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IMPROVING SOLUBILITY OF CURCUMIN IN AQUEOUS SYSTEM BY THE SELF-ASSEMBLY OF HYPERBRANCHED POLY(AMIDE-ESTER) AND CURCUMIN

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ABSTRACT: In order to improve the water-solubility of curcumin, the complex between curcumin and hyperbranched poly(amide-ester) was formed by dispersing the solid curcumin in hyperbranched poly(amide-ester) aqueous solution. The interaction process was characterized by UV-vis absorption spectroscopy, Fluorescence quenching method, Fourier transformation-infrared spectroscopy, thermogravimetric analysis. Fluorescence quenching analysis reveal that the fluorescence quenching of curcumin is due to the formation of ground state complex between curcumin and hyperbranched poly(amide-ester) and the binding constant was $3.98 \times 10^5 \text{ L} \cdot \text{mol}^{-1}$. The other analysis also can prove the formation of complex between curcumin and HBPAE. The solubility of curcumin in aqueous solution was greatly increased more than 1000-fold at the presence of hyperbranched poly(amide-ester).

INTRODUCTION: Curcumin (CUR) (Scheme 1) containing keto-enol-enolate equilibrium is a kind of pigment that isolated from the rhizomes of turmeric. It could be commonly utilized as food dye and spice in curries.^{1, 2, 3} In recent years, many researchers discovered that CUR and its derivatives exhibited some amazing properties such as antioxidant, anti-inflammation, anti-microbial, anti-cancer and anti-mutagenic with low intrinsic toxicity.^{4, 5} However, its poor water-solubility restricted its practical application in biology to a great degree. Thus, it is urgent to improving the water-solubility of CUR. Recently, scholars have made great efforts to develop new methods to increase the water-solubility of CUR, such as nanoparticles,⁶ nanogels,^{7, 8} conjugates^{9, 10} and bilayers.^{11, 12}

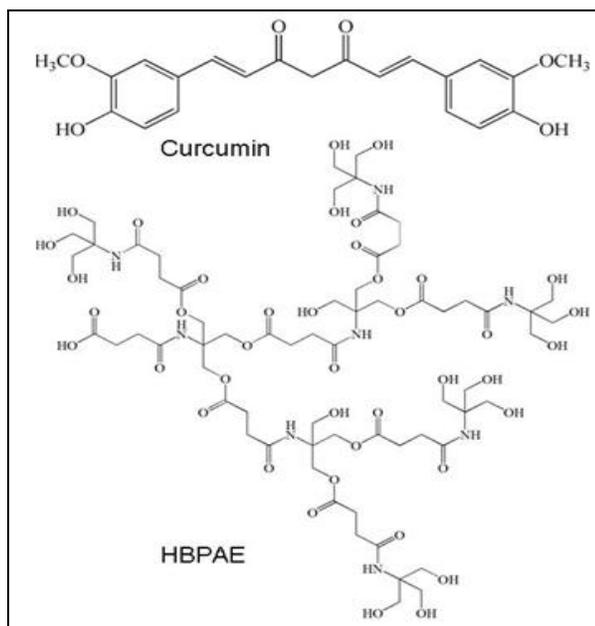
For example, Bhawana⁶ have successfully prepared the nanoparticles of CUR with a narrow particle size distribution in the range of 2-40nm by a process based on a wet-milling technique, which can freely dispersible in water in the absence of any surfactants. Ahmad Safavy¹⁰ have synthesized two kind of water-soluble CUR conjugates and detailly studied their cytotoxicity as potential anticancer agents.

In recent years, hyperbranched polymers have received more and more attention because of their unique three-dimensional structure and characteristics. Distinct from their linear analogs, hyperbranched polymers have structures and topologies similar to the dendrimer, and they could show some outstanding properties such as low solution/melt viscosity, abundance in terminal groups and enhanced solubility, etc.^{10, 11} The preparation of dendrimers require tedious synthetic procedures, but the hyperbranched polymers could be easily synthesized through simple one-pot approach.¹⁵ This kind of polymers with highly branched globular structures can be used as host molecules to combine with guest molecules

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through intermolecular interactions.^{16, 17} If the prepared hyperbranched polymers could easily dissolved in water and could complex with curcumin, the solubility of CUR in aqueous solution might be effectively enhanced.

To our knowledge, improving the water-solubility of CUR by the formation of complex with hyperbranched polymers was scarcely reported. Therefore, in this study, we aimed to increase the water-solubility of CUR by the formation of complex between CUR and water-soluble hyperbranched poly(amide-ester)(HBPAE) with amount of hydroxyl groups. HBPAE was synthesized by a one-pot condensation reaction through the reaction of the commercially available succinic anhydride and Tris(hydroxymethyl) methyl aminomethane. The chemical structure of HBPAE was given in **Scheme 1**. HBPAE could be easily dissolved in water because of the presence of many hydroxyl groups. When solid CUR was dispersed into HBPAE aqueous solution, the complex could be formed by intermolecular interaction, which could effectively improve the water-solubility of CUR. The complex process was analysed by UV-vis absorption spectroscopy, fluorescence quenching method, Fourier transformation-infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA). All of the results could prove the formation of complex. The water-solubility of CUR was increased more than 1000-fold at the presence of HBPAE.



SCHEME 1: THE CHEMICAL STRUCTURES OF CUR AND HBPAE

MATERIALS AND METHODS:

Chemical and reagents: Tris (hydroxymethyl) methyl aminomethane and Succinic anhydride were obtained from Tianjin GuangFu Fine Chemical Industry Research Institute, P-Toluenesulfonic acid methanol and curcumin(CUR) were all purchased from Tianjin Kermel Chemical Reagent Co., Ltd. All of the purchased reagents were in analytical grade.

Preparation of HBPAE: HBPAE have been firstly synthesized and reported by our groups^{18, 19, 20}. Following is the detailed procedure. Tris (hydroxymethyl) methyl aminomethane (0.1mol), absolute methanol (15mL) and 1mL pyridine were added into dried reaction bulb. The mixture was stirred and cooled to 0°C. Succinic anhydride (0.1mol) was added to reaction system in bathes. Then reaction was continued for 6h and methanol was removed by vacuum distillation to obtain monomer. The monomer was polymerized for 10h using *p*-toluene sulfonic acid as catalyst at 120 °C. In reaction process, water was excreted in order to increase the molecular weight. The final polymer was light yellow glassy solid. The molecular weight was measured by GPC. $M_n = 0.83 \times 10^4$; PDI:2.02.

Preparation of HBPAE/CUR complex: HBPAE (0.625g) was dissolved in deionized water (5 mL) in a glass vial containing a magnetic bar. Solid CUR (5 mg) was added to the HBPAE aqueous solution and the as-formed mixture was then stirring for 24 h at room temperature. After filtering, the supernatant containing highly water-soluble HBPAE/CUR complex was obtained. A series of complex were obtained by dispersing CUR in different concentration of HBPAE aqueous solution. These complex was collected by freeze-drying the supernatant and stored in desiccator for use.

Characterization methods: UV-vis spectra were recorded on Shimadzu UV-2550 spectrophotometer equipped with 1.0cm quartz cells and water-bath with temperature controlled. The measured slit width was 1nm. The Fourier transform infrared spectra (FT-IR) were obtained in powder form by using Varian 640-IR over the wave number range of 4000-400 cm^{-1} with KBr pellets.

Measurement of fluorescence quenching were performed on RF-5301PC at room temperature. Thermal gravimetric analysis (TGA) were carried out on Perkin-Elmer Pyris 6 under nitrogen atmosphere with a heating rate of 10°C/min.

RESULTS AND DISCUSSION:

The solubility of CUR in HBPAE aqueous solution: In order to study the solubility of CUR in HBPAE aqueous solution, CUR solid was dispersed in different concentration of HBPAE aqueous solutions. Then the solution was stirred for 24 h at room temperature and filtered with filter membrane. The HBPAE/CUR complex was obtained when filtrate was freeze-dried. To quantify the exact amount of CUR in different concentration of HBPAE, the calibration curve of UV absorption vs. CUR concentration must be plotted. To avoid the measurement error in different solvent, methanol was chosen as solvent to plot standard curve, which can dissolve both pure CUR and HBPAE/CUR complex. The absorption spectrum of HBPAE/CUR complex in methanol are different with that in water, which may be caused by the different polarity. The dielectric constant of water was 81, but 33.7 for methanol. Here, the absorption spectrum of HBPAE/CUR in aqueous and methanol was also given in **Fig. 1**. Complex in aqueous solution featured three absorption peaks at 324 nm, 364 nm and 425 nm. But only one broad absorption peak at 425 nm was observed in methanol. The absorbance of HBPAE/CUR at 425nm in methanol and CUR was fitted with the following equation obtained by calibration curve:

$$A = 0.04 + 0.11C$$

A is the absorbance of HBPAE/CUR at 425nm in methanol; C is the concentration of CUR combined in HBPAE aqueous solution.

The absorption spectra of freeze-dried HBPAE/CUR complex with different concentration HBPAE in methanol were measured under the same conditions and the curve of absorbance at 425nm in methanol vs. HBPAE concentration are shown in **Fig. 2(A)**. From the spectra, when the HBPAE concentration changed from 0.025g/mL to 0.175g/mL, the solubility of CUR in HBPAE aqueous solution increased from 1.081×10^{-3} g/mL to

7.565×10^{-3} g/mL calculated by the standard curve equation.

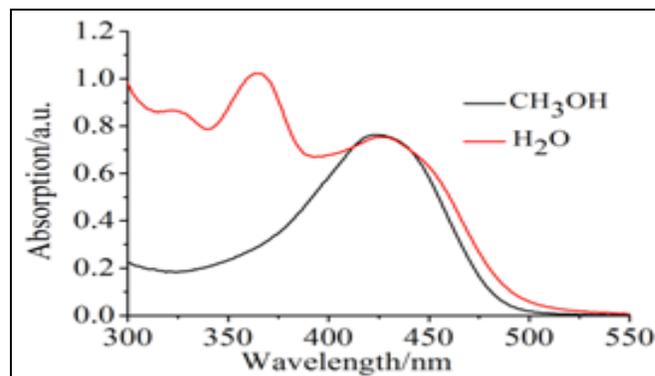
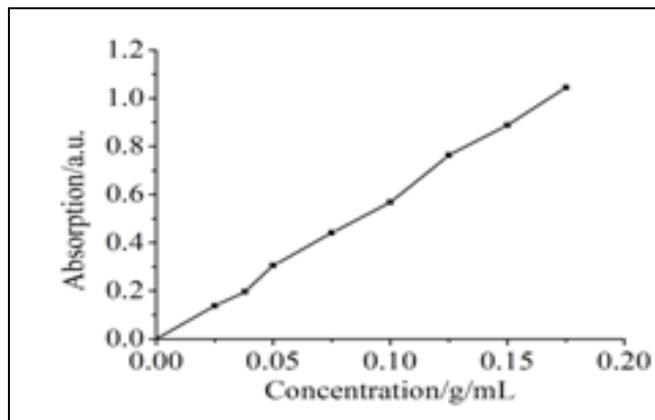


FIG. 1: UV-VISIBLE ABSORPTION OF COMPLEX IN WATER AND METHANOL

However, the dissolubility of CUR in pure water is only 1×10^{-6} g/mL in literature.²¹

HBPAE with a large number of internal cavities can complex with CUR by non-covalent interaction between HBPAE and CUR molecules. Thus, the addition of HBPAE in water can greatly raise the solubility of CUR through the formation of HBPAE/CUR complex.

The photograph of solid CUR in HBPAE aqueous solution after stirring for 24h at room temperature was given in **Fig. 2(B)**. From left to right, first is the pure HBPAE aqueous solution, others is respective corresponding to the concentration in (A) from 0.025g/mL to 0.175g/mL. From the photograph, the transparent solution was obtained for all concentration. The HBPAE aqueous solution itself was almost no color, but the color was more yellow with the higher concentration of HBPAE. The result show that the more CUR was dissolved when the concentration of HBPAE was increased.



(A)



(B)

FIG. 2 (A) CHANGE IN PEAK INTENSITY OF ABSORBANCE AT 425NM VS. HBPAE CONCENTRATION (B) THE PHOTOGRAPH OF CUR IN DIFFERENT CONCENTRATION OF HBPAE SOLUTION

Fluorescence quenching experiments: CUR had a maximum fluorescence emission at wavelength about 541nm when it was excited at 423nm. Fig. 3 showed the change of the fluorescence emission spectra of CUR. It could be observed that the fluorescence of CUR was obviously quenched after addition of different concentration HBPAE.

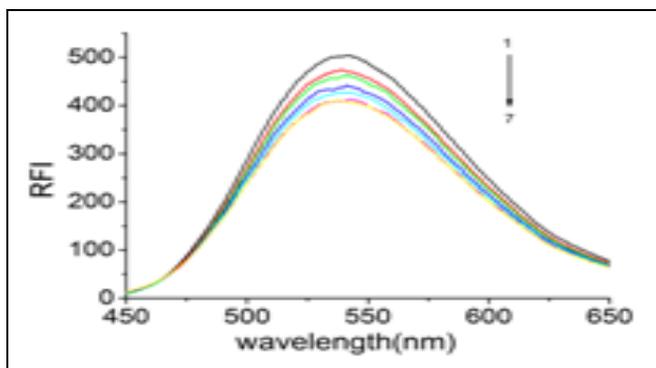


FIG. 3: FLUORESCENCE EMISSION SPECTRA OF CUR IN ABSENCE AND PRESENCE OF DIFFERENT HBPAE

The fluorescence quenching mechanism was usually classified to static quenching and dynamic quenching. Static quenching is caused by the formation of a ground state complex between the quencher and fluorophore. Whereas, dynamic quenching is the collision between the excited-state fluorophore with the quencher. In order to confirm the possible fluorescence quenching mechanism, the Stern-Volmer equation was used to analyze the fluorescence quenching data:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{sv} [Q]$$

where, F_0 and F are the fluorescence intensities in absence and presence of quencher(HBPAE), K_{sv} is the Stern-Volmer quenching constant, $[Q]$ is the concentration of HBPAE, k_q is the biomolecular

quenching rate constant, t_0 is the average lifetime of the biomolecule without quencher($t_0=10^{-8}$ s). The plot of F_0/F versus quencher concentration was found to be linear. k_{sv} could be obtained from the slope of the linear plot and the value was $4.93 \times 10^4 \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$. The biomolecular quenching rate constant can be calculated and the value was $4.93 \times 10^{12} \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$. For dynamic quenching, the maximum biomolecular quenching rate constant is about $2.0 \times 10^{10} \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$. In this system, the larger value reveal that the quenching is due to the formation of ground state complex, not initiated by collision process. The quenching data was further analyzed basis on the Lineweaver-Burk equation:

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{1}{[Q]F_0K}$$

The binding constant k_A can be calculated from the slope of the plot of $1/(F_0-F)$ versus $1/[Q]$ and the value was $3.98 \times 10^5 \text{ L}\cdot\text{mol}^{-1}$. The data analysis show that ground state complex can be formed between HBPAE and CUR. This may be the main reason for enhanced solubility of CUR in HBPAE aqueous solution.

UV-Vis absorption spectra analysis: For the better understand of complex formation, the UV-Vis absorption of complex in aqueous solution at different temperature from 25°C to 90°C were further measured (Fig. 4A). When the solution temperature was increased from 25°C to 90°C, the intensity of the absorption peak at 425 nm was decreased and the absorption peak at 324 nm was raised. But for the absorption at 364nm, there was no obvious change. This result is absolute different with the change of CUR in pure water reported in literature.²¹ In pure water, the three absorption were situated at 237nm, 345nm and 419nm. The change of absorption peak was caused by the change of solvent polarity because of the addition of HBPAE in pure water. The three peak intensity were obviously increased because the solubility in water was enhanced when temperature is changed from 25°C to 95°C. These difference further proved that the complex may be formed by intermolecular force between HBPAE and CUR molecules when HBPAE was added. When temperature was raised, the intermolecular interaction may be destroyed.

In order to determine the speculations, the absorption of repeated heating and cooling cycle process were performed (Fig. 4B). Plot a and c are respective the curve heated to 90°C and cooled to 25°C for the first time. Plot b and d are respective the curve heated to 90°C and cooled to 25°C for the second time. Plot a and b are basically the same, which show that the intermolecular interaction may be changed when temperature was heated to 90 °C. Plot c and d are basically the same, which show that the intermolecular interaction was reconstructed when temperature was cooled to 25°C. Furthermore, the transition is reversible.

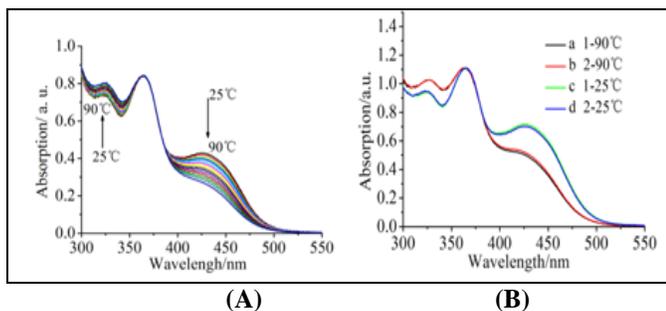


FIG. 4: UV-VIS SPECTRA OF COMPLEX IN AQUEOUS SOLUTION AT DIFFERENT TEMPERATURE

FT-IR spectra analysis: The formation of the HBPAE/CUR complex can be ascertained by FT-IR spectroscopy. The solid samples of CUR(a), HBPAE/CUR mixture(b), HBPAE/CUR complex (c) and HBPAE(d) were recorded on Varian 640-IR spectrometer and the results were shown in Fig. 5. The FT-IR spectrum of CUR exhibited an absorption peak at 3510 cm^{-1} belonging to the phenolic O-H stretching vibration. The sharp absorption bands at 1605 cm^{-1} , 1502 cm^{-1} , 1435 cm^{-1} and 1285 cm^{-1} were ascribed to the stretching vibrations of benzene ring of CUR, C=O and C=C vibrations of CUR, olefinic C-H bending vibration and aromatic C-O stretching vibrations. FT-IR spectrum of HBPAE showed the characteristic peaks at 3379 cm^{-1} , 1739 cm^{-1} and 1662 cm^{-1} due to the O-H stretching vibration and C=O stretching vibration. In the spectrum of HBPAE/CUR mixture, the absorption of HBPAE and CUR were appeared, but some absorption were coincide.

In the case of HBPAE/CUR inclusion complex spectrum, similar to that of HBPAE, all the sharp peaks belong to HBP have appeared and only few characteristic peaks of CUR are visible. Because of complexing with CUR, the peaks related to

HBPAE were shifted to higher/lower wave numbers, i. e., 3379-3382 cm^{-1} , 1739-1738 cm^{-1} , 1662-1660 cm^{-1} .

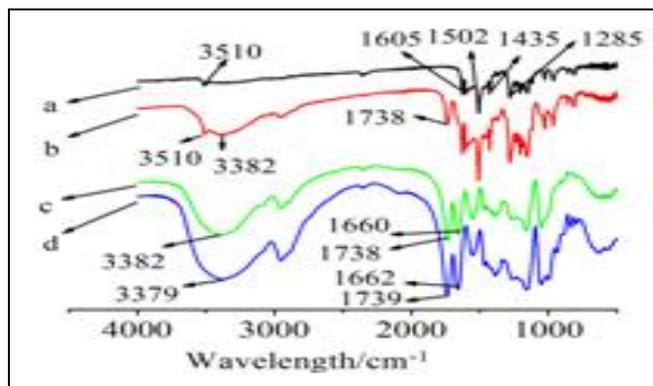


FIG. 5: FT-IR SPECTRUM OF CUR, HBPAE/CUR MIXTURE, HBPAE/CUR COMPLEX AND HBPAE

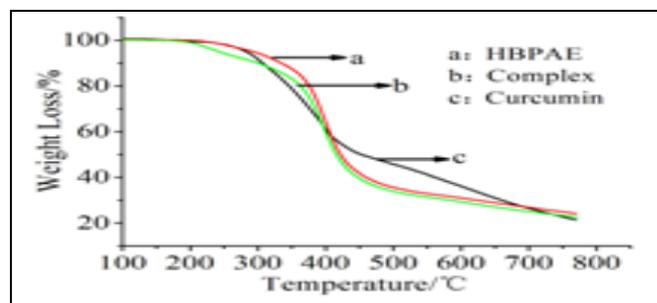


FIG. 6: THERMOGRAVIMETRIC CURVE OF HBPAE, CUR AND COMPLEX

Thermogravimetric curve analysis: Fig. 6 was the thermogravimetric analysis of HBPAE, curcumin and complex. From the spectra, the TGA curve of complex is obviously different from that of curcumin. Comparing the curve of HBPAE and complex, the weight loss of complex is greater than HBPAE at temperature from 200°C to 400°C, which may be the loss of curcumin complexed with HBPAE. Above 400°C, the weight loss of complex was similar with that of HBPAE, which may be the skeleton construction of HBPAE.

CONCLUSIONS: In summary, the complex of hyperbranched polymer (HBPAE) and curcumin was prepared by dispersing solid curcumin in HBPAE aqueous solution. Clearly, the solubility of curcumin was effectively enhanced in HBPAE aqueous solution, which is beneficial for its potential application in biology. This complex procedure was investigated through fluorescence quenching method, FT-IR spectra, UV-Vis spectra and TGA. We do believe that this primary study provides more room to approach the design and

synthesis of novel hydrophilic hyperbranched polymers and extend the bioavailability of curcumin.

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