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Polyhydric polymer-loaded pyrene composites as powerful adsorbents and fluorescent probes: highly efficient adsorption and test strips-based fluorimetric analysis of curcumin in urine and plant extracts†

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Polyhydric poly (vinyl alcohol) was covalently loaded with a 1-pyrenecarboxyaldehyde fluorophore. The yielded PVA-Pyr composites can serve as powerful adsorbents and strong fluorescent probes for the highly efficient adsorption and sensitive fluorimetric detection with test strips of curcumin in samples of urine and plant extracts.

Curcumin (Cur), a natural yellow pigment that widely exists in some plants like curry and mustard, is often applied as a natural seasoning, a food coloring agent, and especially a drug showing strongly antioxidant, antibacterial, antifungal, and anti-HIV biological–pharmacological activities.[1–5] Moreover, recent studies have demonstrated that an excessive Cur dose in the human body due to food intake or medicine treatment may bring about pro-oxidant DNA damage and decrease intracellular adenosine triphosphate levels so as to trigger cell necrosis.[6] A variety of conventional detection methods have been developed to date for Cur analysis; the most well known are UV-vis spectrophotometry,[7,8] liquid chromatography,[9] capillary electrophoresis,[10] and mass spectrometry.[11] These traditional analysis methods may, however, generally suffer from some disadvantages such as time consumption, on-site detection inability, complex sample pretreatment, and bulky infrastructure requirement. Therefore, exploring a rapid, simple, sensitive, and field-applicable analysis method for the determination of Cur levels in Cur-rich plants or Chinese herbal medicine, and the on-site monitoring of Cur levels in the human body or excreta like urine is highly desirable in the clinical pharmacology and disease diagnostic fields. Fluorimetric analysis methods with high sensitivity and easy operation properties have attracted increasing interest, where the detection performances largely depend on the optical properties of the fluorescent probes.[12–14] Although Cur species themselves can display green fluorescence that may rapidly disappear in water, it is hard to be applied as fluorescent probes in aqueous media like plant extracts and body fluids or excreta. To date, many efforts have been devoted to the development of fluorimetric methods for the evaluation of Cur in various aqueous media by alternatively using different kinds of fluorescent probes.[15,16] For example, Chen and co-workers reported a resonance light scattering assay for Cur in human urine using Cu(II) ion as the spectral probe.[17] Shuang *et al.* developed a ratiometric fluorescent probe of functional ZnS quantum dots and nitrogen-doped carbon dots separately for the monitoring of Cur in urine.[18,19] Nevertheless, these fluorescent probes may encounter with either heavy metal toxicity and poor aqueous solubility, or low luminance emission. Therefore, it is still highly desirable to develop more sensitive and water-dispersible fluorescent probes, especially highly enrichment-absorbable probes for the detection of Cur in aqueous samples as well as the Cur accumulated in plant extracts. Recent years have witnessed the rapid development of water-soluble functional polymers serving as efficient adsorbents or carriers of fluorescent probes, featuring several important advantages like low toxicity, easy fabrication, good aqueous solubility, and strong adsorption capacities.[20,21] As an important polymer representative, polyhydric poly (vinyl alcohol) (PVA) features some outstanding merits such as good water solubility, ease of use, and biological affinity and degradability.[22,23] In particular, PVA exhibits a wonderful film-forming and adsorption ability to serve as a favorable carrier for extensive adsorption and detection applications.[24,25] Nevertheless, the application of PVA simultaneously serving as carriers of fluorescent probes has hardly been explored.

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Inspired by the previous pioneering studies mentioned above, in the present work, we initially tried to endow the polyhydric PVA chains with tracking fluorescence by covalently loading a strong fluorophore of 1-pyrenecarboxyaldehyde (Pyr) through the acetal reaction. As expected, the yielded PVA-Pyr composites can present powerful absorption and enrichment to Cur. More interestingly, they could display extremely strong cyan fluorescence that could be specifically quenched by Cur through the inner filter effect (IFE). Herein, Cur possesses a wide absorption peak around 300–550 nm. The prepared PVA-Pyr composites as the fluorophores can present excitation and emission spectra overlapping with the absorption band of Cur. Consequently, Cur could not only shield the excitation light from PVA-Pyr composites but also absorb their emission light, leading to the quenching of the fluorescence intensities of the PVA-Pyr composites toward the fluorimetric detection of Cur. A PVA-Pyr-based accumulation and fluorimetric detection strategy has therefore developed for Cur separately in human urine and plant extracts. The main synthesis reactions of PVA-Pyr for the aggregation-based Cur adsorption and fluorimetric analysis procedure are schematically illustrated in Fig. 1. As shown in Fig. 1A, the PVA-Pyr composites were prepared by a single step of condensation of PVA and Pyr. The so-yielded PVA-Pyr particles consist of 98.15% hosting polyhydric PVA chains and 1.85% pyrene moieties, showing powerful cyan fluorescence in aqueous media (Fig. 1B). As a result, the fluorescence of the PVA-Pyr probes was quenched effectively due to the IFE. By taking advantage of the outstanding film-forming ability of PVA, a test strips-based fluorimetric method was thereby developed for the highly efficient accumulation and rapid detection of Cur in the practical samples afterwards.

Fig. 2A presents a visual comparison of the aqueous products of PVA-Pyr, Cur, and PVA-Pyr with adsorbed Cur under white light. It was observed that benefiting from the combined advantages of polyhydric PVA, the PVA-Pyr adsorbent could be well dispersed in water to form an almost transparent solution with a high stability (**tube a**). Yellow Cur could also be suspended in water (**tube b**). Upon the addition of the PVA-Pyr adsorbent, interestingly, Cur was largely aggregated out of the aqueous media to produce yellow precipitates (red arrow in **tube c**). The results demonstrate the powerful adsorption and accumulation ability of the PVA-Pyr adsorbent for Cur presumably due to the specific intermolecular hydrogen bonding between Cur and the numerous hydroxyl groups of the PVA chains. Furthermore, as can be observed in Fig. 2B, the fluorescence of the resulted PVA-Pyr probes could be selectively quenched by Cur, presumably resulting from the IFE between Cur and pyrene. Moreover, the fluorescent PVA-Pyr probes were coated onto the test strips for the fluorimetric detection of Cur simply by using a dipping method (Fig. 2C).

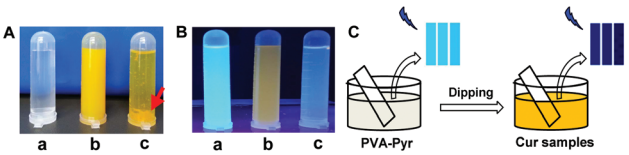

Fig. 2 Photographs of the aqueous products of (a) PVA-Pyr, (b) Cur, and (c) PVA-Pyr with adsorbed Cur towards the accumulating aggregation (red arrow) under (A) white light and (B) UV light (ex = 365 nm). (C) Schematic illustration of the dipping-based analysis procedure of the test strips coated with the PVA-Pyr probes for the visual evaluation of Cur samples.

UV-vis and fluorescence spectral studies were conducted for PVA-Pyr in the presence and absence of Cur, taking native Pyr and PVA as the controls (Fig. 3). As can be seen in Fig. 3A, the fluorescent PVA-Pyr probes exhibit the strong monomer emission peak separately at 376 and 396 nm, including an excimer emission peak at 472 nm (**curve c**), which may correspond to that of the Pyr included (**curve a**). Such a result implies that the majority of the pyrene units of the probes were monodispersed even in the aqueous system. After adding Cur, the fluorescence intensities of the PVA-Pyr probes could be dramatically quenched (**curve d**), thus serving as a visual indicator of Cur. Moreover, Fig. 2B shows the comparison of UV-vis spectra among these substances. One can note that the PVA-Pyr probes could exhibit absorption peaks in the 232–340 nm region (**curve c**). Notably, a large blue-shift of the absorption wavelength (about 60 nm) was observed as compared to the typical absorption peaks of Pyr alone (**curve a**), which indicated the covalent bond formation of the polymer-based fluorescent probes of PVA-Pyr. Besides this, after the addition of Cur, the PVA-Pyr probes could display basically similar characteristic

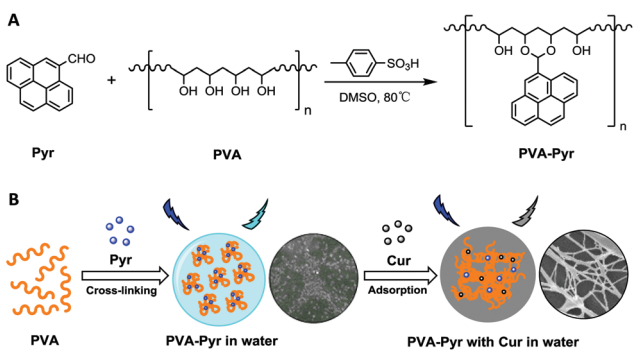

Fig. 1 Schematic illustration of (A) the reaction for the synthesis of the polymer-based adsorbent and fluorescent probe and (B) the preparation and "turn-off" fluorescence procedure of PVA-Pyr triggered by the selective PVA-Pyr–Cur interaction towards the probe aggregation.

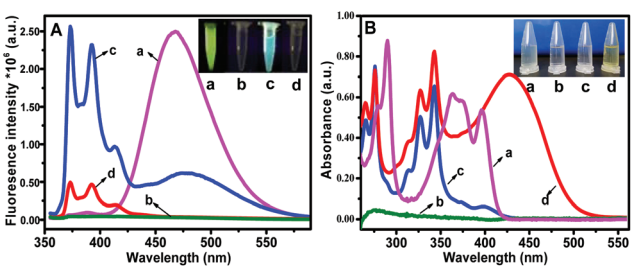

Fig. 3 (A) Fluorescence spectra and (B) UV-vis spectra of (a) Pyr (0.02 mg L^{-1}), (b) PVA, (c) PVA-Pyr (1.0 mg L^{-1}), and (d) PVA-Pyr with Cur (2.0 μM) (inserts: the corresponding photographs of the products).

absorption spectra, suggesting that no clear interaction might occur between Cur and pyrene (**curve d**).

Fluorescence microscopy was employed to investigate the morphology change of PVA-Pyr in the absence and presence of Cur (Fig. S1†). It can be seen in Fig. S1A† that the PVA-Pyr probes could be well dispersed on the glass slide after drying, showing the bright blue light emitted in the dark-field image (Fig. S1B†). Upon the addition of Cur, the PVA chains of the PVA-Pyr composites (the adsorption agent) would produce strong intermolecular hydrogen bonds with Cur leading to the aggregation or precipitation of Cur-loaded composites, thus showing a drastic conformation change (Fig. S1C†). As a result, the fluorescence intensities of PVA-Pyr composites would be greatly quenched towards the fluorimetric analysis of Cur (Fig. S1D†). This phenomenon indicates that the developed PVA-Pyr composites could feature the unique double functions: acting as the adsorbent and detectable probes for Cur. Furthermore, the change of the morphological structures of the PVA-Pyr composites in the absence and presence of Cur was examined by transmission electron microscopy (TEM) imaging (Fig. 4). As expected, the PVA-Pyr composites were witnessed to be well dispersed in the aqueous media with a uniform olive-like shape, showing a particle size of about 100 × 50 nm (Fig. 4A). Again, the addition of Cur could cause the PVA-Pyr particle to largely aggregate towards the formation of network polymers (Fig. 4B), as shown in Fig. S1C,† that is, the PVA chains of the PVA-Pyr composites could act as strong adsorbents for accumulating Cur from the media.

The fluorimetric selectivity of the PVA-Pyr probes in sensing Cur was investigated by comparing with various common ions and small molecules (*i.e.*, amino acids) (Fig. S2†). It was discovered that the fluorescence of PVA-Pyr could be quenched only by Cur in aqueous solutions. In contrast, negligible variations of the fluorescence intensities of the composites were observed for the other tested 34 kinds of common ions (Fig. S2A†) and other common compounds or biomolecules (Fig. S2B†), even though their concentrations were three times higher than that of Cur, including the 1000 and 100 equivalent concentrations of urea and UA, respectively. Furthermore, the fluorescent probes could present basically the same responses to Cur in the separately competitive co-existence of these tested ions or molecules (red histograms). The data indicate that the prepared PVA-Pyr probes could exhibit excellent selectivity in sensing Cur.

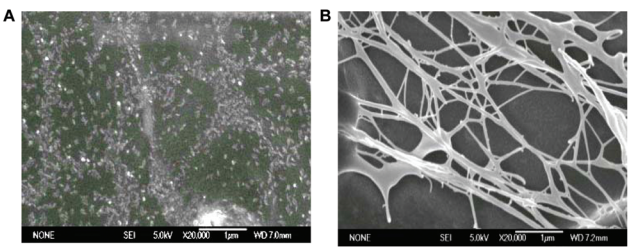

Fig. 4 SEM images of PVA-Pyr (1.0 mg mL^{-1}) (A) before and (B) after the addition of Cur (0.10 mM).

Next, the optimization of the main detection conditions was carried out for the PVA-Pyr-based fluorimetric Cur analysis (Fig. S3†). At first, the concentrations of the probes were optimized (Fig. S3A†), suggesting that 1.0 mg L^{-1} of the probes was the most suitable for the experiments. Moreover, the pH-dependent fluorimetric responses of the PVA-Pyr probes to Cur were studied (Fig. S3B†). It was clear that the optimal pH value for the Cur sensing should be 6.0. Additionally, using NaCl as the ionic strength regulator, no significant effect was found on the fluorimetric Cur analysis (Fig. S3C†). Lastly, we observed that the reaction between the PVA-Pyr probes and Cur could be completed within 20 min, after which the fluorescence intensities would tend to be stable (Fig. S3D†).

Under the optimized conditions, the fluorimetric analysis with the PVA-Pyr probes was performed for different levels of Cur in a buffer (Fig. S4†). As described in Fig. S4A,† the fluorescence intensities of the PVA-Pyr probes could rationally decrease with incremental amounts of Cur. A relationship between the fluorescence intensities of PVA-Pyr and Cur concentrations was obtained over the linear Cur concentrations ranging from 0.01 μM to 2.0 μM (R^2 = 0.9898), with a limit of detection of 3.5 nM, estimated by using the 3σ rule (Fig. S4B†). Moreover, a test strips-based fluorimetric strategy was developed by coating the PVA-Pyr probes onto the test strips by taking advantage of the outstanding film-forming ability of PVA. Subsequently, the feasibility of the practical application of the as-prepared test strips was explored for Cur spiked in urine samples simply by using the dipping method (Fig. 5). As can be noted from Fig. 5A, the fluorescence spectra of the PVA-Pyr probes could change rationally with the increase in Cur levels in urine. The plots of the fluorescence quenching efficiencies can fit linearly with the Cur concentrations ranging from 0.05 to 4.0 μM, with a correlation coefficient of 0.9800, of which the detection limit was obtained to be as low as 8.3 nM (Fig. 5B). Moreover, a comparison of the Cur analysis results was conducted among the developed fluorimetric method and other analysis techniques reported elsewhere (Table S1†). It can be seen that the developed fluorimetric strategy could exhibit better or comparable performances for Cur detection in terms of linear range and detection limit.

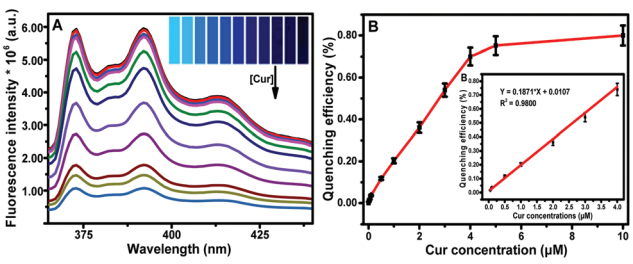

Fig. 5 (A) Fluorescence spectra of the test strips-based fluorimetric responses to Cur samples of different concentrations spiked in urine (0, 0.01, 0.05, 0.10, 0.25, 1.0, 2.0, 3.0, 4.0, 5.0, and 10.0 μM). (B) The relationship between the quenching efficiencies *versus* the concentrations of Cur samples, where the solid-state fluorescence intensities of the fluorescent test strips were measured at λ_{em} = 392 nm.

Taking all these results together, the data indicate that the PVA-Pyr-based test strips show promising potential in practical applications for the sensitive and selective detection of Cur in samples.

In order to further explore the practicality, the developed fluorimetric test strips were employed to probe Cur in human urine, and the detection results were compared to those obtained by using the classic HPLC method (Table S2†). One can find that the developed fluorimetric method can produce analysis results that are in good agreement with those obtained by using the HPLC technique in terms of recovery percentages. Moreover, the test strips-based fluorimetric method was used to probe Cur in plant extracts of curry and mustard, and the results are summarized in Table S3.† Accordingly, the developed fluorimetry can facilitate the detection of Cur in plant samples with the measured levels consistent with those of the added ones of which the recovery percentages so calculated can range from about 96.0–105.0%. Furthermore, the storage stability and analysis reproducibility of the developed fluorimetric test strips for probing Cur were investigated separately by using the test strips stored over different time intervals and different batch productions (Fig. S5†). The results indicate that there is no significant change in the fluorimetric responses to Cur. The results demonstrate that the developed test strips-based fluorimetric method show promising practical applicability for detecting Cur in various kinds of real samples.

In summary, adsorbent-detectable polymer composites were successfully synthesized by the covalent coupling of the Pyr fluorophore onto the polyhydric PVA chains using a facile one-step acetal reaction. The yielded PVA-Pyr composites can serve as powerful adsorbents and strong fluorescent probes, so as to allow the accumulation and fluorimetric detection of Cur. Moreover, PVA-Pyr composites were coated onto test strips towards a rapid and visual fluorimetric analysis method for Cur by taking advantage of the unique film-forming ability of PVA. In particular, it may promise in-field applications for the fast onsite monitoring of Cur in urine and plant extract samples. Besides this, such a facile and effective polyhydric polymer-based probe fabrication route may open a new door toward the extensive design of a variety of organic fluorescent probes, showing improved aqueous solubility, highly efficient adsorption, and selective recognition for a variety of targets of biomedical and environmental importance.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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