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A Nontoxic Reversible Thermochromic Binary System via π - π Stacking of Sulfonephthaleins

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The mechanisms of thermochromic materials are mostly based on Bragg reflection, surface plasmon absorption, molecular structure changes, conformational stretching or aggregation. Here, a simple class of dye-solvent binary systems with clear and reversible thermochromism around ambient temperatures is reported with an alternative mechanism. This system comprises of commercially available sulfonephthaleins dissolved in liquid linear chain esters, acids or alcohols which demonstrate colour changes at different temperatures when the solvents transition from solid to liquid states. The colour changing mechanism is based on π - π stacked aggregation or disaggregation of the sulfonephthalein dyes. Cell toxicity testings confirmed the sulfonephthalein dyes are less toxic to NIH-3T3 cells than Cyanidin chloride, which is the only reported nontoxic food dye affording thermochromism, while this is based on an alternative mechanism. With the choice of nontoxic solvent, this binary system can form a nontoxic thermochromic material.

1. Introduction

There are many examples of commercial products which change colour under an external stimulus, such as pressure (piezochromic), light (photochromic), temperature (thermochromic) and those affected by changes in pH. Thermochromism was first discovered in 1888 by Reinitzer¹ with the discovery of the properties of cholesteryl benzoate liquid crystals. Thermochromic products have had great commercial success in the past with colour changing products such as beer cans, coffee mugs, nail polish, costumes and hair dyes. To date, scientists have developed a large range of thermochromic materials and systems with a number being applied into industrial applications.² Examples of thermochromic materials include liquid crystals,^{3, 4} photonic crystals,⁵ gold or silver nanoparticles,⁶ leuco dye systems,⁷ polydiacetylene derivatives⁸ and aggregachromic dyes.⁹ Bragg reflection,^{5, 10} surface plasmon absorption⁶, ring-opening and closing of molecular structures,⁷ protonation and deprotonation of dye materials¹¹ or conformational stretching of spiral structures⁴ have been used to explain the mechanisms of these thermochromic materials. Nevertheless, few reports mention the relative toxicity of these thermochromic materials and concern about their safety limits their application in the areas of food or health care products packaging.¹² Currently, the most utilized commercial thermochromic products are based on leuco dye systems. The leuco dye system, consists of three-components comprising of dye,

developer and solvent.¹³ The thermochromic mechanism of the leuco dye system relies on the molecular structure of the dye component switching between two isomeric forms in the presence of a developer, and this process is triggered by a phase change of the solvent.¹⁴ The solvent components of the system are typically relatively non-toxic long chain esters, alcohols or fatty acids.¹⁵ The dye components are one of the following class of compounds including triphenylmethane phthalides, rhodamine lactams, fluorans, phenothiazines, indolylphthalides, spiropyrans and leucoauramines, while the developers are typically phenolic compounds, thioureas, guanidines, benzothiazoles or benzothiazolyls.¹⁶ Due to concerns of the toxicity of the dyes and developers, industry is reticent to use these formulations on food product labels without significant barriers between the dye system and the food itself. A common consensus in the packaging area is that these leuco dye materials are recommended to only be applied to glass, metal or thick plastic substrate surfaces to avoid direct food contact.¹² Hence, solving the toxicity issue of dyes and developers is the key to reducing the toxic limitations associated with the leuco dye system.

Sulfonephthalein dyes manifest low toxicity according to their Material Safety Data sheets. These commercially available dyes are well-known pH indicators which change colour reversibly, based on pH triggered ring-opening and ring closing of their chemical structures.¹⁷ Based on their low toxicity and pH-chromogenic properties, sulfonephthalein dyes have been applied in colour changing hydrogels^{18, 19} and time-temperature indicators for monitoring food quality.²⁰ Recently, they have been used to fabricate thermochromic systems, for example, one sulfonephthalein dye (bromocresol purple) was used as a colour former in a three-component thermochromic system, in which stearic acid was the developer and long-chain

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alcohols were described as solvents with the reversible ring-opening mechanism similar to that of the leuco dye system.²¹

π - π stacking, a non-covalent interaction between molecules, has been reported to be the source of reversible thermochromism in conjugated polymers^{22, 23} and crystals²⁴⁻²⁶. It has also been used to explain colour changing phenomenon of aggregachromic dyes with highly conjugated planar structures^{9, 27}, such as triphenylenes²⁸ and perylene bisimides²⁹, which form dimers or other agglomerates in solution with an increase in dye concentration. Theoretical models were developed to explain or predict these aggregation behaviours using UV-Vis and ¹H NMR spectroscopy.³⁰ Based on π - π stacking theory, aggregachromic dye-polymer binary systems have been extensively studied with regard to their thermoresponsive colour changing properties.³¹⁻³⁶ There are two main areas in aggregachromic dye-polymer studies. The first involves mixing fluorescent dyes into polymer matrices to fabricate binary systems that show reversible or irreversible fluorescence change at different temperatures. However, these colour changes can only be observed under applied

conditions, such as UV light^{31-33, 35, 36}. The second area involves, dye-polymer binary systems which show thermochromism observable to the naked eye. However, their colour changes are irreversible and only occur above the glass transition temperatures of the polymer matrix³⁷⁻³⁹. Hence there is a need for thermochromic materials which are both fully reversible and are observable to the naked eye, for applications in the food packaging and health care industries which can be observed by the consumer.

Herein, we report the first example of a dye-solvent binary system that shows clear reversible thermochromism near room temperature resulting from the solubility-dependent π - π stacked agglomeration of sulfonephthalein dyes controlled by melting/crystallisation processing of the solvent. This simple binary thermochromic system is based purely on dye (sulfonephthalein) and solvent (linear chain esters, acids or alcohols). We further demonstrate that this thermochromic binary system is nontoxic. The mechanism study for thermochromism is further investigated using various analytical techniques including NMR, UV-vis absorption, XRD and FTIR-ATR analysis.

Figure 1. The chemical structures of A) sulfonephthalein dyes: chlorophenol (CPR), bromothymol blue (BTB), bromochloro purple (BCP), bromocresol green (BCG), bromophenol blue (BPB), thymol blue (TB); B) comparative dyes: methyl green (MG), crystal violet (CV), crystal violet lactone (CVL), and dimethyl yellow (DMY); and C) solvents: straight chain aliphatic esters, acids and alcohols.

2. Results and discussion

2.1 Thermochromism of binary sulfonephthalein and model dye systems

In the study of the proposed binary systems, we firstly used a dye dissolved in either aliphatic linear chain acids, esters or alcohols. Six sulfonephthalein 'dyes', chlorophenol red (CPR), bromothymol blue (BTB), bromocresol purple (BCP), bromocresol green (BCG), bromophenol blue (BPB) and thymol blue (TB), and 'solvents' with varying end group functionality (ester, alcohol, acid) and melting points were then used and

their structures are shown in Figure 1A and C respectively. In addition, examples of charged triarylmethane dyes, methyl green (MG) and crystal violet (CV); leuco dye, crystal violet lactone (CVL); and azo dye, dimethyl yellow (DMY) were used for comparison in a binary system (Figure 1B).

The dyes were dissolved in heated dodecanoic acid (DA) at 70°C at typical concentrations of 0.07 mol %, to form homogenous solutions showing different colours depending on the dye, as shown in Figure 2A and 2C. Upon cooling to 25°C, distinct colour changes were observed for sulfonephthalein samples resulting in non-uniform crystalline materials (Fig 2A).

Quantification of the colour change according to CIE2000⁴⁰ indicates the sample containing CPR (Fig. 2A, sample 'a') had a significantly high $\Delta E = 37.8$, with a distinctive contrast in colour. For this reason, the subsequent investigations used CPR as an example. The colour changes happen between melted state and solid state of solvents. Therefore, the threshold temperature of colour change is related to the melting point or freezing point of solvent in the system. A plot of the absorbance (541nm) versus temperature of CPR-DA (0.07 mol%) upon heating (Fig. S1), shows a steep decrease in intensity to a constant value around 43-44°C. The red colour disappearance is accompanied by the melting process of DA

occurring at its melting point of $43.77 \pm 0.03^\circ\text{C}$. The CIE chromaticity chart (Fig. 2B), illustrates the colour shift of six sulfonephthalein dye-DA samples (Fig. 2A, samples a-f). Similarly, six sulfonephthalein dye-methyl dodecanoate (MDD) samples (Fig. S2) also show colour change between solid (0°C) and liquid (25°C) state. In contrast, four common dyes (MG, CV, CVL and DMY) when combined in a binary system (Fig. 2C, samples g-j) showed no colour change between solid and liquid states. The chemical structure of the sulfonephthalein dyes is therefore important in the thermochromism of the binary system.

Figure 2. A) Photographic images of the colour change between solid (25°C) and liquid (70°C) state of six sulfonephthalein dyes (0.07 mol%) in DA. B) Standard CIE 1931 chromaticity chart showing the x-y values in colour transition between solid (25°C) and liquid (70°C) state of samples a-f. C) Photographic images of solid (25°C) and liquid (70°C) state of MG, CVL, DMY and CV (0.07 mol%) in DA.

Figure 3. A) Photographic images of the colour change between solid (25°C) and liquid (70°C) state of CPR (0.07 mol%) in a) hexadecanoic acid, b) methyl hexadecanoate and c) hexadecanol. B) The corresponding UV-Vis absorption spectra of all solid CPR-solvent samples (a-c) at 25°C , showing strong absorption peaks at 541nm compared to liquid samples (a'-c') at 70°C independent of the functionality of the solvent.

The functionality of the solvent molecules and their importance for thermochromism was studied using acid, alcohol and ester moieties of linear chain hexadecane, with CPR as the dye molecule. Figure 3 shows the colour change between solid (25°C) and liquid (70°C) states and their corresponding UV-Vis absorption spectra of samples of CPR-hexadecanoic acid (CPR-HA) (a-a'), CPR-methyl hexadecanoate (CPR-MH) (b-b') and CPR-hexadecanol (CPR-HD) (c-c'). It is interesting that CPR exhibits slightly different colour in each liquid solution due to solvatochromism phenomenon⁴¹, yet all yellow liquid solutions turned red upon solidification at 25°C , whether the solvent is acid, ester or alcohol. Correspondingly, the UV-Vis absorption peak or shoulder of liquid CPR-hexadecanoic acid, CPR-methyl hexadecanoate and CPR-hexadecanol solutions are at 396 nm, 385 nm and 423 nm

respectively. After solidifying at 25°C, a peak at 541nm appears in the UV-Vis spectra of all three samples which is indicative of the emerging red colour.

In order to investigate the effect of hydrophobicity and hence solubility on the thermochromic nature of the binary system, a series of mixtures containing CPR (0.07 mol %) in hexadecanol (HD), tetradecanol (TD) or dodecanol (DD) were prepared. As the aliphatic chain length increases along with an increase in hydrophobicity, the solubility of the hydrophilic CPR in the solvent decreases, leading to phase separation. At CPR concentrations of 0.07 mol%, the solid binary systems of CPR-HD and CPR-TD are heterogeneous and exhibit a strong red appearance with $\lambda_{\text{max}} = 541 \text{ nm}$ (Fig. 4A and 4B: a, b). At this concentration however, CPR is fully solubilized in DD, even below its freezing point (24°C) and shows no thermochromism (Fig. 4A and 4B: c). As the concentration of CPR is increased to

0.2 mol% in DD, phase separation occurs in the solid state and the characteristic red colour at $\lambda_{\text{max}} = 541 \text{ nm}$ is observed (Fig. 4A&4B: d). Microscopic images of sample 'a' in the solid state shows strong red coloured regions of aggregated dye within the crystalline hydrophobic solvent regions. Video analysis (Fig. 4C) monitoring the thermochromism of sample 'a' upon cooling (Supporting Information), reveals agglomeration of the dye compounds forming between the regions of crystalline growth of the solvent. These observations reveal that the thermochromic behaviour of this binary system is dependent on the solubility of the dye in the solvent matrix. For effective thermochromism in this system the concentration of the dye must be such that dye is able to agglomerate below the freezing point of the solvent and be fully dissolved above the melting point of the solvent.

Figure 4. A) Photographic images of CPR-HD (0.07 mol%), CPR-TD (0.07 mol%), CPR-DD (0.07 mol%) and CPR-DD (0.2 mol%) at solid state (20°C, a-d) and liquid state (70°C, a'-d'). B) The corresponding UV-Vis absorption spectra of solid (20°C) samples a-d and liquid (70°C) samples a'-d' and microscopic image of solid sample 'a'. C) Captured images from microscopic video of solidification of sample 'a' upon cooling.

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Figure 5. A) Plots of UV-Vis absorbance at 541nm of CPR-DA vs. time upon cooling from 70°C to 35°C. The concentrations of CPR in binary systems were 0.07 mol%, 0.03 mol%, 0.02 mol%, 0.01 mol% and 0.007 mol%, respectively. B) Plots of colour change equilibrium time vs. concentration of dye in CPR-DA binary system.

The concentration of dye in the binary system plays a very important role in its thermochromic behaviour, therefore, the effect of dye concentration on the colour changing time of this binary system is also examined. Here, CPR-DA solutions with five different CPR concentrations of 0.07, 0.03, 0.02, 0.01 and 0.007 mol% were cooled from 70°C to 35°C under the same conditions. The freezing points of these binary systems at varying concentrations are very similar ($F_p: 41.7 \pm 0.1^\circ\text{C}$, Table S1) with the change in CPR concentration having little effect.

However, the effect of concentration on the time for the colour to change upon cooling is significant. A plot of the absorbance (541nm) versus colour changing time of these samples upon cooling (Fig. 5A), shows a steep increase in intensity to a constant value. The colour change equilibrium time was taken as the intersection of the extrapolated baseline and the tangent to the plot at the inflection point (Fig. 5A). From the comparison of colour change equilibrium time of each sample (Fig. 5B), it is clear that higher concentrated CPR-DA solution showed a faster colour change upon the same rate of cooling. This phenomenon can be explained in this way: the solubility of the dye in solvent decreases as the temperature drops, with the dye being excluded from the solvent phase; as a result, the more concentrated solution reaches its saturated point faster, forming a more coloured solid than those at lower concentrations. Therefore, the colour changing speed of this binary system can be adjusted by varying the dye concentration.

2.2 Cell toxicity testing

The relative cytotoxicity of these six sulfonephthalein dyes were examined by using the CCK-8 approach⁴². NIH-3T3 (noncancerous fibroblasts) cell lines were incubated with different concentrations of dyes for 24 hours and then processed with fresh medium containing CCK-8 solutions for a further 3 hours to determine cell viability. As shown in Figure S3, TB is more toxic to NIH-3T3 cells than other sulfonephthalein dyes and the viability of cells decreases to 70% at concentration of 200 $\mu\text{g/mL}$ TB. Except for TB, a negligible cytotoxicity effect on NIH-3T3 was observed when the concentration of all sulfonephthalein dyes ranges from 0 to 200 $\mu\text{g/mL}$, and all viabilities are more than 90% after 24 hours incubation. Especially, toxic effects of BTB, BCP and CPR on cells just have been seen at very high concentration (>500 $\mu\text{g/mL}$).

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Figure 6. (A) Cytotoxicity of CPR, cyanidin chloride and rhodamine B base toward NIH-3T3 cells incubated for 24 h. (B) Comparison of the IC₅₀ values of the CPR, cyanidin chloride and rhodamine B base in NIH-3T3 cells. *P<0.05 and **P<0.01.

Figure 7. A) Digital pictures of CPR-MDD binary system with concentration of 0.07 mol% after being treated by ammonium or hydrochloride vapour for 1 minute at 25°C and corresponding solids at 0°C. B) The corresponding UV-Vis spectra of sample a, a', b and b' and ring-closed or ring opened molecular structures of CPR.

Triarylmethane fluorans such as rhodamine B has been studied as colour former in Leuco dye system for decades and is one of the most commonly used ingredients in current thermochromic products.⁴³⁻⁴⁶ On the other hand, the food dyes are usually regarded as safe additives⁴⁷ and have gradually been used in thermochromic area.⁴⁸ Therefore, it is necessary to compare the relative toxicities of the Leuco dyes, food dyes and sulfonephthaleins used in this work. Three dyes were chosen from each class, namely rhodamine B base, cyanidin chloride and chlorophenol red (CPR) respectively.

As shown in Figure 6A, both cyanidin chloride and rhodamine B base are statistically toxic to NIH-3T3 cells in the concentration range of 0-1000 μg/mL. The viability of cells decreases to 45 % and 36 % at concentration of 200 μg/mL cyanidin chloride and rhodamine B base, respectively. These results indicate all sulfonephthalein dyes including TB are less toxic to NIH-3T3 cells than Cyanidin chloride or Rhodamine B base. The calculated half-maximum inhibitory concentration value (IC₅₀) for cyanidin chloride and rhodamine B base were 172 and 163 μg/mL, respectively, which were significantly lower than CPR which was 786 μg/mL (Figure 6B). Cyanidin chloride had a surprisingly similar IC₅₀ to that of rhodamine B base, which may inhibit its use in commercial applications. Considering the low toxicity and very low dosage of sulfonephthalein, this binary thermochromic system can be regarded as nontoxic when it is used with a non-toxic solvent, e.g. dodecanoic acid,^{49,50} and has potential to be applied to real commercial food or health care products packaging.

2.3 Mechanism study

Sulfonephthalein are commonly used as pH indicators due to their strong colour and transition over a wide pH range. The mechanism for this equilibrium is well known with the opening of the sulfoxide ring leading to the highly coloured resonance structure shown in Fig. 7.^{17, 51} For example, CPR has a characteristic purple colour above pH 10 when ring opened and a yellow colour below pH 4.6 in the ring closed form.⁵² In order to investigate the chemical structure of CPR in this binary system, melted CPR-methyl dodecanoate (CPR-MDD, Fig. 7a) was treated with basic ammonium hydroxide vapor (aq. NH_4OH , 25 °C) for 1 min. The material became purple in appearance with a $\lambda_{\text{max}} = 596 \text{ nm}$ (Fig. 7b) and upon cooling led to a stronger purple colour with a $\lambda_{\text{max}} = 585 \text{ nm}$ (Fig. 7b'). The CPR-MDD solution returned to a pale-yellow solution (Fig. 7a) after being reacidified with hydrochloric acid vapor (aq. HCl , 25 °C) for 1 min which formed a red solid (Fig. 7a') upon cooling. This is in contrast to the thermochromic colour change observed by the solid CPR-solvent binary systems (Fig. 3B, 4B and 7B) with an absorbance peak of around $\lambda_{\text{max}} = 541 \text{ nm}$ (Fig. 7a') in UV-Vis spectra. In other words, ring-closed CPR resulted in a unique absorbance peak at 541nm, while the ring-opened CPR shifted from 596nm to 585nm after solidification. Therefore, for reversible thermochromism, CPR must not remain in the ring opened state. Further evidence of the CPR structure in our binary thermochromic system comes from FTIR analysis (Figure S4 of Supporting Information). FTIR measurements indicated that the binary system of CPR-TD (10 mol%) at both hot liquid (70 °C) or cool solid (25 °C) showed similar peaks attributed to the sulfone group at 1345 and 1191 cm^{-1} which indicates a ring-closed CPR structure²¹ at both temperatures and different colours. After treatment with base however, peaks at 1180 and 1630 cm^{-1} due to the benzene sulphonate and *p*-quinoid groups respectively appear^{53,54} which are consistent with the ring opened structure of CPR. This evidence confirms the thermochromic mechanism of this binary system is not related to a ring-opening of dye molecules and that reversible thermochromism only occurs when the dye remains in the ring closed state.

Figure 8. XRD patterns of pure (a) 100% solid TD, (b) CPR-TD solid binary system with 10 mol% CPR, (c) CPR-TD solid binary system with 25 mol% CPR and (d) 100% solid CPR single crystal.

Furthermore, the XRD patterns of solid CPR-TD binary system (Fig. 8) indicate CPR is crystallized in its agglomeration. Compared to TD (Fig. 8a), the pattern of CPR-TD binary system with 10 mol% CPR (Fig. 8b) exhibits an extra small diffraction peak at 7.8°. As CPR is concentrated to 25 mol%, this diffraction peak dominates the XRD pattern (Fig. 8c) and is along with appearance of other peaks (●). However, the peaks from CPR agglomeration do not match the peaks of pure CPR single-crystalline form (Fig. 8d). Therefore, CPR molecules within the solid mixture agglomerate into another crystalline form, different from pure CPR. Macroscopically, this is consistent with the fact that CPR agglomeration appears red rather than greenish-brown, reported for pure CPR powder.⁵⁵ Proton NMR was used to further elucidate the molecular structure of CPR in the binary system. Analysis was performed on mixtures of CPR dissolved in d_3 -methanol at 10⁻⁴, 0.1 and 2 mol % (sample α - γ in Fig. 9). As shown in Fig. 9A, the colour of the methanol solution became darker and changed colour, with an increase in CPR concentration. The ¹H NMR spectra of CPR at increasing concentrations in d_3 -methanol showed exclusive up-field shift of the aromatic hydrogens (Fig. 9B, signals a, b, b' and c) connected to the sulfoxide ring. This effect is attributed to aromatic electron shielding and is always observed in π - π stacking of conjugated planar molecules.^{26, 30, 56-58} It is well established that electron withdrawing groups attached to aromatic rings enhance π - π stacking.^{59, 60} In the case of sulfonephthalein dyes, the electron withdrawing sulfoxide (SO_3^-) group connected to the benzene ring, leads to an increase in the π - π stacking and increased intensity due to the agglomeration of the dye molecules.

The UV-Vis absorption spectrum of CPR in methanol sample at low concentration (Fig. 9A: α : 0.1 mmol %) shows insignificant shift in wavelength between the liquid and solid states. Also, at a high concentration of CPR (Fig. 9A: γ : 2 mol %), no peak shift in wavelength is observed. In both of these cases no thermochromism is observed upon solidification and the CPR molecules remain either homogeneously dissolved or completely aggregated in both solid and liquid states. The UV-Vis spectrum of CPR in methanol at medium concentration (Fig. 9A: β : 0.1 mol%) shows a significant shift in wavelength between the solid and liquid states, with an obvious thermochromism being observed for the sample. At this concentration CPR is fully solubilized in the liquid methanol and is insoluble enough in the solid methanol to lead to π - π stacking and thermochromism.

This observation is analogous to the thermochromism seen for the binary systems of dye and long chain hydrophobic solvents shown earlier. The UV-Vis absorption spectra of red solid CPR-HA, CPR-MH, CPR-HD, CPR-TD and CPR-DD in Fig. 3 or Fig. 4 show the peak at 541nm. Although the UV-Vis absorption peaks in spectra of liquid and solid CPR-Methanol (2 mol%) and solid CPR-Methanol (0.1 mol%) are broad due to high intensity of absorbance, their locations are still around 541nm (Fig. 9A). Therefore, CPR shows similar colour change and same behaviour of UV-Vis spectra both in methanol and long chain hydrophobic solvents. Here the dye structures agglomerate between the regions of the solvent crystal

boundaries. The schematic diagram in Figure 9C illustrates the proposed mechanism of thermochromism in solution: 1) a low concentration of dye molecules are surrounded by solvent molecules and show the colour of diluted liquid solution; and 2) with an increase in dye molecules, they agglomerate in solution resulting in a stronger colour; and 3) dye molecules are excluded from the solvent crystal structure as the solvent solidifies and forms agglomerated structures at the solvent crystal boundaries.

To further illustrate the π - π stacking mode of CPR in the binary system, molecular dynamics (MD) simulations were used to model the preferential alignment of the CPR molecules in methanol at 25°C and -196°C. The eight CPR molecules quickly (within a few picoseconds) approach each other forming a rather compact cluster at -196°C (Fig. S5B at 10 ns), contrary to room temperature that the CPR molecules are dispersed in methanol (Fig. S5A at 10 ns). Figure S6A shows a snapshot of the benzene rings connected to the sulfoxide group (highlighted pink atoms) of eight CPR molecules in 200 methanol molecules at -196°C. Three pairs of benzene rings are shown, two of which (rings 1 & 2 and rings 2 & 3) exhibit edge-to-face π - π stacking.⁶¹ Specifically, at $t = 10$ ns the geometric centre of the planes of rings 1 & 2 have a distance of 4.94 Å and interplanar angle of 65.2° (Fig. S6B: green line), while those of rings 2 & 3 are 4.96 Å and 85.4°, respectively (Fig. S6B: black line). Even though rings 2 and 4 (Fig. S6B: red line) have a shorter plane-to-plane distance of 4.75 Å, they do not form π - π stacks as their interplanar angle is 48.9°.⁶¹ In

fact, the π - π stacking formation is maintained throughout the simulation ($t > 0$ ns) as evidenced by the time evolution of the interplanar distance and angle (Fig. S6B) of pair rings 1 & 2 (green diamonds) and 2 & 3 (black circles).

Additionally, the π - π stacking formation is also confirmed by the calculated UV/Vis absorption spectra (Fig. 10) of CPR molecules at 25°C (broken line) and -196°C (solid lines). At -196°C, increasing the number of CPR molecules from 1 (Fig. 10: red line) to 4 (Fig. 10: blue line) causes a red-shift in the absorbance from 215 nm to 400 – 500 nm, respectively, due to increasing length of π -electron conjugation.⁶² At 25°C, however, the 8 CPR molecules are dispersed in methanol (Fig. S5A at 10 ns), acting as single molecules that result in absorption peaks between 200 – 300 nm in the UV/Vis spectrum (Fig. 10: broken line).

In summary, the thermo-induced solvent phase change results in agglomeration or dis-agglomeration of sulfonephthalein molecules and is accompanied with changes in the localised concentration of dye molecules, both combining to affect a strong colour change of the samples. Unlike other thermochromic π - π stacking systems, sulfonephthaleins do not conjugate uniformly on a plane and are further enhanced by aggregachromic changes. The solvent crystal boundaries during crystallisation of the binary system provide limited space for dye π - π stacking, which enables them to be reversed upon melting, a reduction in π - π stacking, reversal of the colour and full homogenous solvation of the dye molecules.

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Figure 9. A) The photographic images of CPR-methanol samples with concentration of (α) 0.0001 mol%, (β) 0.1 mol% and (γ) 2 mol% at 25 °C and -196 °C. The corresponding UV-Vis absorption spectra indicate only sample β shows thermochromism. B) ^1H NMR spectra of CPR (0.1 mol% and 2 mol%) in CD₃OD showing an upfield shift for peaks a, b, b' and c. C) A schematic representation of CPR molecules in solvent in diluted liquid state, concentrated liquid state and diluted solid state.

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Figure 10. Absorbance spectra of 1 (red solid line), 2 (green solid line), 3 (black solid line) and 4 CPR molecules (blue solid line) at -196°C and 8 CPR molecules at 25°C (black broken line).

Conclusions

In conclusion, a new class of reversible and nontoxic dye-solvent binary thermochromic materials is reported. These binary systems with sulfonephthaleins as dyes and linear chain esters, acids or alcohols as solvents show clear colour changes from the solid to liquid states. The colour change is attributed to changes in π - π stacking, caused by different dye solubilities in the liquid or solid solvent. Contrary to the mechanism associated with pH indicator dyes, this binary system involves ring-closed sulfonephthaleins exhibiting aggregachromic phenomenon. The relative low toxicity and concentration of the sulfonephthalein dyes, make this system promising for real commercial food or health care products packaging applications where thermochromic observations can be utilized. Although the fabrication is simple and rapid, incorporating this binary system into a processable sealed film will be the next challenge for further study.

Experimental section

Materials

Chlorophenol red (CPR, indicator grade, Aldrich), bromothymol blue (BTB, 99%, Ajax Finechem), bromocresol purple (BCP, 99%,

Ajax Finechem), bromocresol green (BCG, 99%, Chem-supply), bromophenol blue (BPB, 99%, Ajax Chemicals), thymol blue (TB, 99%, Sigma-Aldrich), crystal violet lactone (CVL, 97%, Sigma-Aldrich), dimethyl yellow (DMY, 98%, Sigma-Aldrich), crystal violet (CV, 99%, Sigma-Aldrich), methyl green (MG, 85%, Sigma-Aldrich), Cyanidin chloride (98%, BOC Sciences, USA), rhodamine B Base (97%, Aldrich), 32 wt.% hydrochloric acid solution (Ajax Finechem), aqueous ammonium hydroxide solution (28% w/w%, Sigma-Aldrich), chloroform (99.8%, RCI Labscan), sodium chloride (NaCl, Emsure), sulfuric acid (H_2SO_4 , 98%, RCI Labscan), methanol (99.8%, Chem-supply), deuterated methanol (CD_3OD , 99.8 atom %D, Aldrich), dodecanoic acid (98%, Sigma-Aldrich), 1-dodecanol (98%, Sigma-Aldrich), 1-tetradecanol (97%, Aldrich), methyl dodecanoate (98%, Sigma-Aldrich), methyl hexadecanoate (97%, Sigma-Aldrich), hexadecanoic acid (99%, Sigma) and 1-hexadecanol (99%, Aldrich) were used as received without further purification.

Instrumentation

Differential Scanning Calorimetry (DSC). Full Heat flow curves were measured on a Perkin Elmer Differential Scanning Calorimeter 8500 under a nitrogen atmosphere. Melting points or freezing points were taken at the intersection of the extrapolated baseline and the tangent to the heat flow curve at the inflection point of appropriate side of the peak⁶³ when heating or cooling rate is $1^{\circ}\text{C}/\text{min}$.

Fourier Transform Infrared Spectroscopy (FTIR-ATR). FTIR spectra were recorded on a Bruker TENSOR II FTIR-ATR spectrometer at a resolution of 4 cm^{-1} over the range of 4000 cm^{-1} to 400 cm^{-1} . Measurements were determined at both 25°C and 70°C controlled by the Specac ATR assembly.

X-ray diffraction (XRD). XRD patterns of the samples were recorded at 25°C on a Bruker D8 Advance diffractometer with $\text{Cu K}\alpha$ radiation (40 kV, 40 mA) and a nickel filter, and the samples were exposed at a scanning rate of $2\theta = 0.020^{\circ}/\text{s}$ in the range of 5° – 85° .

^1H Nuclear Magnetic Resonance (NMR) spectroscopy. ^1H NMR spectroscopy was carried out at 25°C on a Varian Unity 400 MHz spectrometer, using deuterated methanol (CD_3OD) as solvent. CPR (0.1 mol% in CD_3OD) ^1H NMR (400 MHz): δ 7.92 (d, $J=7.6\text{ Hz}$, 1H), 7.77 (t, $J=7.6\text{ Hz}$, 1H), 7.71 (t, $J=7.6\text{ Hz}$, 1H), 7.46 (d, $J=8\text{ Hz}$, 1H), 7.18 (d, $J=2.4\text{ Hz}$, 2H), 7.04 (dd, $J=8.8$, 2.4 Hz, 2H), 6.89 (d, $J=8.8\text{ Hz}$, 2H). CPR (2 mol% in CD_3OD): ^1H NMR (400 MHz): δ 7.85 (d, $J=7.6\text{ Hz}$, 1H), 7.66 (t, $J=7.6\text{ Hz}$, 1H), 7.60 (t, $J=7.6\text{ Hz}$, 1H), 7.40 (d, $J=7.6\text{ Hz}$, 1H), 7.20 (d, $J=2.4\text{ Hz}$, 2H), 7.03 (dd, $J=8.8$, 2.4 Hz, 2H), 6.89 (d, $J=8.4\text{ Hz}$, 2H).

Chromaticity. Photographs of bulk samples were calibrated for white balance using Adobe Photoshop CS6 as Camera Raw

format⁶⁴. The x and y coordinates of the CIE chromaticity were determined by conversion from RGB values of the colour balanced images⁶⁵.

Ultraviolet visible (UV-Vis) Spectroscopy. UV-Vis absorption analysis was performed on a Shimadzu UV-1800 spectrophotometer combined with an Ocean Optics DH-2000 optical fibre light source and Flame UV-VIS-ES miniature spectrometer. The thickness of the quartz cuvette containing samples for analysis was 1 mm.

Light Microscope. Optical microscope images and videos were captured using an Olympus CKX41 microscope with AnalySIS getIT software.

UV-Vis Kinetic Analysis

The samples chambers of a Pharmacia LKB • Novaspec II spectrometer connected to a thermostatic water bath was kept at 35°C. A quartz cuvette (optical path length of 1 mm) containing sample was pre-heated at 70°C for 30 mins prior to being placed rapidly into the thermostatted spectrometer chamber. The absorbance was recorded every 0.5 sec at a single wavelength of 541 nm.

Dye Toxicity Study.

Normal NIH-3T3 cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), GlutaMAX™ (2 mM), and Penicillin (100 units/mL). Cells were passaged every 3–4 days using 0.25% trypsin-EDTA at subconfluence and incubated at 37°C, 5% CO₂, and 90% humidity. Cell passages 5–15 were used for cell experiments⁶⁶. The cell viability tests were analyzed by the standard cell counting kit-8 (CCK-8) assay method. NIH-3T3 cells were seeded into 96-well plates (1 × 10⁴ cells per well) in cell culture medium. After 12 hours, the medium was replaced with 100 µL of fresh medium containing various concentrations of dyes (0–1000 µg/mL) and incubated for a further 24 h. The cells were then washed three times with PBS and incubated with 110 µL fresh medium containing 10 µL CCK-8 solutions for a further 3 hours. Finally, the medium was removed and the absorbance at 460 nm was measured using a microplate reader (TECAN M200 infinite Pro). Note that all experiments were conducted in triplicate, and error bars represent the standard error of independent experiments. The cell viability (%) was calculated by the following formula, where [A] is the average absorbance:

$$\text{cell viability(\%)} = \frac{[A]_{460(\text{sample})} - [A]_{460(\text{blank})}}{[A]_{460(\text{control})} - [A]_{460(\text{blank})}}$$

Statistical Analysis: Data are shown as averages and standard deviations. The student's t tests were used to analyze the statistical differences between samples for cytotoxicity and were considered significant at $p < 0.05$.

Single crystal of CPR growth.

Chloroform was kept with activated molecular sieves for 48 hours. HCl gas generated from the reaction of solid NaCl and 98% H₂SO₄ was injected into the chloroform. 20 mg CPR was dissolved in 5 g acidified chloroform and the dye solution was kept in a glass vessel that covered by a layer of aluminium foil with several holes. The glass vessel was surrounded by 30 g

hexane in a sealed glass jar at room temperature (25°C) until single crystals growth finished.

Computational Methods.

Molecular dynamics (MD) simulations were used to model the preferential alignment of the CPR molecules in methanol at 25°C and −196°C. Eight CPR molecules were placed at the centre of a 30 × 30 × 30 Å simulation cell surrounded by 200 methanol molecules (initial configuration is shown at the top of Fig. S5A&B of the Supporting Information). The equations of motion are integrated by the velocity-Verlet algorithm⁶⁷ with a timestep of 1 fs in the NVT (constant number, volume, and temperature) ensemble using the polymer consistent force field (PCFF). The MD simulations were performed on the University of Melbourne's High-Performance Computing system for up to 10 ns using the LAMMPS MD code.⁶⁸ The UV/Vis absorption spectra were calculated using ORCA's UV/Vis spectra simulator.^{68–70}

Conflicts of interest

There are no conflicts to declare.

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Table of Contents

The first example of dye-solvent binary system that shows clear reversible thermochromism near room temperature.