A two component thermoreversible hydrogel of riboflavin and melamine: Enhancement of photoluminescence in the gel form†

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A new thermoreversible hydrogel of riboflavin and melamine supramolecular complex (⩾0.02%, w/v) shows enhanced photoluminescence properties through H-bonding.

Small molecular hydrogels have intense research interest because of their potential use as biocompatible scaffolds for tissue engineering, pollutant capture and in drug delivery.[1–7] In most of the examples the hydrogelators are one component small molecules but recently interest in two component hydrogels has increased.[1,2] An initial interaction occurs between the two components to produce a complex, which subsequently self assembles into a fibrous supramolecular structure.[1] The fibrillar network entraps the solvent to produce the gel, which may be thermally reversible for transition into the sol state. We have chosen (−)- riboflavin (R) ($[\alpha]^{21} = -114.9^{\circ}$) and melamine (M) as the two components because there is the possibility of a hydrogen bonding interaction through the carbonyl oxygen of the former and the hydrogen atoms of the amine group of the latter to produce the complex. Besides, riboflavin is a well-known vitamin and its action is closely associated with the function of flavin nucleotides in biochemical reactions.[8,9] It also behaves as a photoreceptor in the phototropism of plants.[10] The ribityl side chain aids the solubility of riboflavin in water and may also induce solubility of its melamine complex in water. Due to the possibility of multiple hydrogen bond formation the complex may have a rigid structure, which can aggregate to produce the fibril and hence gel formation.

Self-assembly induced enhancement of photoluminescence of organogel systems has been explored recently.[11] Here we are reporting for the first time the photoluminescence enhancement of a new two component hydrogel. The photoluminescence properties of riboflavin are well reported[8,12] and its efficiency depends on the polarity of the solvent. Hydrogen bond forming solvents reduce the photoluminescence efficiency.[8] Most of the studies on photoluminescence of riboflavin deal with quenching by a variety of substances *e.g.* metal ions, electrolytes, proteins and aromatic compounds.[13,14] In the proposed complex all the H-bonding sites may be used up so there would be a tendency to increase the photoluminescence efficiency of riboflavin in the aqueous medium.

A mixture of melamine and riboflavin in a 1 : 3 mole ratio was taken in a glass tube and water was added to make the total complex concentration 0.02% (w/v). It was homogenized at 120 °C and quenched to room temperature when a yellow coloured gel was produced. The TEM picture (Fig. 1) of the dried gel indicates a fibrillar network structure of fibrillar diameter 20–70 nm. Gel formation is confirmed by inverting the tube as shown in the inset of Fig. 1. The gel has birefrigent properties and an optical micrograph (Fig. S1†) in the gel state also exhibits a fibrillar network structure. The DSC thermogram of the gel shows a reversible first order phase transition (Fig. S2†) during the heating and cooling processes. This concludes the formation of thermoreversible hydrogel of the R–M complex.[15]

The structure of the complex was studied by FTIR spectroscopy of the freeze-dried gels using KBr pellets (Fig. S3†). For the 3 : 1 R–M complex (molar ratio), the >C=O peak has shifted from 1732 cm^{-1} to 1710 cm^{-1} indicating the formation of hydrogen bonds[16] between the >C=O groups of R and the amino hydrogen atoms of M. A comparison of the 3100–3600 cm^{-1} region indicates that in the 3 : 1 complex the N–H vibration peaks[16] are broadened indicating that all the N–H bonds are used for hydrogen bond formation, but for the 1 : 1 complex the peaks corresponding to the N–H vibrations are satisfactorily prominent indicating that some of the N–H bonds of melamine are not used up. These results, therefore, suggest that the components produce complete complexation at the 3 : 1 molar ratio.

Fig. 2(a) shows the UV-vis absorption spectra of the 3 : 1 R–M gel (0.07% w/v) at the indicated temperatures. There are two peaks at 363 and 446 nm in the gel state. These two peaks are attributed to the π–π* transition of riboflavin[12] as melamine has no absorption peak in that region. The 363 nm peak may be attributed to the mixing of the n–π* transition with the π–π*

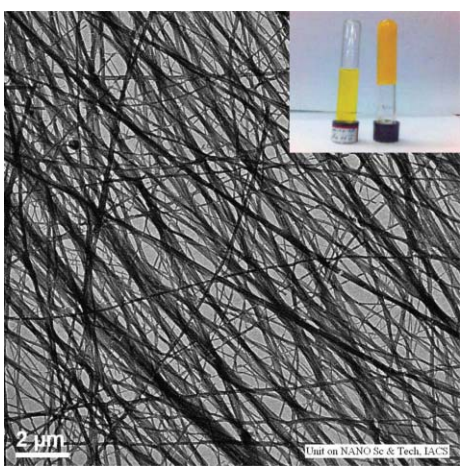

Fig. 1 TEM picture of dried riboflavin–melamine hydrogel (0.02%, w/v). (Inset: Photograph of 0.2% solution of the complex in sol and gel form.)

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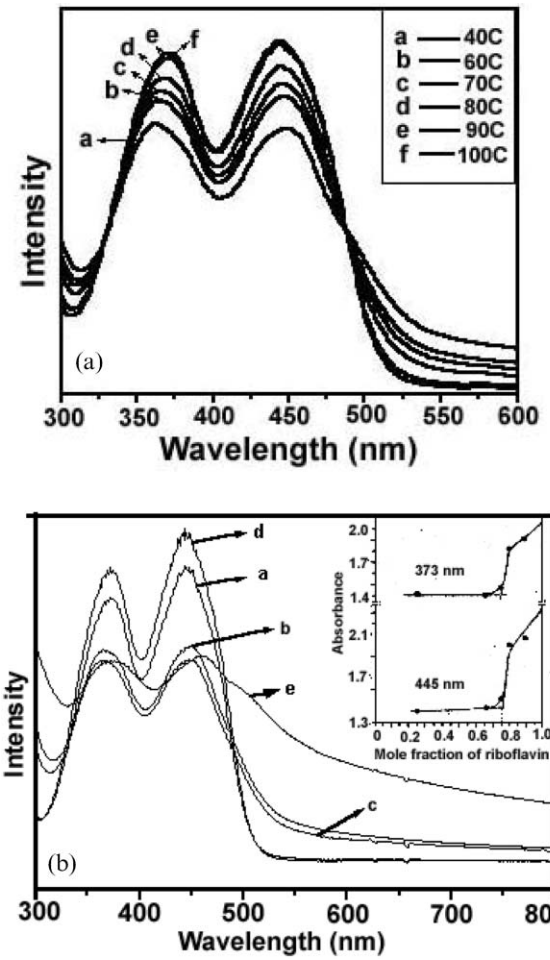
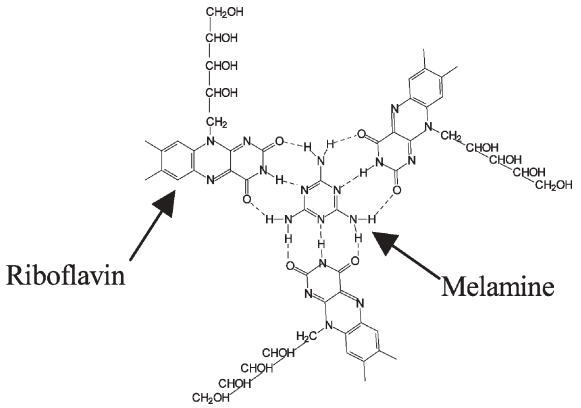


Fig. 2 (a): UV-Vis spectra of 3 : 1 R–M gel (0.07%) at indicated temperatures. (b): UV-Vis spectra at 30 °C of R–M gels at different molar ratios of R and M and at constant concentration of R (0.09% w/v): (a) R–M 4 : 1, (b) R–M 3 : 1, (c) R–M 2 : 1, (d) R, (e) R–M 1 : 3. (Inset: Absorbance *vs.* mole fraction of riboflavin in the R–M system.)

transition. With an increase in temperature the increasing intensity of these transitions may be due to the increasing 'allowedness' of these transitions. One important observation is that the 363 nm peak shows a red shift with increasing temperature, but the 446 nm peak is almost nonvariant. A probable cause for the red shift of the 363 nm peak is the gradual breaking of H-bonds between R and M freeing R molecules from the hydrophobic pocket in the gel state. This causes greater solvation of the nonbonding electrons, as a result a red shift occurs in the 363 nm band and it reaches the characteristic value of pure R in water at 100 °C (373 nm).

In Fig. 2(b) the UV-Vis spectra at 30 °C of the R–M system at different molar ratios of R and M and at a constant concentration of R (0.09% w/v) is presented. Fig. 2(b) clearly indicates that with increasing melamine content the intensity of the π–π* transition decreases and it falls sharply from 4 : 1 to 3 : 1 R–M content. For molar compositions (R–M) 3 : 1, 2 : 1 and 1 : 3 the intensities are almost same. In the inset of Fig. 2(b) the UV-Vis intensities of the π–π* transitions are plotted with the mole fraction of riboflavin and from the titration curve it is apparent that the composition of the supramolecular complex is 3 : 1 supporting the FTIR observation. A schematic model of the 3 : 1 complex is shown in



Scheme 1 Schematic model of the riboflavin–melamine supramolecular complex.

Scheme 1. The complex then self assembles to produce the fibrillar network structure.

The wide angle X-ray diffraction patterns of the R–M gel is quite different from that of the pure components (Fig. S4†). New peaks at lower angles *i.e.* at *d* spacings of 39.4, 22.3, 13.02, 11.28 Å appear in the gel state. Probably the one at 39.4 Å is for the lateral dimension and that at 22.3 Å is for the interlayer stacking distance of the complex. The other d_{hkl} values might arise for the lesser order reflections. An amorphous halo at $2\theta \sim 28^{\circ}$ is also observed and it may arise due to the entrapped water in the fibrillar network.

Fig. 3 shows the photoluminescence spectra of a R–M gel (0.07% w/v) at indicated temperatures. The photoluminescence spectra of a pure riboflavin solution of the same concentration as in the gel state is also shown for comparison. Two differences are noted: (i) the luminescence intensity of the R–M gel is about 15 times greater than that of the pure riboflavin solution and (ii) there is a red shift in the gel of the photoluminescence peak position from 535 nm to 545 nm. The fifteen times increase in intensity of the photoluminescence spectra may be related to the complexation of the H-bonding moieties *e.g.* >C=O and >NH groups of riboflavin with amino hydrogens and nitrogen of the

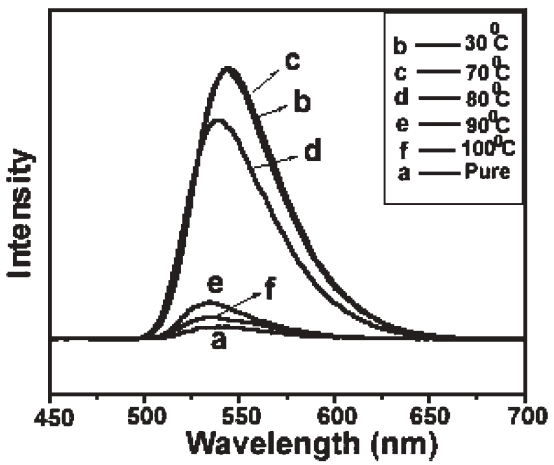


Fig. 3 Photoluminescence spectra of 3 : 1 R–M gel (0.07%) at indicated temperatures (curve 'a' for the emission of pure riboflavin solution with equal concentration to that in the gel).

triazine ring of melamine, respectively, thereby reducing the hydrogen bonding ability with water. In other words melamine blocks the hydrophilic part of the isoalloxazine ring inhibiting quenching due to solvent polarity.[12] Also the dynamic quenching in the gel form is highly inhibited compared to riboflavin solution due to the formation of a network structure of infinite mass. These two factors add to a very large increase in photoluminescence intensity in the gel form. The red shift of the photoluminescence peak in the gel state may be caused by the π stacked self assembly of the complex.[11d] This is also supported by the blue shift of the emission spectra and decrease of intensity with increase in temperature. The results suggest that the gel starts to melt at 70–80 °C and at 100 °C it melts completely showing almost equal emission to that of riboflavin solution.

The 3 : 1 (R–M) composition produces a physically rigid gel, but in the other compositions *e.g.* 1 : 1, 1 : 2, 1 : 3, 4 : 1 and 8 : 1 physical softness gradually appears. At 1 : 3 and 8 : 1 compositions the system is discontinuous. However, the complex is produced at all concentrations as evidenced from FTIR, but the concentration of the complex for the extreme compositions is very low. Consequently fibril concentrations are also much lower (required for overlapping) inducing discontinuity in the gel structure.

The contribution of the ribityl chain to the self-assembly of the π-stacked complex has been explored here. The hydroxyl groups of the ribityl chain are capable of intermolecular hydrogen bonding facilitating the π- stacking process to produce the gel. But on acetylation of the four-hydroxyl groups of the ribityl chain the gel does not form. Probably the absence of intermolecular hydrogen bonds in the ribityl chain disfavors the formation of the π-stacked self-assembly inhibiting the fibril formation required for gel formation.[15] Also there is a possibility that there is a change of specific rotation between the sol state and the gel state due to a probable change in the conformation of the ribityl chain during gelation;[17] this will be addressed later.

The effect of pH on the gelation of the R-M system is interesting. At acidic pH (pH = 4) the system produces a gel in water but in basic medium (pH = 9.2) the system does not produce any gel. A probable explanation is that in basic medium the imino hydrogen between the two >C=O groups of riboflavin is abstracted by the base preventing H-bond formation with melamine. So this study concludes the formation of a new two component hydrogel through supramolecular organization with enhanced photoluminescence properties. This significantly enhanced emission may be useful for producing temperature and pH sensitive sensors.

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