggregates and Hydrogels

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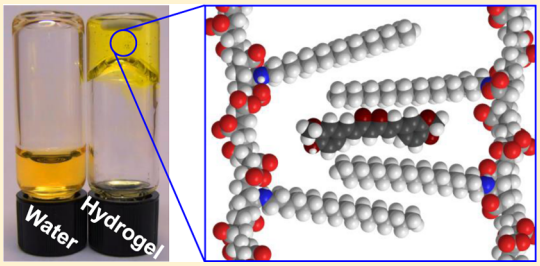
Supporting Information

ABSTRACT: Hydrophobically modified polyacrylates are shown to suppress the degradation of the medicinal pigment curcumin under physiological conditions. In aqueous solution, the 3% octadecyl randomly substituted polyacrylate, PAAC18, forms micelle-like aggregates at a concentration of <1 wt % and a hydrogel at >1 wt %. Under both conditions, PAAC18 shows a remarkable ability to suppress the degradation of curcumin at pH 7.4 and 37 °C such that its degradation half-life is increased by 1600−2000-fold. The suppression of degradation is attributed to hydrophobic interactions between curcumin and the octadecyl substituents of PAAC18 within the micelle-like aggregates and the hydrogel, as indicated by 2D NOESY ^{1}H NMR spectroscopy. UV−visible absorption titration results are consistent with the interaction of curcumin with five octadecyl substituents on average, which appears to substantially exclude water and greatly decrease the curcumin degradation rate. Dynamic light scattering and zeta potential measurements show the average hydrodynamic diameters of the PAAC18 aggregates to be 0.86−1.15 μm with a negative surface charge. In contrast to the octadecyl substitution, the 3% dodecyl randomly substituted polyacrylate, PAAC12, shows a negligible effect on slowing the degradation of curcumin, consistent with the dodecyl substituents being insufficiently long to capture curcumin in a adequately hydrophobic environment. These observations indicate the potential for PAAC18 to act as a model drug delivery system.



INTRODUCTION

Curcuminoids are a group of naturally occurring pigments extracted from the rhizomes of *Curcuma longa*, commonly known as turmeric.[1,2] The most abundant active agent is curcumin (Figure 1a), which constitutes 77% of curcuminoids.[3] Other major curcuminoids include demethoxycurcumin and bisdemethoxycurcumin, which compose 17% and 3%, respectively.[3] The medicinal effects of curcumin have been demonstrated for cancer,[4−8] inflammation,[8,9] Alzheimer's disease,[10−12] and cystic fibrosis.[13,14] As a result, clinical trials are either underway or have been completed with an aim to develop curcumin as a therapeutic agent.[15−18] However, there are two key challenges that have hindered the development of curcumin as a readily usable drug. These are the low aqueous solubility of curcumin which limits its availability *in vivo*,[19,20] and its susceptibility to hydrolysis and fragmentation which result in substantial degradation within 15 min.[21−23] Seeking to address these problems, recent studies have explored a number of delivery systems which include micelles,[24−28] plasma proteins,[22,29,30] cyclodextrins,[21,31,32] and polymer nanoparticles.[33−35]

An attractive approach is to capture and deliver curcumin in hydrogels, which have been utilized in pharmaceutical and biomedical fields including drug delivery and tissue engineering.[36−41] Hydrogels possess the ability to hold large quantities of water and biological fluids by forming three-dimensional networks.[42] Polyacrylate is an attractive starting material for hydrogel construction because of its well-established applications in drug delivery and tissue engineering.[43−46] The synthesis and characterization of the 3% octadecyl and dodecyl randomly substituted polyacrylates, PAAC18 and PAAC12, respectively (Figure 1b), were reported in previous studies.[47−49] In aqueous solution, hydrophobic interactions between the alkyl substituents of these polyacrylates result in intra- and interstrand associations, depending on the polyacrylate concentration in weight percent (wt %) as follows. Aqueous solutions of PAAC18 at 0.5 wt % exhibit a low viscosity,[48,49] suggesting the formation of micelle-like aggregates due to the dominant presence of intrastrand associations. Increasing the PAAC18 concentration up to 1 wt % results in a gradual increase in viscosity,[48,49] showing a greater propensity to form cross-linking between different strands. At a concentration >1 wt %, PAAC18 aqueous solutions form a hydrogel. Similarly, >2.7 wt % PAAC12 aqueous solutions form a hydrogel.[48] Thus, the spontaneous cross-linking between polyacrylate strands conveniently allows a variation of viscosity up to the formation of hydrogels as concentration is varied.

In this study, we investigate the capture of curcumin by hydrophobically modified polyacrylates in aqueous solutions. Both the micelle-like aggregates and hydrogels formed by PAAC18 show a remarkable ability to increase the solubility of curcumin and suppress its degradation under physiological

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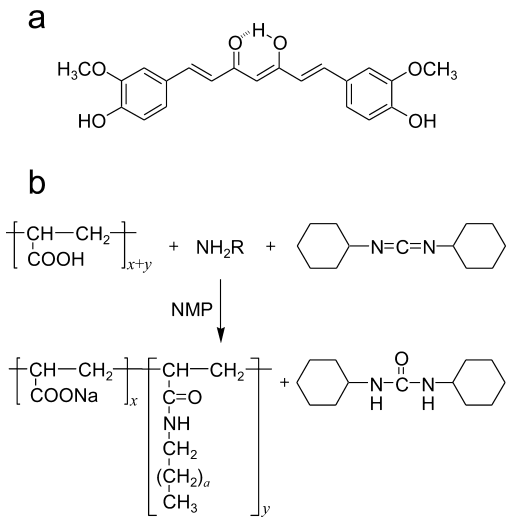

Figure 1. (a) The keto−enol form of curcumin. (b) The synthetic scheme for the random substitution of polyacrylate with either octadecyl or dodecyl substituents to give PAAC18 and PAAC12, respectively, where NMP is 1-methyl-2-pyrrolidone and R represents either the octadecyl (a = 16) or dodecyl (a = 10) substituent. The degree of random substitution is presented as x:y = 97:3 for 3% substitution and x:y = 90:10 for 10% substitution.

conditions, while PAAC12 shows little effect. A strong binding of curcumin by the octadecyl substituents is revealed by UV−visible absorption titration studies, and a similar binding is shown to occur by 2D NOESY ^{1}H NMR spectroscopy. Dynamic light scattering studies reveal average hydrodynamic diameters of 0.86−1.15 μm for the PAAC18 micelle-like aggregates, of which zeta potential results show to have negatively charged surfaces.

MATERIALS AND METHODS

Materials. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) was obtained from LKT Laboratories (purity 98%). Methanol (AR grade, 99.5%), dimethyl sulfoxide (DMSO, ACS grade), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$, AR grade) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$, AR grade) from Merck Pty Ltd. were used as received, and D_2O and DMSO-d_6 were purchased from Cambridge Isotope Laboratories (D, 99.9%). Polyacrylic acid (M_w = 250 000, $M_w/M_n \approx 2$) was obtained from Sigma-Aldrich as a 35 wt % aqueous solution and freeze-dried to constant weight. Dodecylamine (99%), octadecylamine (99%), 1-methyl-2-pyrrolidone (NMP) (99.5%) and dicyclohexylcarbodiimide (DCC) (99%) were used as received from Sigma-Aldrich. Sodium hydroxide (NaOH) pellets (AR grade, 97.0%) from Ajax Finechem were used to make a 40% NaOH solution with water deionized using a Millipore Milli-Q NANOpure system. The 50 mM phosphate buffer solution at pH 7.4 ([$NaH_2PO_4{\cdot}H_2O$] = 0.156% w/v and [$Na_2HPO_4{\cdot}2H_2O$] = 1.04% w/v) was also prepared in deionized water.

Syntheses of Hydrophobically Modified Polyacrylates. The 3% octadecyl and dodecyl randomly substituted polyacrylates, namely PAAC18 and PAAC12, respectively, were prepared and characterized as previously described.[48,49] Briefly, octadecylamine and DCC solutions in NMP were added to a solution of polyacrylic acid in NMP with vigorous stirring. After 48 h at 60 °C, the solution was cooled to room temperature, and a 40% NaOH aqueous solution was added to induce precipitation of the polyacrylate product. After washing and filtering the precipitate with NMP at 60 °C and methanol at room temperature, the crude PAAC18 was dialyzed (molecular weight cutoff of 7500 Da) against deionized water for 5 days. The solution in the dialysis tube was then freeze-dried, and PAAC18 was obtained as a white solid. A similar method in which dodecylamine replaced octadecylamine was used to synthesize PAAC12. The 10% octadecyl and dodecyl randomly substituted polyacrylates, 10%-PAAC18 and 10%-PAAC12, respectively, were also synthesized by methods similar to those described above except that the amounts of octadecylamine and dodecylamine used were proportionately increased. The degrees of substitution for the substituted polyacrylates were determined from ^{1}H NMR spectra as described previously.[48,49]

UV−Visible Absorption Spectra of Curcumin in Aqueous Solutions of PAAC18, PAAC12, and 10%-PAAC12, and the Half-Lives of Curcumin Degradation. All UV−visible absorption measurements were carried out in solutions made up in 50 mM phosphate buffer solutions at pH 7.4, and temperature was kept at 37 °C. The concentrations of PAAC18 were 0.3, 1.0, and 2.0 wt %, those of PAAC12 were 1.0 and 2.9 wt %, and that of 10%-PAAC12 was 0.5 wt %. Solutions of 10%-PAAC18 became cloudy as a result of precipitation and were not further studied. For the experiments in which the curcumin half-lives, $t_{1/2}$, were determined, the samples were prepared by adding ~4 μL of curcumin stock (5.4 mM in methanol) to either 700 μL of buffer solution alone or solutions of PAAC18, PAAC12, or 10%-PAAC12 to give a curcumin concentration of 30 μL. Absorption spectra were acquired from these solutions in 0.2 cm quartz cells thermostated at 37 °C using a Varian Cary 5000 UV−vis/NIR spectrophotometer. In the experiments where degradation of curcumin occurred in phosphate buffer solution alone or in the presence of 1.0 wt % PAAC12, the UV−visible absorption spectra were collected over 30 min at 1 min intervals in the wavelength range of 300−700 nm. For the experiments where degradation of curcumin was much slower in the presence of PAAC18 (at 0.3, 1.0, or 2.0 wt %), 10%-PAAC12 (at 0.5 wt %) and PAAC12 (at 2.9 wt %), the UV−visible absorption spectra were collected over 18 h at 60 min intervals in the wavelength range of 300−700 nm. In addition, evolution of absorbance around 350 nm, which is attributed to generation of curcumin degradation products was analyzed by normalizing it to the peak absorbance around 430 nm to show the release kinetics of curcumin from PAAC18, PAAC12, and 10%-PAAC12 (Figure S2, Supporting Information).[21−23]

Binding Constant of the PAAC18-Curcumin Complex. The binding constant, K_n, for the PAAC18-curcumin complex, $(C18)_n$-Cur, was determined from the UV−visible absorbance changes (eq 1 below), which occurred on addition of aliquots in the range of 2−5 μL of the 2.5-mM curcumin stock solution in DMSO in a stepwise fashion to 700 μL of a 0.3 wt % PAAC18 aqueous solution in three separate titrations, in which curcumin concentrations ranged from 8 μM to 248 μM in 50 mM phosphate buffer at pH 7.4, at 37 °C. Thus, the DMSO concentration was kept to <10% v/v in the PAAC18 solution to minimize any effect of DMSO on the binding of curcumin with the octadecyl substituents of PAAC18, for each titration. Each titration was carried out in a 0.2 cm path-length quartz cell over 45 min during which time curcumin degradation was insignificant and no precipitation of either curcumin or

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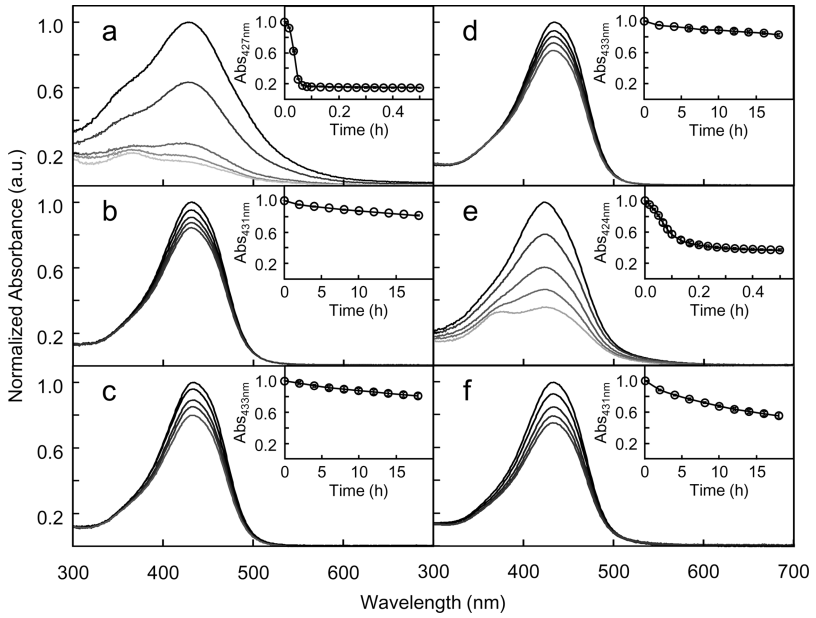

Figure 2. Time-dependent UV−visible absorption spectra of 30 μM curcumin in (a) pH 7.4 phosphate buffer solution, those in PAAC18 at (b) 0.3 wt %, (c) 1.0 wt % and (d) 2.0 wt %, and those in (e) PAAC12 at 1.0 wt % and (f) 10%-PAAC12 at 0.5 wt %, at 37 °C. Spectra were recorded for 0.5 h in the panels (a) and (e) and 18 h in the panels (b−d) and (f). The insets show the decays of the absorption maxima due to degradation of curcumin, which are fitted to a mathematical function to estimate the values of half-lives. A 0.5 wt % solution of 10%-PAAC12 possesses the equivalent number of substituent carbons to 1.0 wt % PAAC18. Note that only selected data are shown for clarity purposes.

PAAC18 occurred. The binding constants were determined by best-fitting an algorithm derived through eqs 2−6 (discussed below) for the variation of molar absorbance at 0.5 nm intervals over the wavelength range 400−500 nm with the ratio of the total octadecyl substituent concentration of PAAC18 to the total curcumin concentration, $[C18]_n/[Cur]$, as the average number of octadecyl substituents binding to each curcumin, n, was varied from 1 to 10 using a nonlinear least-squares program (HypSpec).[50,51]

2D NOESY ^{1}H NMR Spectra of Curcumin in PAAC18 and 10%-PAAC12. The 2D NOESY ^{1}H NMR spectra were recorded with a Varian-Inova 600 spectrometer operating at 599.602 MHz using a standard pulse sequence with a mixing time of 300 ms and acquisition time of 150 ms with 8 repetitions. The first sample was prepared by adding 6.4 μL of a 40 mg/mL stock solution of curcumin in DMSO-d_6 to 700 μL of a 1.0 wt % solution of PAAC18 in D_2O to achieve a curcumin concentration of 1.0 mM, which resulted in an approximately 1:3 molar ratio of curcumin to the octadecyl substituents of PAAC18. It is noted that the curcumin concentration in the NMR study is about 30 times higher than that used in the UV−visible absorption study. The second sample was prepared by adding 6.4 μL of a 80 mg/mL stock solution of curcumin in DMSO-d_6 to 700 μL of a 1.0 wt % solution of 10%-PAAC12 in D_2O, which resulted in an approximately 1:7 molar ratio of curcumin to the dodecyl substituents of 10%-PAAC12. The rate of degradation of curcumin in the presence of PAAC12 was too rapid for spectra of adequate reliability to be collected.

3D Molecular Illustration and Molecular Size Estimation of Curcumin in PAAC18 and PAAC12. The chemical structures of curcumin, PAAC18 and PAAC12 were energy-minimized and illustrated using CS Chem3D Ultra MM2 protocol.[52] The 3D molecular illustration of curcumin in PAAC18 is shown in the table of content graphic. The size of curcumin was calculated using the distance between methoxy carbon atoms. Furthermore, assuming that the octadecyl substituent is present as a straight chain, its length was calculated using the distance between the terminal carbon atoms. Similarly, the length of a straight dodecyl substituent was calculated.

Dynamic Light Scattering and Zeta Potential Measurements on PAAC18, PAAC12, and 10%-PAAC12. Hydrodynamic diameters and zeta potentials of PAAC18, PAAC12, and 10%-PAAC12 were determined using a Malvern ZetaSizer Nano S. The instrument settings were automatically set by Malvern dispersion technology software with a 633 nm laser. Hydrodynamic diameter and zeta potential measurements were made on degassed aqueous solutions of either 0.3 and 1.0 wt % PAAC18, 1.0 wt % PAAC12, and 0.5 wt % 10%-PAAC12 alone or in the presence of 30 μM curcumin in low volume disposable cuvettes. Zeta potential measurements were made on these solutions alone in disposable zeta potential cuvettes (DTS1060). The ionic strength varied over the range of approximately 30 mM to 60 mM, depending upon the weight percent of the polyacrylate solution. The refractive index (RI) of each polyacrylate solution was assumed to be similar to that of a sodium dodecyl sulfate (SDS) micellar solution (material RI = 0.11 and water dispersant RI = 1.33) due to their structural similarities. The width of a distribution for hydrodynamic diameter is represented as a standard deviation (SD). Hydrodynamic diameter measurements for a 2.0 wt % PAAC18 solution showed inconsistent results due to a combination of the instrument hydrodynamic diameter detection limit of 6 μm and its unsuitability for measurements of viscous solutions.

RESULTS AND DISCUSSION

Degradation of Curcumin in Phosphate Buffer and Stabilization Effects of Hydrophobically Modified Polyacrylates. High levels of the aqueous stability of curcumin are important to its availability *in vivo*. The rapid changes in the absorption spectra of curcumin in a pH 7.4 phosphate buffer

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Table 1. Modified Polyacrylate Concentrations, Half-Life ($t_{1/2}$) of Curcumin Degradation, Hydrodynamic Diameters and Zeta Potential of Micelle-Like Aggregates and Fluid Hydrogels of PAAC18, PAAC12, and 10%-PAAC12

polyacrylate	[curcumin] (μM)	$t_{1/2}$ (h)[a]	diameter ± SD (μm)[b,c]	zeta potential (mV)[c]
–	30	0.04 ± 0.01	–	–
0.3 wt % PAAC18	–	–	0.86 ± 0.14	−70 ± 6
0.3 wt % PAAC18	30	71.6 ± 4.0	0.98 ± 0.22	–
1.0 wt % PAAC18	–	–	1.12 ± 0.17	−73 ± 5
1.0 wt % PAAC18	30	74.0 ± 9.4	1.15 ± 0.22	–
2.0 wt % PAAC18	–	–	>6[d]	–
2.0 wt % PAAC18	30	98.6 ± 16.2	>6[d]	–
1.0 wt % PAAC12	–	–	1.40 ± 0.29	−70 ± 7, −35 ± 5
1.0 wt % PAAC12	30	0.07 ± 0.02	1.35 ± 0.38	–
0.5 wt % 10%-PAAC12	–	–	1.42 ± 0.44	−64 ± 6
0.5 wt % 10%-PAAC12	30	22.3 ± 4.0	2.26 ± 0.92	–

[a]In aqueous 50 mM phosphate buffer at pH 7.4 and 37 °C. [b]Mean hydrodynamic diameter and standard deviation (SD) of a distribution. [c]At 25 °C (ionic strength of 30−60 mM). [d]Meaningful data was not obtained for 2.0 wt % PAAC18 solutions due to the instrument detection limit of 6 μm and its unsuitability for use with viscous samples.

solution at 37 °C over 30 min are shown in Figure 2a. The decay at the maximum absorbance at 427 nm with a half-life of 0.04 ± 0.01 h (Table 1) is shown in the inset of Figure 2a. This result, together with the growth of the absorbance around 350 nm, is consistent with the degradation of curcumin.[21−23] Previous studies have shown that degradation of curcumin by hydrolysis and fragmentation led to possible formation of *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal (*half*-curcumin), ferulic acid, feruloyl methane, and vanillin.[23,53]

The time-dependent UV−visible absorption spectra of curcumin in 0.3 wt % PAAC18 over 18 h are shown in Figure 2b. The shape of the initial absorption spectrum is similar to that of curcumin in a SDS micellar solution and polar organic solvents including methanol and DMSO,[25,28] which suggests a similarity between the hydrophobic microenvironments in these solvents and that of PAAC18. Suppression of rapid curcumin degradation was characterized by an estimated half-life of 71.6 ± 4.0 h in the 0.3 wt % PAAC18 solution, which is ≥1600 times longer than that in the absence of PAAC18 (0.04 ± 0.01 h, Table 1). The time-dependent UV−visible absorption spectra of curcumin in 1.0 and 2.0 wt % PAAC18 solutions are shown in Figures 2c and 2d, respectively. Curcumin degradation is also decreased in these hydrogels as shown by estimated half-lives of 74.0 ± 9.2 h and 98.6 ± 16.2 h, respectively, which are 1700−2000 times longer than that observed in the absence of PAAC18 (0.04 ± 0.01 h, Table 1). It is interesting that the degradation of curcumin in the presence of PAAC18 also contains useful information regarding the release of curcumin from these aggregates and hydrogels. We argue that degradation occurs when curcumin is released from PAAC18 to the aqueous environment owing to hydrolysis and fragmentation, as mentioned above. As a consequence, the rate of curcumin release can be inferred from the rate of curcumin degradation. The results are shown in the Supporting Information (Figure S2).

To gain insight into the effect of the alkyl substituent length on the stabilization of curcumin degradation, the 3% dodecyl substituted polyacrylate, PAAC12, was also investigated. A previous study has shown that the viscosities of PAAC12 aqueous solutions at 1.0 wt % (∼2 cP) and 2.9 wt % (∼8 cP) are similar to those of PAAC18 solutions at 0.5 and 1.0 wt %, respectively,[49] which infers that similar levels of cross-linking between polyacrylate strands are present. Thus, PAAC12 at these concentrations should exhibit similar abilities to suppress curcumin degradation. However, the time-dependent UV−visible absorption spectra showed that solutions of PAAC12 at 1.0 and 2.9 wt % are ineffective in inhibiting curcumin degradation, as shown in Figure 2e (0.07 ± 0.01 h, Table 1) and Figure S1 in Supporting Information. These results indicate that the alkyl substituent must be sufficiently long to capture curcumin to suppress its degradation. The effect of 10%-PAAC12 solution at 0.5 wt % was also investigated to give insight into the effect of the degree of polyacrylate substitution on the aqueous stabilization of curcumin. At this concentration, 10%-PAAC12 possesses the same number of substituent carbons as does PAAC18 solution at 1.0 wt %. The time-dependent UV−visible absorption spectra of curcumin in 0.5 wt % 10%-PAAC12 solution show that the rate of curcumin degradation is slowed (Figure 2f), which is consistent with a considerable increase of its half-life (22.3 ± 4.0 h, Table 1). However, this suppression of curcumin degradation is less than that achieved in 1.0 wt % PAAC18 solution (Figure 2c, Table 1) despite the equivalent number of substituent alkyl carbons. This result indicates that the length of the substituent has a more substantial effect on the aqueous stability of curcumin than the effect of the extent of polyacrylate substitution or the concentration of the polyacrylate. Similarly, the rate of release of curcumin from PAAC12 can be inferred from the rate of degradation, and the results are shown in Figure S2 in the Supporting Information. Overall, the results show the presence of strong interactions between curcumin and the octadecyl substituents of PAAC18.

Binding Constant of the PAAC18-Curcumin Complex. A systematic variation in the molar absorbance maximum of curcumin, ε, and its wavelength, λ, occur as a curcumin stock solution is titrated into an aqueous PAAC18 solution at pH 7.4 and 37 °C (Figure 3a), consistent with the binding of curcumin by the octadecyl substituents of PAAC18, as follows. This binding may be represented by eq 1 where a group of n octadecyl substituents of PAAC18, $(C18)_n$, binds to a single curcumin molecule, Cur, to form a complex, $(C18)_n$-Cur:

$$(C18)_n + \text{Cur} \rightleftharpoons (C18)_n\text{-Cur} \quad (1)$$

The binding constant, K_n, at equilibrium is given by

$$K_n = \frac{[(C18)_n\text{-Cur}]}{[(C18)_n][\text{Cur}]} \quad (2)$$

Given that $[(C18)_n]_0$ and $[\text{Cur}]_0$ are the initial concentrations:

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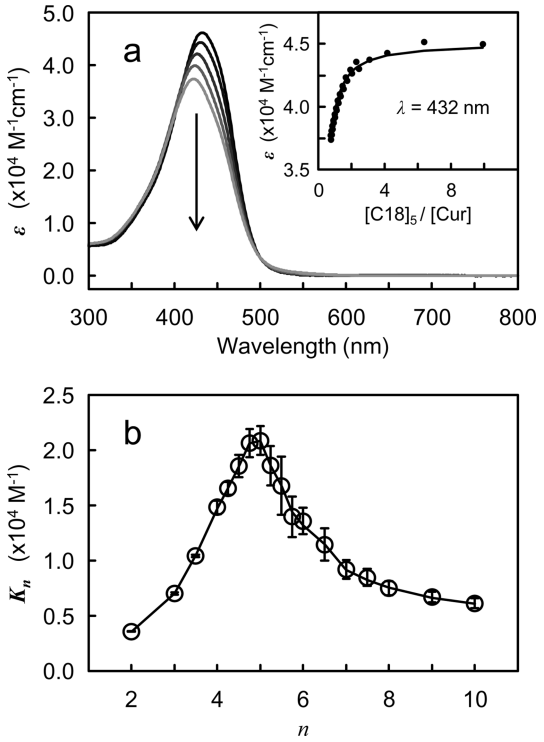

Figure 3. (a) Molar absorptivity (ε) as a function of λ for curcumin in titration studies in pH 7.4 phosphate buffer at 37 °C, where the arrow shows that direction of change in λ_{max} and ε with a decrease in $[C18]_5/[Cur]$ ratio. The inset shows the variation of ε with $[C18]_5/[Cur]$ ratio at 432 nm and the solid line represents the best fit of the algorithm obtained in the range 400−500 nm. (b) Binding constants (K_n) of the $(C18)_n$-curcumin complex as a function of n. Note that only selected data are shown for clarity purposes.

$$[Cur]_0 = [(C18)_n\text{-Cur}] + [Cur] \quad (3)$$

$$[(C18)_n]_0 = [(C18)_n\text{-Cur}] + [(C18)_n] \quad (4)$$

It follows that the molar absorbance at a particular wavelength, $A(\lambda)$, is given by

$$A(\lambda) = \varepsilon(\lambda)\cdot l\cdot[Cur]_0 \quad (5)$$

$$= \varepsilon_{Cur}(\lambda)\cdot l\cdot[Cur] + \varepsilon_{(C18)_n\text{-Cur}}(\lambda)\cdot l\cdot[(C18)_n\text{-Cur}] \quad (6)$$

where ε, ε_{Cur} and $\varepsilon_{(C18)_n\text{-Cur}}$ are the observed molar absorptivity and the molar absorptivities of Cur and $(C18)_n$-Cur, respectively. The magnitude of K_n was determined by best-fitting an algorithm derived through eqs 2−6 to the variation of the molar absorbance, A, at 0.5 nm intervals over the range 400−500 nm with the ratio $[(C18)_n]/[Cur]$ for n = 1−10.

The number of substituents involved in complexation may vary with the total [C18] such that complexations of different stoichiometries arise, but it is not possible to carry out a calculation to determine the exact nature of this variation. Thus, several calculations were carried out for the equilibria shown in eqs 1 and 2 where a group of n octadecyl substituents for the complex $(C18)_n$-Cur was varied in the range of $1 \leq n \leq 10$, using the quantities in eqs 3 and 4 to derive an algorithm based on eqs 5 and 6 to best fit to the absorbance data. The variations of λ_{max} and ε_{max} as increasing the total curcumin concentrations are shown in Figure 3a. The variations indicate changes in the complexation of curcumin by the octadecyl substituents of PAAC18. A satisfactory fit could not be obtained for the binding of single octadecyl substituent and single curcumin (n = 1), but good fits were obtained for n = 2−10 for which K_n are plotted in Figure 3b. (The binding constants, K_n for other n values appear in Supporting Information, Table S1.) The largest derived binding constant of $(2.1 \pm 0.1) \times 10^{-4}$ M^{-1} corresponds to n = 5, which implies that the most favorable binding involves a single curcumin and five octadecyl substituents of PAAC18 on average. It is probable that there is a small variation of n around n = 5 in a dynamic equilibrium, but the binding model used here does not allow an exploration of this. The binding constant of $(C18)_5$-curcumin complex is comparable to those of curcumin in micellar solutions.[24,27] Moreover, analysis of the results revealed that the molar absorptivity of $(C18)_n$-curcumin complex is 4.47×10^4 M^{-1} cm^{-1} at λ_{max} = 432 nm, while curcumin alone in buffer possesses an ε_{Cur} of 2.20×10^{-4} M^{-1} cm^{-1} at λ_{max} = 427 nm.

2D NOESY ^{1}H NMR Study of PAAC18-Curcumin Complex. The 2D NOESY ^{1}H NMR spectrum of a 1.0-mM solution of curcumin in a 1.0 wt % solution of PAAC18 in D_2O, shown in Figure 4, provides evidence for curcumin binding by

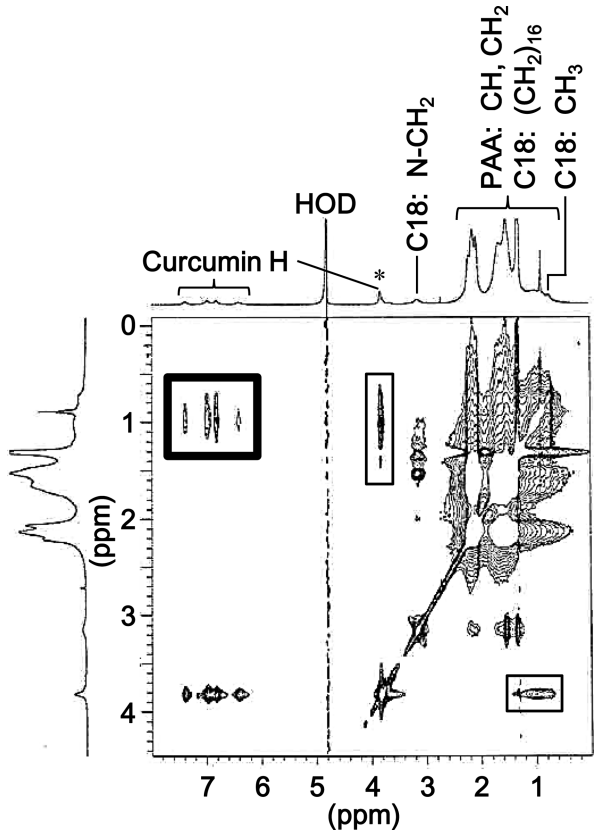

Figure 4. 2D NOESY ^{1}H NMR spectrum of 1.0-mM curcumin in a 1.0 wt % PAAC18 solution in D_2O at pD 7. The cross-peaks arising from the interactions between the octadecyl substituent protons and the curcumin CH and CH_3O protons are enclosed in bold and plain rectangles, respectively. The asterisk indicates the curcumin CH_3O resonance.

the octadecyl substituents of PAAC18. The ^{1}H resonances arising from the curcumin CH protons appear in the range 6.3−7.4 ppm and those of methoxy (OCH_3) protons appear at 3.8 ppm in this solution. These proton peaks are broadened and show upfield shifts by comparison with curcumin alone in either deuterated chloroform or DMSO-d_6 solutions (Support-

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ing Information, Table S2).[54,55] Within the PAAC18 spectrum, the polyacrylate backbone CH and CH_2 resonances appear in the range 1.3−2.4 ppm, while the octadecyl substituent CH_3, CH_2 and $N-CH_2$ resonances appear at ∼0.6, ∼1.5 and 3.1 ppm, respectively. Strong cross-peaks arise from the through-space interactions of the CH protons of curcumin with the CH_2 and CH_3 protons of the octadecyl substituents of PAAC18 (enclosed in a bold rectangle) and those from interactions between the OCH_3 protons of curcumin and those of the octadecyl substituents of PAAC18 (enclosed in plain rectangles). These results indicate an interaction distance of ≤400 pm between these protons. However, no cross-peaks between curcumin and either the octadecyl $N-CH_2$ protons or the polyacrylate backbone CH and CH_2 protons were observed, which is consistent with the dominant interaction between the octadecyl substituents and curcumin. The 2D NOESY ^1H NMR spectrum of a slightly viscous sample of 2.0-mM curcumin in 1.0 wt % 10%-PAAC12 solution shows substantial broadening of the proton resonances of both curcumin and 10%-PAAC12 (Supporting Information, Figure S3). This result is consistent with decreased transverse proton relaxation times arising from a lengthened tumbling time and a consequently decreased effectiveness of through-space dipolar interactions, resulting in the absence of significant cross-peaks (Supporting Information, Figure S3). These observations are consistent with the effectiveness of the suppression of curcumin degradation through binding by PAAC18, PAAC12 and 10%-PAAC12 being substantially dependent on the relative lengths of the octadecyl and dodecyl substituents and curcumin, which are approximately 2160, 1400, and 1920 pm, respectively. Although increasing the local concentration of the dodecyl substituent in 10%-PAAC12 offsets this disadvantage of the shorter substituent to some extent, curcumin is more likely to be fully encapsulated in hydrophobic domains of octadecyl substituent aggregates formed by PAAC18 rather than those of dodecyl substituent aggregates formed by PAAC12.

Hydrodynamic Diameters of PAAC18, PAAC12, and 10%-PAAC12 Micelle-Like Aggregates. It was suggested earlier that PAAC18 and PAAC12 exist as micelle-like aggregates at low concentrations in aqueous solutions due to the presence of intrapolyacrylate cross-links formed by association between either octadecyl or dodecyl substituents.[48,49] In this study, hydrodynamic diameters and zeta potentials of PAAC18, PAAC12, and 10%-PAAC12 provide insight into the formation of micelle-like aggregates and their differing abilities to suppress the degradation of curcumin (Table 1). The 0.3 wt % solution of PAAC18 forms aggregates with a mean hydrodynamic diameter of 0.86 μm (Figure 5a). The incorporation of curcumin causes a small change in the hydrodynamic diameter of the micelle-like aggregates to 0.98 μm and an increase in the width of the distribution (as its standard deviation) from 0.14 to 0.22 μm, as shown in Figure 5b. Similarly, Figures 5c and 5d show that the mean diameter of the PAAC18 micelle-like aggregates is 1.12 μm at 1.0 wt % and that the addition of curcumin causes a negligible change (Table 1). These results suggest that the increased concentration of PAAC18 allows more extensive cross-linking among octadecyl substituents and an increase in aggregate size. Thus, the octadecyl intrastrand associations of PAAC18 are sufficiently strong to maintain the micelle-like structure of the hydrophobic substituents in the presence of curcumin. A more viscous solution of 2.0 wt % PAAC18, however, produced inconsistent size distributions (data not shown), probably due to a high level

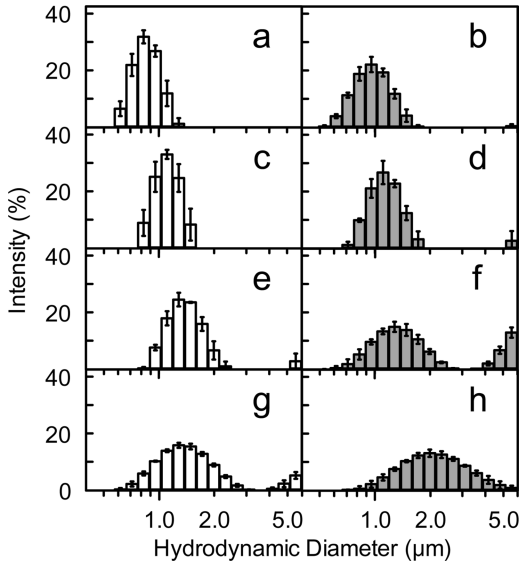

Figure 5. Hydrodynamic diameter distributions of (a−b) 0.3 wt % PAAC18, (c−d) 1.0 wt % PAAC18, (e−f) 1.0 wt % PAAC12, and (g−h) 0.5 wt % 10%-PAAC12 aqueous solutions, in the absence (unfilled) and presence (filled) of 30 μM curcumin.

of interstrand cross-linking resulting in large aggregates with hydrodynamic diameter over the detection limit of 6 μm, consistent with the formation of a hydrogel network.

Figure 5e shows that a solution of PAAC12 at 1.0 wt % has a mean hydrodynamic diameter of 1.40 μm, which is larger than those of PAAC18 at 0.3 and 1.0 wt %. This is consistent with the shorter dodecyl substituents forming looser aggregates than do the octadecyl substituents. The incorporation of curcumin increases the width of the distribution from 0.29 to 0.38 μm for the smaller aggregate and causes the formation of larger aggregates, as shown in Figure 5f. The overall effect may be that curcumin is more exposed to water, which is consistent with the rapid degradation of curcumin observed under these conditions (Figure 2e). Figures 5g and 5h show that the mean hydrodynamic diameter of the 10%-PAAC12 micelle-like aggregates is 1.42 μm at 0.5 wt % and the presence of curcumin significantly shifts the mean hydrodynamic diameter to 2.26 μm, with a corresponding change in the distribution width from 0.44 to 0.92 μm. Thus, addition of curcumin appears to reduce dodecyl intrastrand polyacrylate associations of 10%-PAAC12, resulting in the wider hydrodynamic diameter distribution of the micelle-like aggregates. Nevertheless, the degradation of curcumin is greatly slowed ($t_{1/2}$ = 22.3 h) by comparison with that in PAAC12 at 1.0 wt % ($t_{1/2}$ = 0.07 h) as a consequence of the greater concentration of dodecyl substituents in 10%-PAAC12. These results suggest that the reduced intrastrand associations of dodecyl hydrophobic substituents of PAAC12 and 10%-PAAC12 are related to their lesser ability to suppress curcumin degradation by comparison with PAAC18. It has been shown in previous studies that SDS and dodecyl trimethylammonium bromide (DTAB) micelles are effective in stabilizing curcumin.[25,28] However, in this study, PAAC12 exhibits no stabilizing effects even though it also has dodecyl groups. It should be noted that the number of dodecyl groups per curcumin in a SDS or DTAB micelle is >60.[56] Although a similar ratio of dodecyl groups to curcumin is found in 1.0 wt % PAAC12, it is clear that not all dodecyl groups are involved in binding of curcumin due to the

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large aggregate size, as shown in Figures 5e and 5f. It is highly likely that the available dodecyl groups for curcumin binding is significantly less than 60 in 1.0 wt % PAAC12, which contributes to its inability to stabilize curcumin. From these observations, it is clear that the greater ability of the octadecyl substituents to aggregate is the origin of PAAC18 being more effective in suppressing curcumin degradation than PAAC12 and 10%-PAAC12. The smaller hydrodynamic diameters of the micelle-like aggregates of PAAC18 than those of PAAC12 are attributed to the formation of more strongly bound aggregates, which is consistent with the significant decrease in the curcumin degradation rate in the presence of PAAC18 (Figures 2b–2d).

Zeta Potentials of PAAC18, PAAC12, and 10%-PAAC12 Micelle-Like Aggregates. The zeta potentials determined for the PAAC18, PAAC12, and 10%-PAAC12 systems, which are attributable to the negatively charged polyacrylate carboxylate groups, are shown in Figure 6. The

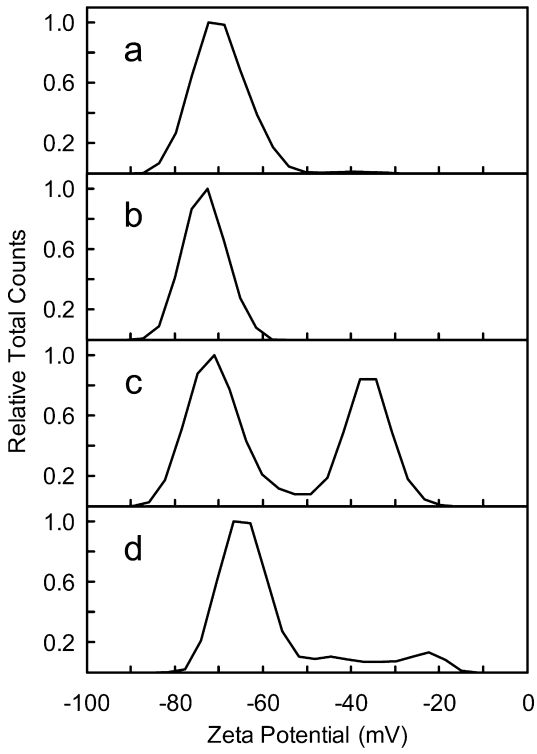

Figure 6. Zeta potential measurements of aqueous solutions of PAAC18 at (a) 0.3 wt % and (b) 1.0 wt %, (c) PAAC12 at 1.0 wt %, and (d) 10%-PAAC12 at 0.5 wt %.

high zeta potential values of PAAC18 at 0.3 and 1.0 wt % of −70 ± 6 mV and −73 ± 5 mV, respectively (Table 1 and Figures 6a–6b), are consistent with the formation of stable colloidal micelle-like aggregates as indicated by no precipitation occurring over 30 days. This is similar to the behavior of other highly stable colloidal systems of similar zeta potentials.[57] Two zeta potentials of −70 ± 7 mV and −35 ± 5 mV were determined for a PAAC12 solution at 1.0 wt % (Figure 6c). The main zeta potential peak of 10%-PAAC12 is located at −64 ± 6 mV, and minor broad peak arises around −40 mV (Figure 6d). The zeta potential values of −70 mV and −64 mV for PAAC12 and 10%-PAAC12, respectively, correspond to the micelle-like aggregates, while the smaller zeta potential peaks may be attributable to the presence of nonaggregated substituents of PAAC12 and 10%-PAAC12, which are consistent with their distributions of hydrodynamic diameters (Figures 5e and 5g). The much greater nonaggregated substituent component for PAAC12 is probably related to its inability to suppress curcumin degradation as shown in Figure 2e.

CONCLUSIONS

This study highlights the potential applications of curcumin micelle-like aggregates and hydrogels formed using hydrophobically modified polyacrylates as model curcumin delivery systems. The capture of the medicinal pigment curcumin by the hydrophobically modified polyacrylates PAAC18 and 10%-PAAC12 helps identify some of the factors that stabilize curcumin in aqueous solution. The length of the octadecyl substituent of PAAC18 and its aggregation with curcumin in both micelle-like aggregates and hydrogels show a remarkable combined ability to suppress its degradation, as indicated by an increase in the curcumin half-life by a factor of 1600–2000. At the molecular level, results from 2D NOESY ^{1}H NMR spectroscopy indicate that the association of curcumin with octadecyl substituents of PAAC18 occurs through hydrophobic interactions, where those from UV–visible absorption titration show that it occurs dominantly through five octadecyl substituents binding curcumin with a $K_5 = (2.1 \pm 0.1) \times 10^4$ M^{-1}. These molecular interactions underpin the macroscopic observations of hydrodynamic diameters and zeta potentials, which indicate the formation of micelle-like aggregates and hydrogels of PAAC18. The shorter dodecyl substituent of PAAC12 is ineffective in suppressing curcumin degradation. While the more highly substituted 10%-PAAC12 shows an improved stabilization of curcumin, it is less effective than PAAC18. In conclusion, the octadecyl hydrophobic substituents of PAAC18 possess a significant ability to stabilize curcumin in both micelle-like aggregates and hydrogels under physiological conditions.

ASSOCIATED CONTENT

Supporting Information

The time-dependent UV–visible absorption spectra of curcumin in 2.9 wt % PAAC12 over 18 h at 37 °C, release kinetics of curcumin from PAAC18, PAAC12 and 10% PAAC12, binding constants, K_n for $n = 1–10$, ^{1}H NMR signal assignments of curcumin in chloroform and DMSO-d_6,[54,55] and 2D NOESY ^{1}H NMR spectrum of curcumin in 10%-PAAC12 in D_2O. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Leung, M. H. M.; Harada, T.; Kee, T. W. Delivery of Curcumin and Medicinal Effects of the Copper(II)−Curcumin Complexes. *Curr. Pharm. Des.* **2013**, *19*, 2070−2083.
(2) Sa, G.; Das, T. Anti-Cancer Effects of Curcumin: Cycle of Life and Death. *Cell Div.* **2008**, *3*, 1−14.
(3) Anand, P.; Thomas, S. G.; Kunnumakkara, A. B.; Sundaram, C.; Harikumar, K. B.; Sung, B.; Tharakan, S. T.; Misra, K.; Priyadarsini, I. K.; Rajasekharan, K. N.; et al. Biological Activities of Curcumin and Its Analogues (Congeners) Made by Man and Mother Nature. *Biochem. Pharmacol.* **2008**, *76*, 1590−1611.
(4) Hatcher, H.; Planalp, R.; Cho, J.; Tortia, F. M.; Torti, S. V. Curcumin: From Ancient Medicine to Current Clinical Trials. *Cell. Mol. Life Sci.* **2008**, *65*, 1631−1652.
(5) Huang, M. T.; Lou, Y. R.; Ma, W.; Newmark, H. L.; Reuhl, K. R.; Conney, A. H. Inhibitory Effects of Dietary Curcumin on Forestomach, Duodenal, and Colon Carcinogenesis in Mice. *Cancer Res.* **1994**, *54*, 5841−5847.
(6) Rao, C. V.; Rivenson, A.; Simi, B.; Reddy, B. S. Chemoprevention of Colon Cancer by Dietary Curcumin. *Ann. N. Y. Acad. Sci.* **1995**, *768*, 201−204.
(7) Sharma, O. P. Antioxidant Activity of Curcumin and Related Compounds. *Biochem. Pharmacol.* **1976**, *25*, 1811−1812.
(8) Aggarwal, B. B.; Kumar, A.; Bharti, A. C. Anticancer Potential of Curcumin: Preclinical and Clinical Studies. *Anticancer Res.* **2003**, *23*, 363−398.
(9) Lantz, R. C.; Chen, G. J.; Solyom, A. M.; Jolad, S. D.; Timmermann, B. N. The Effect of Turmeric Extracts on Inflammatory Mediator Production. *Phytomedicine* **2005**, *12*, 445−452.
(10) Lim, G. P.; Chu, T.; Yang, F. S.; Beech, W.; Frautschy, S. A.; Cole, G. M. The Curry Spice Curcumin Reduces Oxidative Damage and Amyloid Pathology in an Alzheimer Transgenic Mouse. *J. Neurosci.* **2001**, *21*, 8370−8377.
(11) Ono, K.; Hasegawa, K.; Naiki, H.; Yamada, M. Curcumin has Potent Anti-Amyloidogenic Effects for Alzheimer's β-Amyloid Fibrils in Vitro. *J. Neurosci. Res.* **2004**, *75*, 742−750.
(12) Yang, F. S.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; et al. Curcumin Inhibits Formation of Amyloid β Oligomers and Fibrils, Binds Plaques, and Reduces Amyloid in Vivo. *J. Biol. Chem.* **2005**, *280*, 5892−5901.
(13) Cartiera, M. S.; Ferreira, E. C.; Caputo, C.; Egan, M. E.; Caplan, M. J.; Saltzman, W. M. Partial Correction of Cystic Fibrosis Defects with PLGA Nanoparticles Encapsulating Curcumin. *Mol. Pharmaceutics* **2010**, *7*, 86−93.
(14) Egan, M. E.; Pearson, M.; Weiner, S. A.; Rajendran, V.; Rubin, D.; Glockner-Pagel, J.; Canny, S.; Du, K.; Lukacs, G. L.; Caplan, M. J. Curcumin, a Major Constituent of Turmeric, Corrects Cystic Fibrosis Defects. *Science* **2004**, *304*, 600−602.
(15) Cheng, A. L.; Hsu, C. H.; Lin, J. K.; Hsu, M. M.; Ho, Y. F.; Shen, T. S.; Ko, J. Y.; Lin, J. T.; Lin, B. R.; Wu, M. S.; et al. Phase I Clinical Trial of Curcumin, a Chemopreventive Agent, in Patients with High-Risk or Pre-Malignant Lesions. *Anticancer Res.* **2001**, *21*, 2895−2900.
(16) Sharma, R. A.; Euden, S. A.; Platton, S. L.; Cooke, D. N.; Shafayat, A.; Hewitt, H. R.; Marczylo, T. H.; Morgan, B.; Hemingway, D.; Plummer, S. M.; et al. Phase I Clinical Trial of Oral Curcumin: Biomarkers of Systemic Activity and Compliance. *Clin. Cancer Res.* **2004**, *10*, 6847−6854.
(17) ClinicalTrials.gov, Trial of Curcumin in Advanced Pancreatic Cancer. 2004; http://clinicaltrials.gov/ct2/show/NCT00094445.
(18) ClinicalTrials.gov, Curcumin in Patients with Mild to Moderate Alzheimer's Disease. 2009; http://clinicaltrials.gov/ct2/show/NCT00099710.
(19) Kaminaga, Y.; Nagatsu, A.; Akiyama, T.; Sugimoto, N.; Yamazaki, T.; Maitani, T.; Mizukami, H. Production of Unnatural Glucosides of Curcumin with Drastically Enhanced Water Solubility by Cell Suspension Cultures of Catharanthus Roseus. *FEBS Lett.* **2003**, *555*, 311−316.
(20) Letchford, K.; Liggins, R.; Burt, H. Solubilization of Hydrophobic Drugs by Methoxy Poly(ethylene glycol)-*block*-Polycaprolactone Diblock Copolymer Micelles: Theoretical and Experimental Data and Correlations. *J. Pharm. Sci.* **2008**, *97*, 1179−1190.
(21) Harada, T.; Pham, D.-T.; Leung, M. H. M.; Ngo, H. T.; Lincoln, S. F.; Easton, C. J.; Kee, T. W. Cooperative Binding and Stabilization of the Medicinal Pigment Curcumin by Diamide-Linked Cyclodextrin Dimers: A Spectroscopic Characterization. *J. Phys. Chem. B* **2011**, *115*, 1268−1274.
(22) Leung, M. H. M.; Kee, T. W. Effective Stabilization of Curcumin by Association to Plasma Proteins: Human Serum Albumin and Fibrinogen. *Langmuir* **2009**, *25*, 5773−5777.
(23) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. Stability of Curcumin in Buffer Solutions and Characterization of Its Degradation Products. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867−1876.
(24) Iwunze, M. O. Binding and Distribution Characteristics of Curcumin Solubilized in CTAB Micelle. *J. Mol. Liq.* **2004**, *111*, 161−165.
(25) Leung, M. H. M.; Colangelo, H.; Kee, T. W. Encapsulation of Curcumin in Cationic Micelles Suppresses Alkaline Hydrolysis. *Langmuir* **2008**, *24*, 5672−5675.
(26) Tønnesen, H. H. Solubility, Chemical and Photochemical Stability of Curcumin in Surfactant Solutions - Studies of Curcumin and Curcuminolds, XXVIII. *Pharmazie* **2002**, *57*, 820−824.
(27) Sahu, A.; Kasoju, N.; Bora, U. Fluorescence Study of the Curcumin−Casein Micelle Complexation and Its Application as a Drug Nanocarrier to Cancer Cells. *Biomacromolecules* **2008**, *9*, 2905−2912.
(28) Wang, Z. F.; Leung, M. H. M.; Kee, T. W.; English, D. S. The Role of Charge in the Surfactant-Assisted Stabilization of the Natural Product Curcumin. *Langmuir* **2010**, *26*, 5520−5526.
(29) Barik, A.; Mishra, B.; Kunwar, A.; Priyadarsini, K. I. Interaction of Curcumin with Human Serum Albumin: Thermodynamic Properties, Fluorescence Energy Transfer and Denaturation Effects. *Chem. Phys. Lett.* **2007**, *436*, 239−243.
(30) Barik, A.; Priyadarsini, K. I.; Mohan, H. Photophysical Studies on Binding of Curcumin to Bovine Serum Albumin. *Photochem. Photobiol.* **2003**, *77*, 597−603.
(31) Baglole, K. N.; Boland, P. G.; Wagner, B. D. Fluorescence Enhancement of Curcumin upon Inclusion into Parent and Modified Cyclodextrins. *J. Photochem. Photobiol. A - Chem.* **2005**, *173*, 230−237.
(32) Singh, R.; Tønnesen, H. H.; Vogensen, S. B.; Loftsson, T.; Másson, M. Studies of Curcumin and Curcuminoids. XXXVI. The Stoichiometry and Complexation Constants of Cyclodextrin Complexes as Determined by the Phase-Solubility Method and UV−Vis Titration. *J. Inclusion Phenom. Macrocyclic Chem.* **2010**, *66*, 335−348.
(33) Mohanty, C.; Sahoo, S. K. The in Vitro Stability and in Vivo Pharmacokinetics of Curcumin Prepared as an Aqueous Nanoparticulate Formulation. *Biomaterials* **2010**, *31*, 6597−6611.
(34) Shaikh, J.; Ankola, D. D.; Beniwal, V.; Singh, D.; Kumar, M. Nanoparticle Encapsulation Improves Oral Bioavailability of Curcumin by at Least 9-Fold When Compared to Curcumin Administered with Piperine as Absorption Enhancer. *Eur. J. Pharm. Sci.* **2009**, *37*, 223−230.
(35) Bisht, S.; Feldmann, G.; Soni, S.; Ravi, R.; Karikar, C.; Maitra, A.; Maitra, A. Polymeric Nanoparticle-Encapsulated Curcumin ("Nanocurcumin"): A Novel Strategy for Human Cancer Therapy. *J. Nanobiotechnology* **2007**, *5*, 1−18.
(36) Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. Recent Advances on Chitosan-Based Micro- and Nanoparticles in Drug Delivery. *J. Controlled Release* **2004**, *100*, 5−28.
(37) Dong, L. C.; Yan, Q.; Hoffman, A. S. Controlled Release of Amylase from a Thermal and pH-Sensitive, Macroporous Hydrogel. *J. Controlled Release* **1992**, *19*, 171−177.

(38) Ende, M. T. A.; Peppas, N. A. Transport of Ionizable Drugs and Proteins in Crosslinked Poly(acrylic acid) and Poly(acrylic acid-*co*-2-hydroxyethyl methacrylate) Hydrogels 2. Diffusion and Release Studies. *J. Controlled Release* **1997**, *48*, 47−56.
(39) Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. *Adv. Mater.* **2006**, *18*, 1345−1360.
(40) Suh, J. K. F.; Matthew, H. W. T. Application of Chitosan-Based Polysaccharide Biomaterials in Cartilage Tissue Engineering: A Review. *Biomaterials* **2000**, *21*, 2589−2598.
(41) Yan, Q.; Hoffman, A. S. Synthesis of Macroporous Hydrogels with Rapid Swelling and Deswelling Properties for Delivery of Macromolecules. *Polymer* **1995**, *36*, 887−889.
(42) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in Pharmaceutical Formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27−46.
(43) Tuntikulwattana, S.; Sinchaipanid, N.; Ketjinda, W.; Williams, D. B.; Mitrevej, A. Fabrication of Chitosan-Polyacrylic Acid Complexes as Polymeric Osmogents for Swellable Micro/Nanoporous Osmotic Pumps. *Drug Dev. Ind. Pharm.* **2011**, *37*, 926−933.
(44) Torrado, S.; Prada, P.; de la Torre, P. M.; Torrado, S. Chitosan−Poly(acrylic) Acid Polyionic Complex: In Vivo Study to Demonstrate Prolonged Gastric Retention. *Biomaterials* **2004**, *25*, 917−923.
(45) Grimm, S.; Giesa, R.; Sklarek, K.; Langner, A.; Gosele, U.; Schmidt, H. W.; Steinhart, M. Nondestructive Replication of Self-Ordered Nanoporous Alumina Membranes via Cross-Linked Polyacrylate Nanofiber Arrays. *Nano Lett.* **2008**, *8*, 1954−1959.
(46) Xie, B. J.; Parkhill, R. L.; Warren, W. L.; Smay, J. E. Direct Writing of Three-Dimensional Polymer Scaffolds Using Colloidal Gels. *Adv. Funct. Mater.* **2006**, *16*, 1685−1693.
(47) Wang, K. T.; Iliopoulos, I.; Audebert, R. Viscometric Behaviour of Hydrophobically Modified Poly(sodium acrylate). *Polym. Bull.* **1988**, *20*, 577−582.
(48) Guo, X.; Abdala, A. A.; May, B. L.; Lincoln, S. F.; Khan, S. A.; Prud'homme, R. K. Rheology Control by Modulating Hydrophobic and Inclusion Associations in Modified Poly(acrylic acid) Solutions. *Polymer* **2006**, *47*, 2976−2983.
(49) Wang, J.; Li, L.; Ke, H.; Liu, P.; Zheng, L.; Guo, X.; Lincoln, S. F. Rheology Control by Modulating Hydrophobic and Inclusive Associations of Side-Groups in Poly(acrylic Acid). *Asia-Pac. J. Chem. Eng.* **2009**, *4*, 537−543.
(50) Gans, P.; Sabatini, A.; Vacca, A. Investigation of Equilibria in Solution. Determination of Equilibrium Constants with the HYPERQUAD Suite of Programs. *Talanta* **1996**, *43*, 1739−1753.
(51) Protonic Software. http://www.hyperquad.co.uk/.
(52) Chem3D Ultra. http://www.cambridgesoft.com/.
(53) Tønnesen, H. H.; Karlsen, J. Studies on Curcumin and Curcuminoids. 5. Alkaline-Degradation of Curcumin. *Z. Lebensm.-Unters. Forsch.* **1985**, *180*, 132−134.
(54) Anderson, A. M.; Mitchell, M. S.; Mohan, R. S. Isolation of Curcumin from Turmeric. *J. Chem. Educ.* **2000**, *77*, 359−360.
(55) Jayaprakasha, G. K.; Rao, L. J. M.; Sakariah, K. K. Improved HPLC Method for the Determination of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin. *J. Agric. Food Chem.* **2002**, *50*, 3668−3672.
(56) Griffiths, P. C.; Paul, A.; Khayat, Z.; Heenan, R. K.; Ranganathan, R.; Grillo, I. A Small-Angle Neutron Scattering Study of Biologically Relevant Mixed Surfactant Micelles Comprising 1,2-Diheptanoyl-*sn*-phosphatidylcholine and Sodium Dodecyl Sulfate or Dodecyltrimethylammonium Bromide. *Soft Matter* **2005**, *1*, 152−159.
(57) Jin, Y.; Ye, F.; Wu, C.; Chan, Y.-H.; Chiu, D. T. Generation of Functionalized and Robust Semiconducting Polymer Dots with Polyelectrolytes. *Chem. Commun.* **2012**, *48*, 3161−3163.