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ORIGINAL PAPER

Interaction of homeopathic potencies with the water soluble solvatochromic dye bis-dimethylaminofuchsone. Part 1: pH studies

Steven J Cartwright*

DiagnOx Laboratory, Cherwell Innovation Centre, Upper Heyford, Oxon, OX25 5HD, UK

Introduction: **Previous studies have demonstrated the potential of solvatochromic dyes for investigating the physical chemistry of homeopathic potencies.**
Results: **Following examination of this class of dyes in organic solvents, results obtained using the positively solvatochromic dye Bis-dimethylaminofuchsone (BDF) in aqueous solution are now reported. Spectral changes observed with this dye in the presence of potencies are both substantial and reproducible. Studies across a wide range of pH values reveal an unusual pH dependence for the dye's interaction with homeopathic potencies. Results indicate potency enhances dye protonation at pH values below c. 7.0, whilst protecting the dye from attack by hydroxyl ions above c. pH 7.5.**
Conclusion: **A possible explanation for these observations is offered in terms of a potency – induced electron density shift in BDF. The interaction of homeopathic potencies with solvatochromic dyes, particularly BDF, points towards a possible physico-chemical model for the nature of potencies, how they may be interacting with this class of dyes, and moreover how their biological effects may be mediated.** *Homeopathy* (2017) **106**, 37–46.

Keywords: Solvatochromism; Bis-dimethylaminofuchsone; Water solubility; Homeopathic potencies; pH; pKa values; Excited state

Introduction

Previous studies[1] have indicated that solvatochromic dyes offer a useful way of studying the fundamental nature of homeopathic potencies (serially diluted and succussed solutions). This class of dye has an electronic structure in which an electron moves spatially as well as energetically upon irradiation by light. The exact wavelength (and hence energy) of absorbed light is determined by the chemical structure of a dye and the polarity of the solvent in which the dye is placed.[2] Unfortunately few commercially available solvatochromic dyes are water soluble and this presents a number of challenges. Organic solvents are difficult to work with and legitimate concerns regarding potential water contamination and an inability to easily control the pH of many solvents has led to the search for an appropriate water soluble solvatochromic dye.

Despite interactions having been demonstrated between homeopathic potencies and N,N-dimethylindoaniline (Phenol Blue), 2,6 Dichloro-(2,4,6-triphenyl-pyridinium-1-yl)-phenolate (ET33) and Brooker's Merocyanine in aqueous solution,[1] these three water soluble dyes do not produce large enough spectral changes to allow more detailed studies to begin. An appropriate dye needs to fulfil a number of criteria if dye/potency studies are to be taken further. These include, as stated above, water solubility, and if pH buffers are to be used, solubility that is relatively unaffected by increased ionic strength. In addition positive solvatochromicity is necessary, such that distinct and

*Correspondence: Steven J Cartwright, DiagnOx Laboratory, Cherwell Innovation Centre, Upper Heyford, Oxon, OX25 5HD, UK.
E-mail: steven.cartwright@oxford-homeopathy.org.uk
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characteristic absorbance peaks in the visible region of the electromagnetic spectrum are seen in aqueous solution. Negatively solvatochromic dyes produce hypsochromically shifted spectra as solvent polarity increases and their spectra in water are usually narrower with a much reduced extinction coefficients, making them less useful for detecting potencies. In contrast positively solvatochromic dyes produce more detailed bathochromically shifted spectra with increased extinction coefficients as solvent polarity increases. These features make the latter dyes better suited for investigating homeopathic potencies.

Consequently a detailed search has been undertaken to identify appropriate water soluble, positively solvatochromic dyes which might be expected to produce significant spectral changes in the presence of homeopathic potencies based on the preliminary studies reported previously.[1] Bis-dimethylaminofuchsone (BDF)[3–5] (Figure 1) fulfils the criteria listed above. It is fully water soluble and can be used in solution with buffers at a concentration of at least 20 mM. It has a significant and characteristic spectrum in water well into the visible region of the spectrum with a high extinction coefficient (40,000 M^{-1}). In addition, in common with other positively solvatochromic dyes it displays aggregation behaviour with a concomitant hypsochromic shift in its spectrum. BDF is also halochromic[2] — it interacts with both metal ions and protons to produce a significantly bathochromically shifted spectrum. All of these features turn out to be important in understanding the nature of the interaction of BDF with potencies.

Experimental protocol

Much of the experimental procedure followed in this study are as stated in Materials and methods and largely follow those published previously.[1] However, there are a number of improvements which it is important to discuss in relation to the results presented below.

Firstly it has been noted over the course of many months that more consistent data has been obtained if dye-control and dye-potency incubations are kept out of ambient light. Consequently, immediately on addition of potency or control to dye solutions an initial scan is made and then the cuvettes are placed in separate black plastic film canisters and kept under these conditions away from light between any subsequent scans. This procedure has been adopted for both control-dye solutions and potency-dye solutions in order to avoid any variations in light exposure between samples. It is unclear at this stage whether light is affecting the dye alone, potency alone or the dye-potency interaction or some combination of these three. The effect of ambient light is only seen over the course of many hours and is a function of light intensity. However, where incubations are carried out over 12 h or more the variation of ambient light intensity from overhead lights etc. under normal laboratory conditions is such that the precaution of incubation in light-excluding film canisters is necessary. This procedure effectively ensures control and potency incubations are comparable. The potential interference by light in potency-BDF interactions is discussed below in the context of results.

Second, whilst BDF has been made up and kept in aqueous solution, potency preparations are supplied by pharmacies in 90% ethanol. Dilution 100-fold into reverse osmosis water was therefore routinely carried out and the new water-based potencies used in all assays. Control solutions involved the 100-fold dilution of 90% un-medicated and un-succussed ethanol into reverse osmosis water. In both cases the bottles into which dilution takes place were from the same batch. Exact material comparability should therefore exist between the control and potency solutions used in this study. ICP-OES analysis of leachates from bottles shows this to be the case (see Materials and methods for details).

BDF chemistry

Figure 1 shows the structure of BDF. In the dye's excited state an electron moves from the electron-rich dimethylamino moieties to the relatively electron-poor carbonyl moiety. This process is solvent sensitive with the absorbance peak/shoulder for BDF moving from 565/523 nm in water to 558/521 nm in ethanol and 540/480 nm in tert-butyl alcohol. This hypsochromic shift with decreasing solvent polarity is consistent with positive solvatochromism.

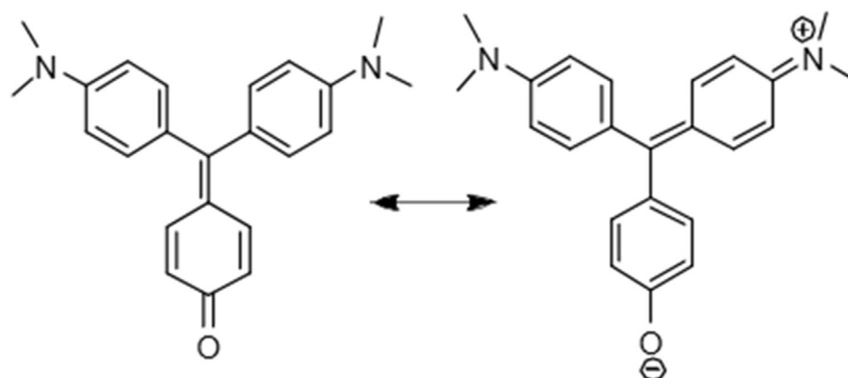

Figure 1 Chemical structure of BDF (4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-Cyclohexadien-1-one) with ground (left) and excited states (right) shown.

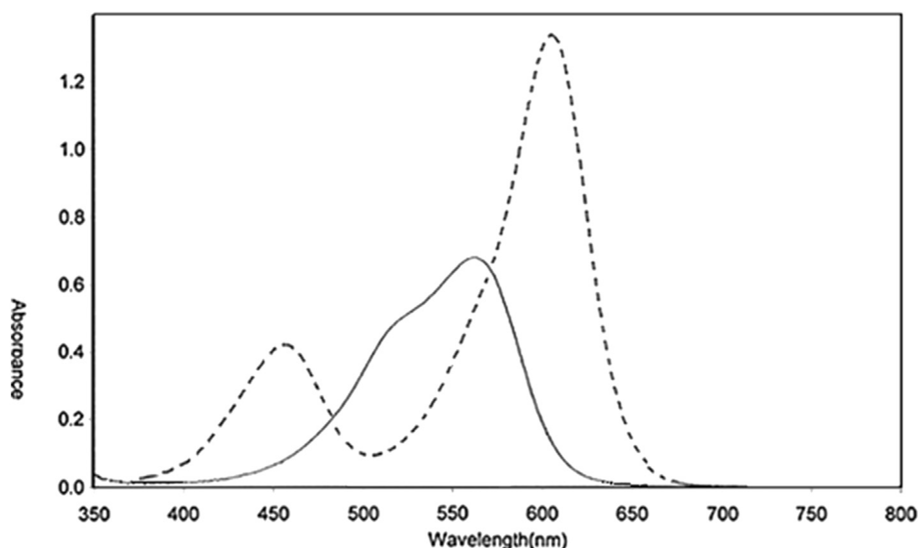

Figure 2 Spectrum of 15 μM BDF in 20 mM borate buffer pH10 (solid line). Spectrum of 15 μM BDF in 20 mM citrate buffer pH4 (broken line).

Figure 2 shows the spectrum of BDF in water buffered at pH 10 (20 mM borate buffer) and at pH4 (20 mM citrate buffer). At pH 10 the dye's absorbance maximum is at 565 nm with a shoulder at 523 nm. This shoulder is seen in all spectra of BDF from c. pH7 upwards. By comparison with the non-solvatochromic dye Crystal Violet and other triphenylmethane dyes where it is known such shoulders represent aggregates,[6] it seems reasonable to conclude the shoulder in BDF also represents aggregated dye. An increase in the 523 nm/565 nm absorbency ratio with increasing BDF concentration supports this view (data not shown).

Protonation of the carbonyl oxygen in BDF (Figure 3) produces new peaks at 605 nm and 458 nm with the concomitant disappearance of the 565 nm peak/523 nm shoulder. Figure 2 shows a typical spectrum at pH4. The pKa for BDF protonation − the pH at which 50% of the dye is protonated − is given as 6.3 in the literature.[3] The current study has produced a figure of 6.5 for the pKa of BDF, in close agreement with this figure. The protonation of BDF is fully reversible at the acid pH values used in this study (Figure 3). In addition there is no evidence of dye aggregation occurring in acidic medium. Protonation appears therefore to correlate with dye disaggregation, whereas un-protonated dye is prone to aggregation (Figure 2).

At high pH BDF is subject to attack by hydroxyl ions[3,5] (Figure 3). The initial reaction is reversible (with an

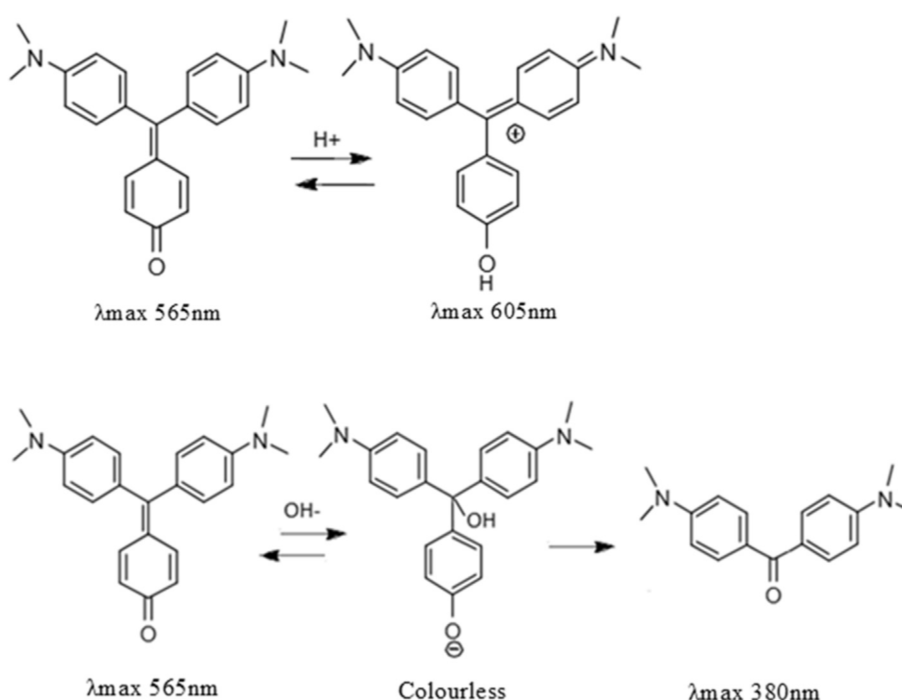

Figure 3 Chemistry of BDF showing the equilibrium between protonated and un-protonated BDF in acidic medium (above) and the more complex equilibrium and slow decomposition of BDF in basic medium (below).

equilibrium lying over to the left) but over time an irreversible decomposition takes place to produce Michler's ketone (MK) with an absorbance peak at 380 nm.

Results

On adding an aliquot of potency solution (*Glycerol* 50 M) to an equilibrated BDF buffered solution at pH values below c. 7.0 and scanning this against an exactly materially equivalent control-BDF solution at the same pH, a difference spectrum appears with peaks at c. 605 nm and c. 458 nm. These spectral differences slowly build up over time and reach a maximum at c. 12 h, after which the differences are stable.

Figure 4a shows a difference spectrum at t = 12 h and at pH 5.5. There are a number of important things to note about this difference spectrum. First the increase at 605 nm and 458 nm, together with a decrease at c. 520−560 nm indicates BDF has become relatively more protonated in the presence of potency at the same time as the level of un-protonated/aggregated BDF has decreased. In other words the level of un-protonated/aggregated BDF

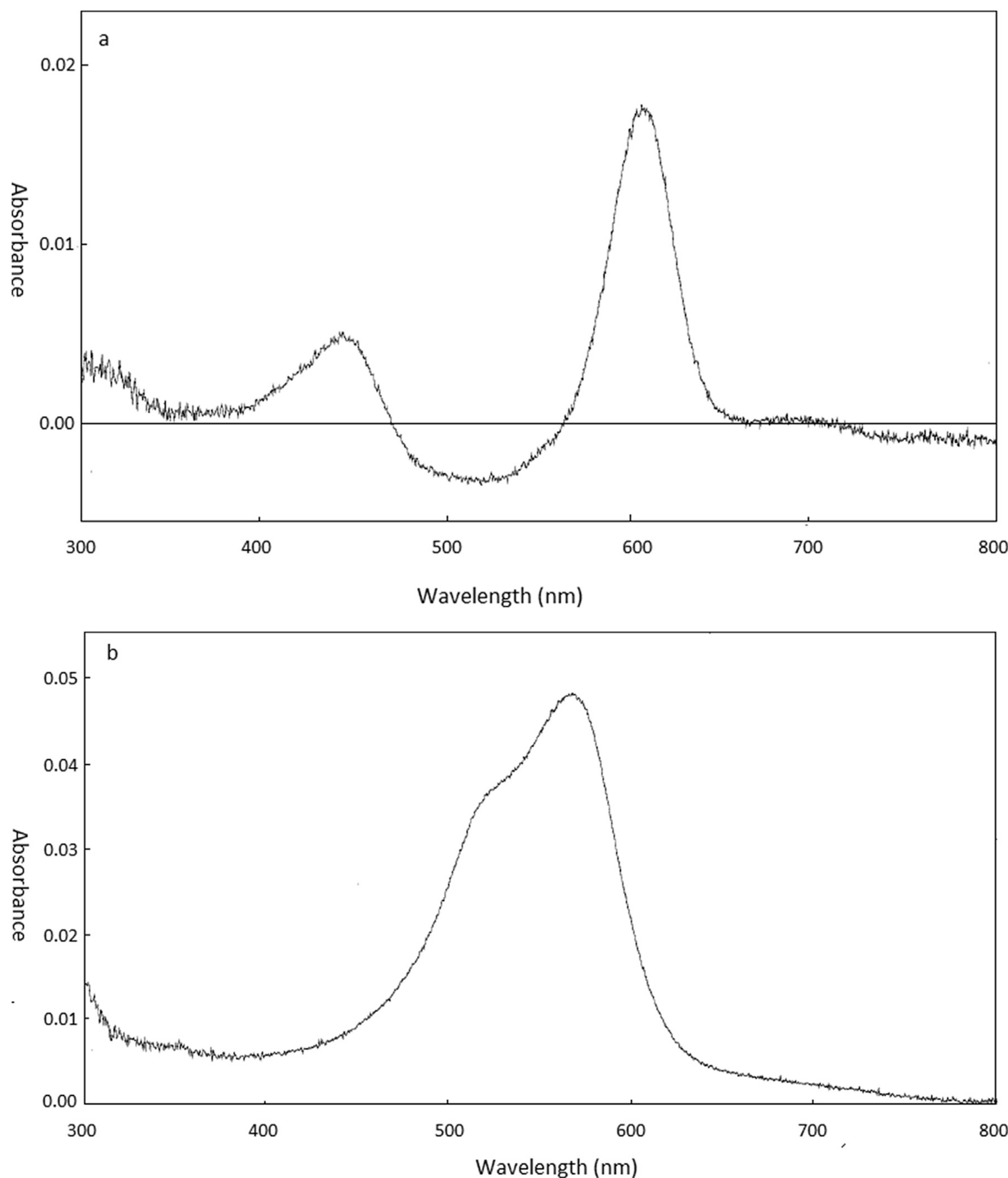

Figure 4 **a** (top). Difference spectrum of BDF in 20 mM citrate buffer pH 5.5 with control added to the reference cuvette and *Glycerol* 50M added to the sample cuvette (see Materials and methods). Difference spectrum consists of the sample spectrum minus the reference spectrum. Concentration of BDF is 15 μM; t = 12 h after mixing. **b** (bottom). Conditions as for (**a**) but BDF difference spectrum is in 20 mM borate buffer pH9; t = 12 h after mixing.

has decreased and the level of protonated BDF has increased (compare with spectra in Figure 2).

The second thing to note is that this process is slow. On acidification of BDF solutions spectral changes occur immediately so the rate of the above process, as well as the process itself, must be due to potency. Either the interaction between potency and dye is slow or the process of potency growth and/or development is slow and only reaches a maximum after c. 12 h. This result should be compared with results obtained with solvatochromic dyes and homeopathic potencies in organic solvents[1] where maximal changes were seen after several hours and then started to decline, often falling back to almost zero overnight. This difference in solution kinetics suggests water may be playing an important part in the propagation or maintenance of the homeopathic signal, a role which ethanol and tert-butyl alcohol appear to be less efficient at.

Difference spectra between potency-BDF and control-BDF solutions are seen in all acidic buffered solutions examined. However, the magnitudes of the differences are pH dependent. Figure 5 shows a plot of absorbance difference at 605 nm *vs* pH (all measurements are at t = 12 h). It is clear that a maximum difference is seen at pH 6.0–7.0 with the difference falling away at pH values either side.

The plot in Figure 5 is one that could be explained by the subtraction of one pH titration curve from another[7] for the BDF + H^+ ↔ $BDFH^+$ equilibrium (Figure 3), and is discussed below.

In contrast to that seen at pH values below c. pH 7.0, a very different effect is seen with potency and BDF at pH values above c. pH 7.5.

When an aliquot of potency solution (*Glycerol* 50 M) is added to an equilibrated BDF buffered solution and scanned against an exactly materially equivalent control-BDF solution, a difference spectrum appears with a maximum at 565 nm/shoulder at 523 nm (Figure 4b; pH 9.0).

The spectrum shown in Figure 4b is at t = 12 h and has a number of important features. As in acidic solution the difference spectrum slowly increases over time but does not reach a maximum at t = 12 h. Rather it continues to increase over several days. Figure 6 shows control-BDF and potency-BDF spectra taken at t = 10 days. What is striking is the marked difference at 565/523 nm. The potency-BDF solution has an absorbance some 70% higher than that of the control-BDF solution. *The difference is actually visible to the naked eye*. Furthermore, the control-BDF solution has a significantly increased peak at 380 nm compared with the potency-BDF solution. This peak is characteristic of MK, a hydroxyl ion mediated decomposition product of BDF (Figure 3).

What these results strongly suggest is that potency protects BDF from hydroxylation and subsequent decomposition.

A plot of absorbance difference at 565 nm *vs* pH at t = 12 h, shows a maximum at around pH 8.5–9.0 (Figure 7). This difference plot, like that in Figure 5, can be explained by the subtraction of a pH titration curve of BDF without potency from a pH titration curve of BDF with potency, but this time for the reversible BDF + OH^- ↔ BDF-OH^- equilibrium, and is also discussed below.

At c. pH 7.0–7.5, the transition pH range where both protonation and hydroxylation take place and neither process predominates, the 605 nm and 565 nm peaks appear together in difference spectra. This indicates that potency

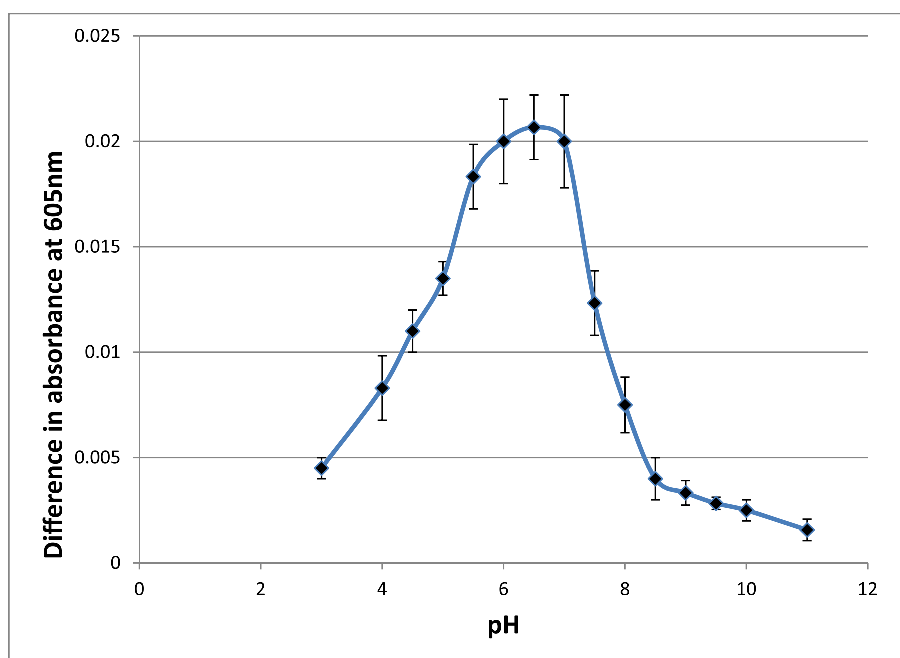

Figure 5 Difference in absorbance at 605 nm between control and *Glycerol* 50M solutions of BDF as a function of pH. BDF is at a concentration of 15 μM. Buffers (citrate pH 3–6; phosphate pH 6–8; borate pH 8–10; CAPS pH 10–11) are at a concentration of 20 mM (see Materials and methods). Difference absorbance is shown at t = 12 h; n = 5 for each pH value.

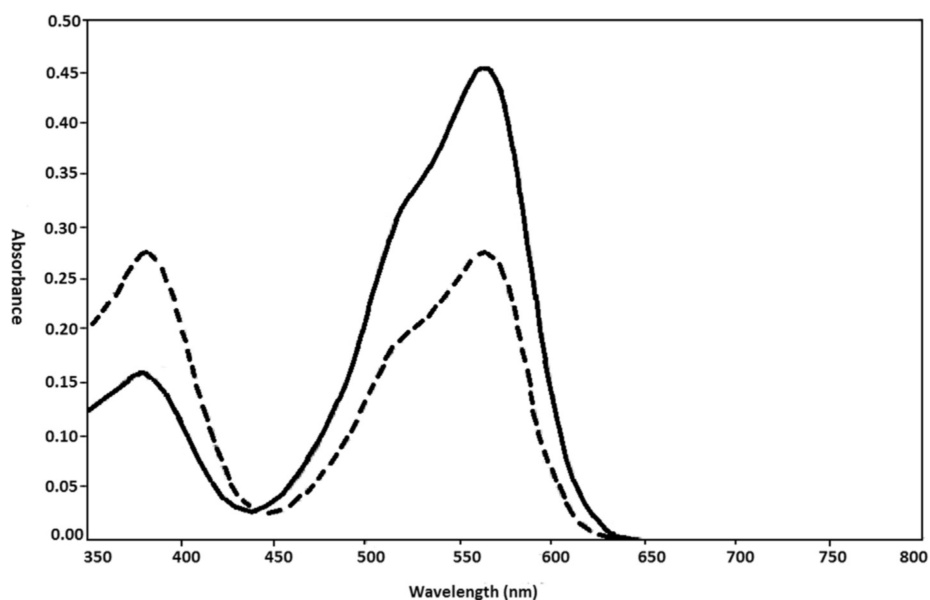

Figure 6 Scan of BDF at 15 μM concentration in 20 mM borate buffer pH 10 incubated with control solution (broken line). t = 10 days. Scan of BDF at 15 μM concentration in 20 mM borate buffer pH 10 incubated with *Glycerol* 50M solution (solid line). t = 10 days. Control-dye and *Glycerol* 50M-dye solutions were started at the same time and kept under identical conditions. (See 'Materials and methods' and text for details).

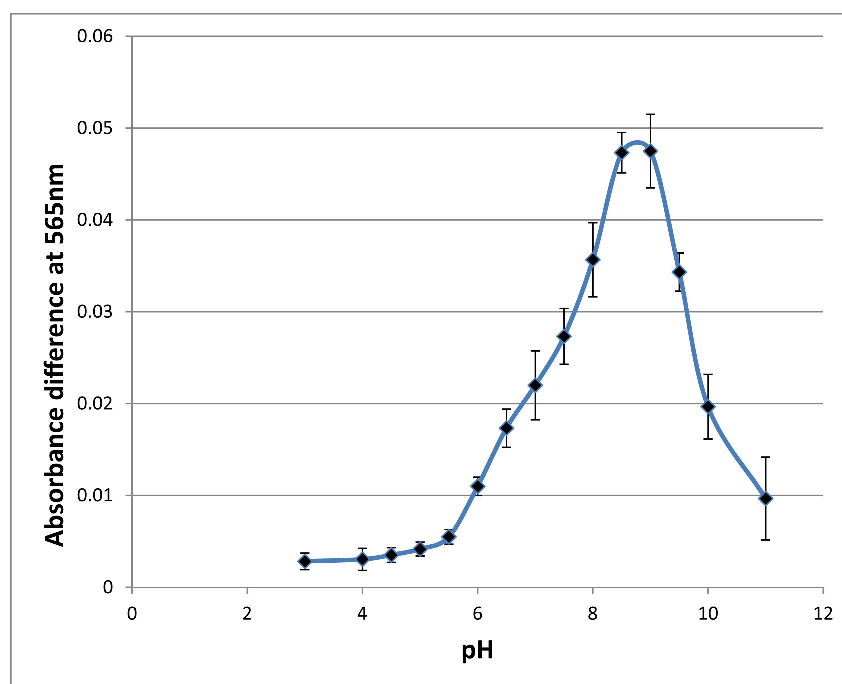

Figure 7 Difference in absorbance at 565 nm between control and *Glycerol* 50M solutions of BDF as a function of pH. BDF is at a concentration of 15 μM. Buffers (citrate pH 3–6; phosphate pH 6–8; borate pH 8–10; CAPS pH 10–11) are at a concentration of 20 mM (see Materials and methods). Difference absorbance is shown at t = 12 h; n = 5 for each pH value.

is having a fundamental action on BDF which results in both enhanced protonation and protection from hydroxylation. It is this fundamental action which is the subject of the following discussion.

Discussion

Before attempting to explain the results presented above it is important to discuss the experimental conditions used in this study in order to address any factors which may potentially skew the results in any way. All assays were carried out in 20 mM buffered solutions. Buffers used were citrate (pH 4–7), phosphate (pH 6–8), borate (8–10) and CAPS (pH10-11) – see Materials and methods – thereby providing overlapping buffering capacity. This addresses any potentially specific effects of particular buffers on the dye system used e.g. specific dye–buffer interactions occurring with one buffer but not another. Essentially identical results were found with citrate and borate buffers, phosphate and borate buffers and

borate and CAPS buffers at pH values where overlapping buffering capacity was available, indicating the results obtained were not being affected by changes in buffer type over the pH range 4–12.

At 20 mM the concentration of buffer in assay solutions is some 10,000 times higher than any leachates from the glassware in which potency and control solutions are stored.[1] It is highly unlikely therefore that the addition of control or potency to dye solutions could be having any effect on solution pH. This was confirmed firstly by ICP-OES analysis which showed the concentration of calcium, sodium, magnesium, boron, silicon (as silicates), iron and potassium leaching out of glassware to be essentially the same (<3 μM) in potency and control bottles following 100× 'dilution' to produce working potency and control solutions (see Materials and methods). A further 60 fold dilution of any leachates occurs on adding potency or control to assay solutions.

In addition non-solvatochromic pH indicators (e.g. Cresol Red and Neutral Red[8]) showed no change in their spectra under identical conditions to those used for the generation of BDF difference spectra. As a further precaution EDTA at a concentration of 1 mM was added to buffered BDF solutions to sequester any divalent cations (e.g. calcium and magnesium) which might specifically interact with BDF. EDTA was found to have no effect on the BDF difference spectra obtained. It seems reasonable therefore to conclude that the BDF difference spectra presented above are not due to any pH shift or specific leachate effect. It goes without saying that as all experiments have been conducted in aqueous solution the results obtained in this present study cannot be assigned to any potential mixed solvent artefact.

How then are the results to be interpreted?

Figure 5 shows that at pH values below c. 7.0, potency enhanced protonation of BDF predominates, with a maximum around pH 6–7. This would suggest that the carbonyl oxygen of BDF has become a slightly stronger Lewis base.[9] In other words it has become better at attracting protons because of increased electron density. If this is the case then the pKa of the BDF carbonyl group should be slightly higher in the presence of potency than in control solutions. As mentioned previously the current study has found BDF without potency to have a pKa of 6.5. If BDF were to have a pKa slightly higher than 6.5 in the presence of potency then a pH titration curve of BDF without potency subtracted from a pH titration curve of BDF with potency would be expected to produce a difference titration curve that looks very similar to that seen in Figure 5. If this conclusion is correct then the data in Figure 5 suggests a potency-induced pKa shift upwards for the BDF carbonyl group of c. 0.03 pH units from 6.5 to 6.53.

It should also be noted that the difference spectrum in Figure 4a shows a trough at c. 520–560 nm. This indicates that potency enhanced disaggregation occurs concomitantly with potency enhanced protonation. It is difficult to know whether these two processes are truly concomitant or one precedes the other, but it would seem likely that protonation occurs first and disaggregation quickly follows, as there is no evidence that protonated BDF aggregates.

Figure 7 shows that at pH values above c. 7.5, potency promoted protection of BDF from hydroxyl ion attack at the central carbon atom predominates. This carbon is an electrophilic centre (electron deficient) and hence a Lewis acid. Consequently any increase in electron density at this central carbon would *decrease* its affinity for hydroxyl ions and would be expected to produce a difference plot at 565 nm very similar to that seen in Figure 7.

The irreversible BDF-OH^- → MK decomposition step (Figure 3), characterised by a 380 nm peak, is not evident at t = 12 h (Figure 4b) and it can consequently be assumed to be much slower than the establishment of the BDF + OH^- ↔ BDF-OH^- equilibrium. The plot in Figure 7 can therefore be confidently assigned to an upward shift in the pKa of the BDF + OH^- ↔ BDF-OH^- equilibrium in the presence of potency.

Over time periods of days the decomposition step BDF-OH^- → MK becomes increasingly significant and this explains the progressively larger difference between potency – BDF and control – BDF spectra (Figure 6).

What the pH plots in Figures 5 and 7 suggest is that both the central carbon atom in BDF and the dye's carbonyl oxygen acquire increased electron density in the presence of potency, resulting in an upward shift in pKa in both cases. Only the two dimethylamino groups of BDF could provide the electron density necessary for this to occur and suggests partial electron movement from the dimethylamino groups towards the dye's carbonyl oxygen. It will be noted that this is what occurs on irradiation of BDF by light, except with light the electron movement is oscillatory (Figure 1).

The inescapable implication arising from the results presented – if the model above is correct – is that potency is partially stabilising some form of BDF which is electronically similar to its excited state. This may explain why more consistent results are obtained with potency and BDF if incubations are conducted in darkness and light exposure is limited, as light also causes an electron density shift in BDF. However, whereas potency appears to protect BDF from hydroxylation, continuous light exposure has the opposite effect, and appears to promote BDF hydroxylation (data not shown). A possible explanation for the opposite effects of light and potency may lie in the ground to excited state oscillatory effect of light on BDF, whereas potency may produce a more continuous electron displacement.

The next question inevitably is, "*What might the nature of the dye – potency interaction be and how could that lead to the postulated shift in electron density away from the dimethylamino groups towards the carbonyl group of BDF?*" One can only speculate at this stage. It may be that potency induces a resonant interaction or electronic coupling with BDF leading to a partial stabilisation of the dye's excited state; it may be that potency induces a small conformational change in BDF that results in both an electron density shift and changes in aggregation levels; it may be that a conformational change leads first to altered

aggregation levels and this in turn leads to increased protonation and decreased hydroxylation of BDF.

Clearly much more work needs to be done to answer this question.

Interaction of potency with BDF, a polar molecule, to produce what appears to be a directed electron density shift suggests that potencies themselves may be polar. In terms of the biology of homeopathic potencies it has long been known that potencies appear to have a sinusoidal action *in vivo*,[10–13] which may also be pointing to some kind of inherent polarity in potencies.

A further consideration arising from the above is that all biochemical reactions proceed via electronic excited states and electron transfers. If potencies were capable of modulating ground state to excited state transitions this would have profound organism-wide effects. However, these suggestions must remain as one of a number of possibilities until further evidence is forthcoming.

What is emerging from working with solvatochromic dyes and homeopathic potencies is that a cautious step by step approach is necessary. There are many factors at play in the action of potencies and the studies reported herein are only beginning to identify what these factors are and their roles. Some factors may work antagonistically or synergistically with respect to others – light being an example illustrated above.

It should be noted in this context that a range of potencies of glycerol from 6c to 50 M have been examined in the current study and found to produce similar effects, differing only in magnitude, but it has been found necessary to confine this investigation to one potency; 50 M. Until the potential role of agents such as water, glass, quartz and succussion in the transfer, propagation, amplification and action of potencies has been examined and clarified, it seems premature to over-complicate an already complex set of interacting factors by looking at a range of potencies or remedies beyond glycerol 50 M at this stage.

Conclusions

The results reported here confirm and extend those reported previously.[1] In particular, it has been demonstrated that homeopathic potencies not only interact with solvatochromic dyes in organic solvents but also in aqueous buffered solution. This dispels any doubts that the effects seen with potencies may be due to low level contamination of organic solvents by water or by uncontrolled shifts in pH due to differential concentrations of contaminants in control and potency solutions. The effects seen with BDF and homeopathic potencies in buffered solution are substantial and reproducible. BDF has demonstrated itself to be amply suited to studying the physico-chemical nature of potencies. Its high extinction coefficient, large spectral changes and good water solubility make it ideal for investigating interactions with homeopathic potencies across a range of conditions.

The results presented show that the effect potency has on the dye is pH-dependent. At low pH where BDF is prone to protonation, potency pushes the equilibrium even further towards the protonated state. Conversely at high pH where BDF is in equilibrium with its carbinol base form (Figure 3), potency favours unsubstituted dye. Several explanations for the effects observed are possible and the following are not exhaustive.

Potency may be interacting with BDF to induce a minor conformational change in the molecule which then leads to a raised pKa for the dye's carbonyl moiety and a raised pKa for the dye's central carbon atom. Alternatively potency may be inducing a conformational change in BDF which affects aggregation levels and it is these altered aggregation levels that are the cause of the dye being more susceptible to protonation and less susceptible to hydroxylation. Finally, potency may be entering into some kind of resonant interaction or electronic coupling with BDF which leads to a partially stabilised excited state and altered pKa values. It seems more likely that aggregation would be an effect of something more fundamental occurring, such as a pKa shift, rather than the cause, and it is difficult to see what kind of conformational change would produce the effects seen. A resonant interaction or coupling between potency and BDF leading to a shifted electron density across the dye molecule and altered pKa values is therefore a distinct possibility.

Results reported here also suggest a role for water in the propagation and/or maintenance of the homeopathic signal. Effects of potency on BDF in aqueous solution are stronger and more stable than results reported in organic solvents previously.[1] This is clearly something which requires further investigation. It is perhaps worth noting in this respect that a number of other studies have found certain physico-chemical characteristics of diluted and succussed solutions, such as conductivity, appear to slowly increase over time.[14,15] The periods involved in those investigations were, however, of the order of months and may therefore represent a phenomenon unrelated to that reported here.

Finally it has been found that more consistent results emerge if potency-dye and control-dye incubations are kept under conditions in which light is excluded. That light may be interfering with the potency-dye interaction brings into stark relief the complexities involved in studying potencies, and the need for chemical systems in which the relative contributions of succussion, water, container materials and light exposure can be separately controlled.

Materials and methods

Materials

The solvatochromic dye 4-[bis[4(dimethylamino) phenyl]methylene]-2,5-Cyclohexadien-1-one (Bis-dimethylaminofuchsone or BDF) was a kind gift from Ana M. Costero, Instituto Interuniversitario de Reconocimiento Molecular y Desarrollo Tecnologico (IDM) Dr. Moliner 50 46100-Burjassot, Valencia, Spain. Structure was confirmed by NMR. Purity was estimated to be >99% based on NMR spectra and HRMS (ESI) data.

pH indicator dyes Cresol Red and Neutral Red, together with sodium citrate, sodium phosphate, sodium borate, sodium N-cyclohexyl-3-aminopropanesulfonate (CAPS) and EDTA were obtained from Sigma Aldrich UK and were of the highest purity available.

Reverse osmosis water (ROW) was used throughout this study and had a resistivity of 15MΩcm (checked daily).

Disposable high purity UV-transparent cuvettes (Brand GmbH) with stoppers were used throughout and are as described previously.[1]

Solution storage

As in previous studies[1] dye solutions were made and stored in HDPE bottles and allowed to equilibrate before use. Buffered dye solutions at pH values above 7 were made up and used within 24 h due to slow hydroxyl ion mediated degradation. All dye solutions were stored in the dark.

BDF was made up in all buffers at a concentration of 15 μM. At this concentration absorbance is of the order of 1.0. Buffer solutions in which dye was dissolved were at a concentration of 20 mM throughout. Where EDTA was used this was at a concentration of 1 mM.

Homeopathic potencies and control solutions

Glycerol 50M along with other potencies of glycerol were obtained from Helios Homeopathy Ltd Tunbridge Wells, UK. All the results presented in this study were carried out with *Glycerol* 50M.

Thirty microlitres of potency (in 90% ethanol) was diluted into 3.5 ml of ROW in an amber moulded glass bottle provided by the Homeopathic Supply Company Ltd, Bodham, UK and stored at room temperature. This 'diluted' aqueous potency was then used for all assays.

Control solutions were either un-medicated and unsuccussed 90% ethanol obtained from Helios Homeopathy Ltd and diluted 100-fold as above into amber moulded glass bottles from the same batch as that used for potency dilutions, or control solutions consisted simply of ROW added to amber moulded glass bottles from the same batch. No difference could be discerned between these two types of control solutions on addition to dye solutions. Control solutions were stored at room temperature. Leachates from both potency and control bottles were analysed by ICP-OES (Oxford-Analytical Ltd, Bicester, UK) and found to be at the same (<3 μM) level for all elements tested (Ca, Mg, Si, K, Na, B, Fe) after storage at room temperature for 6 months. A further 60 fold dilution of leachates occurs on addition of potency or controls to assay solutions.

Instrumentation

Difference spectra were recorded on a Unicam UV500 UV/VIS double-beam spectrophotometer as described previously.[1] pH measurements were made using a Hanna pH210 microprocessor pH meter.

Experimental procedures

All difference spectra were performed as follows. 2.95 ml of buffered dye solution were pipetted into each of two Brand UV-transparent cuvettes with stoppers and the spectrophotometer set to zero across the wavelength range used for scanning (typically 350–800 nm). 50 μl of control solution was then added to the reference cuvette and 50 μl of potency solution added to the sample cuvette. Cuvettes were then inverted three times to mix and scanned (t = 0). After the initial scan both cuvettes were placed in separate black plastic film canisters (Geo-Versand, GmbH) to exclude all light and kept under these conditions between any subsequent scans. Scans were normally performed at t = 0, 10 min, 40 min, 100 min, 6 h, and 12 h after mixing. Subsequent scans were carried out at intervals of days after mixing up to a maximum of 15 days.

Normal (non-difference) scans of dye solutions with potency or control solutions added were against ROW which had been zeroed beforehand.

Conflict of interest statement

No source of funding had any influence on the design, analysis, interpretation or outcome of the research contained within this manuscript, nor on the writing of the manuscript.

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