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Photochemistry in microemulsions: fluorescence quenching of 1- and 2-naphthol by Cu^{2+}

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Abstract

The fluorescence characteristics of 1- and 2-naphthol in the presence of copper ion have been studied in aqueous medium and sodium lauryl sulphate (NaLS)–isobutanol–hexane–water microemulsion. On excitation 1-naphthol (1-ROH) dissociates completely and 2-naphthol (2-ROH) partially in aqueous medium. The dissociation of 2-ROH is suppressed completely and that of 1-ROH partially in the microemulsion. The extent of dissociation of 1-ROH varies with changing microemulsion formulation. The dissociation behaviour and fluorescence quenching of naphthols by Cu^{2+} in the microemulsion suggest a compartmentalization of the fluorophores. Various quenching models have been analysed to explain the quenching efficiency. A generalized model explaining both the dynamic and transient quenching processes has been proposed. The probable number of quenchers present in the quenching sphere of action has been calculated.

Keywords: Microemulsions; Fluorescence quenching; 1-Naphthols; 2-Naphthols; Copper ion

1. Introduction

Synthetic organized assemblies are known to mimic biological aggregates owing to their ability to compartmentalize the solubilizates. These organized assemblies have been exploited extensively for the study of various reactions. Microemulsions are isotropic, transparent and thermodynamically stable dispersions of oil, water and emulsifiers with droplet diameters ranging from 50 to 1000 Å [1–4]. An oil-in-water microemulsion (O/W ME) consists of droplets with a hydrocarbon core surrounded by surfactant and co-surfactant, while a water-in-oil microemulsion (W/O ME) is related to a reversed micelle system. Being a ternary or quaternary system, it has the advantage of offering better compartmentalization than that in other organized assemblies. Fendler [5] has proposed a distribution mechanism of substrates and quenchers in micelles to explain fluorescence quenching.

In view of the biphasic nature of microemulsions providing aqueous and oil phases well separated by surfactant molecules acting as a membrane system, the fluorescence-quenching behaviours of 1-naphthol (1-ROH) and 2-naphthol (2-ROH) are studied with Cu^{2+} as the quencher in O/W microemulsions of the sodium lauryl sulphate (NaLS)–isobutanol–hexane–water system.

2. Experimental details

The microemulsions were prepared by stirring the various components with a mechanical stirrer and the corresponding phase diagram was constructed by visual titration. The order of mixing has no effect on the formation of the microemulsion. Sodium lauryl sulphate (Sigma) was purified by recrystallizing twice from alcohol. Isobutanol (Merck) was dried over K_2CO_3 and distilled twice before use. Hexane (Merck) was distilled twice in a fractionating column. Triply distilled water was used throughout the study. 1-Naphthol and 2-naphthol (Sisco Chem) were purified by subliming twice after crystallization from alcohol. Copper sulphate (Sigma) was recrystallized from water and a solution of $CuSO_4$ (0–0.2 M) was taken instead of water for constructing the phase diagram.

Absorption and fluorescence spectra were recorded with a Shimadzu 160 UV–visible spectrophotometer and a Shimadzu RF-5000 spectrofluorimeter respectively using the same media as references. The excitation wavelength was usually in correspondence with the absorption maximum.

Narrow excitation and emission slit widths (1.5 and 3 nm) were chosen. The fluorescence intensities of 1-naphthol ($\lambda_{ex} = 292$ nm, $\lambda_{em} = 462$ nm in water; $\lambda_{ex} = 297$ nm, $\lambda_{em} = 356$, 459 nm in ME) and 2-naphthol ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 352$, 416 nm in water; $\lambda_{ex} = 330$ nm, $\lambda_{em} = 354$ nm in ME) were monitored at various concentrations of Cu^{2+} at 27 °C.

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The concentrations of 1- and 2-naphthol were 1×10^{-4} M. The Cu^{2+} (quencher) concentration was varied in the range 0–0.2 M. The study was confined only to the molecular form of the naphthols and not to the anionic form at high pH, since the copper ions tend to precipitate at higher pH.

3. Results and discussion

3.1. Phase diagram

The pseudoternary phase diagram constructed by visual titration of the mixtures of NaLS–isobutanol–water–hexane was reported in a previous paper [6]. Addition of $CuSO_4$ shrinks the Winsor IV domain of the phase diagram, where most of it concentrates at the W apex (Fig. 1). There is no isotropic medium observed in the oil-rich region. For the present study we have considered the formulation (A in Fig. 1) as NaLS (1.8%), isobutanol (4.2%), aqueous $CuSO_4$ solution (93.1%), hexane (0.9%) (w/w).

3.2. Absorption and fluorescence spectra

The absorption spectra of 1- and 2-naphthol in water and O/W ME both in the presence and absence of the quencher show no observable change in spectral shape and maxima. However the two compounds behave differently on excitation [7,8]. On excitation in aqueous medium 1-ROH dissociates completely and 2-ROH dissociates partially (33%). Thus in the excited state 1-ROH gives an emission peak due to 1-naphtholate ion (1-RO^{-}*) only, whereas in the case of 2-ROH emission is observed from both neutral and anionic forms (i.e. 2-ROH* and 2-RO^{-}*). The anionic O/W ME suppresses the dissociation: 1-ROH* undergoes partial dissociation and 2-ROH* remains undissociated.

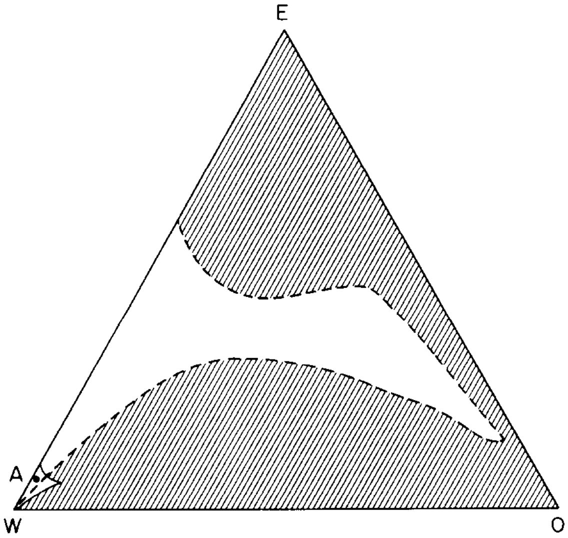


Fig. 1. Pseudoternary phase diagram of NaLS–isobutanol–hexane–water ($[CuSO_4] = 0.2$ M) at 27 °C: full line, in presence of $CuSO_4$; broken line, in absence of $CuSO_4$. Point A indicates the composition of the sample studied.

Although there is appreciable quenching even at low concentration of Cu^{2+} (0.01 M), the shape of the fluorescence spectra remains the same, with no change in the position of the maxima. Further, observation of similar absorption spectra of a solution containing any concentration of the quencher after carrying out the fluorescence indicates that no detectable photoproduct is formed under the experimental conditions. No new fluorescence peak is observed at longer wavelength either. The excitation spectra monitored at different emission wavelengths also remains the same in all the media. These observations indicate that there is no ground state complexation of naphthol and Cu^{2+}. The decrease in the fluorescence intensity of each species (1-ROH*, 1-RO^{-}*, 2-ROH*, 2-RO^{-}* in water and in ME) without the appearance of any new band in the presence of a quencher concentration up to 0.2 M indicates that no emissive exciplex is formed between fluorophores and quencher.

3.3. Deviation from Stern–Volmer (SV) plots

The I_0/I values of all the systems (1-ROH*, 1-RO^{-}*, 2-ROH*, 2-RO^{-}* in water and in microemulsion) have been correlated with $[Cu^{2+}]$ by using the Stern–Volmer equation [9]

$$\frac{I_0}{I} = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \qquad (1)$$

In this equation I_0 and I are the fluorescence intensities of the fluorophores in the absence and presence of the quencher respectively, K_{SV} is the Stern–Volmer constant, k_q is the biomolecular rate constant for quenching, τ_0 is the lifetime of the excited fluorophores in the absence of the quencher and [Q] is the concentration of the quencher. The plots of $I_0/I - 1$ vs. $[Cu^{2+}]$ are found to be linear ($r = 0.99$) up to a limiting concentration of copper ion, the limiting concentration being different for different systems (Fig. 2). Beyond this limiting concentration of Cu^{2+} the plots show an upward deviation both in water and in microemulsion, with the exception of 2-ROH in ME which shows a downward curvature. This suggests that a dynamic quenching process operates at low concentration of the quencher only. Similar results have been reported by Thulborn and Sawyer [10] while investigating the quenching behaviour of Cu^{2+} and dimethyl aniline on anthroyloxy derivatives in a lipid–water system. For the anthroyloxy derivative–Cu^{2+} system where the probe and quencher are in different environments (i.e. probe soluble in lipid and Cu^{2+} soluble in water) they have observed a negative deviation in the SV plot, but for the anthroyloxy derivative–dimethyl aniline system where both probe and quencher are in the same environment they have observed a positive deviation in the SV plot. The downward deviation of the SV plot from linearity for the 2-ROH–Cu^{2+} system in microemulsion thus indicates different environments for the fluorophore and quencher. The probable localization site of copper ion is the interface due to Na^{+}–Cu^{2+} exchange and thus 2-ROH remains inside the microemulsion droplet away

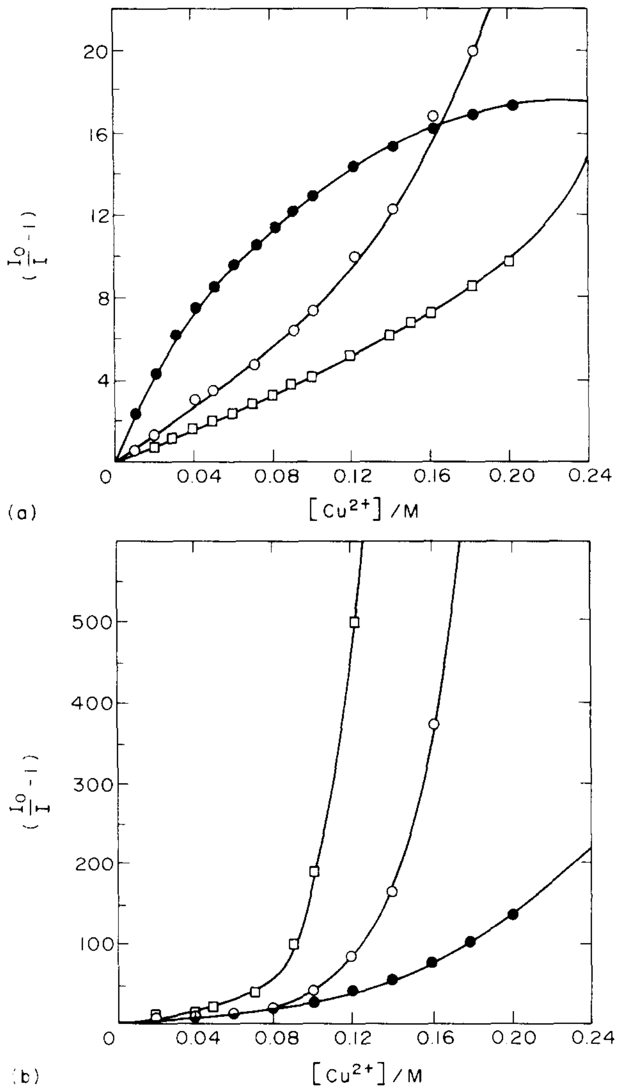


Fig. 2. (a) Stern–Volmer plots of 1- and 2-ROH* in H_2O and O/W ME: ○, 1-ROH* in ME; □, 2-ROH* in H_2O; ●, 2-ROH* in ME. (b) Stern–Volmer plots of 1- and 2-RO^-* in H_2O and O/W ME: ○, 1-RO^-* in H_2O; □, 1-RO^-* in ME; ●, 2-RO^-* in H_2O.

rom the interface. The positive deviation exhibited by the other systems envisages a similar environment for fluorophores and quencher. Hence it is clear that 1-ROH remains n the microemulsion droplet at the interface where $Cu^{2=}$ also resides. This proposition gets support from the emission spectra, which show the ease of ionization in the case of 1-ROH. On excitation 1-ROH ionizes to 1-RO^- which migrates to aqueous medium. Both naphtholates, i.e. 1-RO^-* and 2-RO^-*, reside in aqueous medium with Cu^{2+}.

In the absence of any chemical reactions of ROH* the deviation from linearity may be due to (i) ground state complex formation or (ii) a quenching sphere of action, i.e. transient quenching. However, deviation due to ground state complex formation is discarded on the grounds that no change is observed in the absorption spectra in the presence of quencher. Thus it is clear that fluorescence quenching at high [Cu^{2+}] involves a quenching sphere of action.

While studying the fluorescence quenching of perylene by molecular oxygen, Lacowich and Weber [11] have shown that for effective quenching the oxygen molecule does not have to form a complex with perylene. As long as it is within a distance of 16 Å from the fluorophore, the oxygen molecule is able to quench effectively. From this observation they have proposed a sphere of action within which a molecule can quench the fluorophore effectively.

In the present study the limiting [Cu^{2+}] at which the departure from linearity occurs varies for different species and different environments. Thus, according to the quenching sphere of action model [12,13],

$$\frac{I_o}{I}=\exp(K_T[Q]) \qquad (2)$$

Eq. (2) can be written as

$$\ln\left(\frac{I_o}{I}\right)=VN_a[Q] \qquad (3)$$

where K_T is the transient quenching constant, V is the volume of the transient quenching sphere and N_a is Avogadro's number,

According to Eq. (2), the plots of $\ln(I_o/I)$ vs. [Cu^{2+}] are expected to be linear, passing through the origin, but in the present study the plots are found to be linear only beyond a certain concentration of the quencher (Fig. 3). As discussed

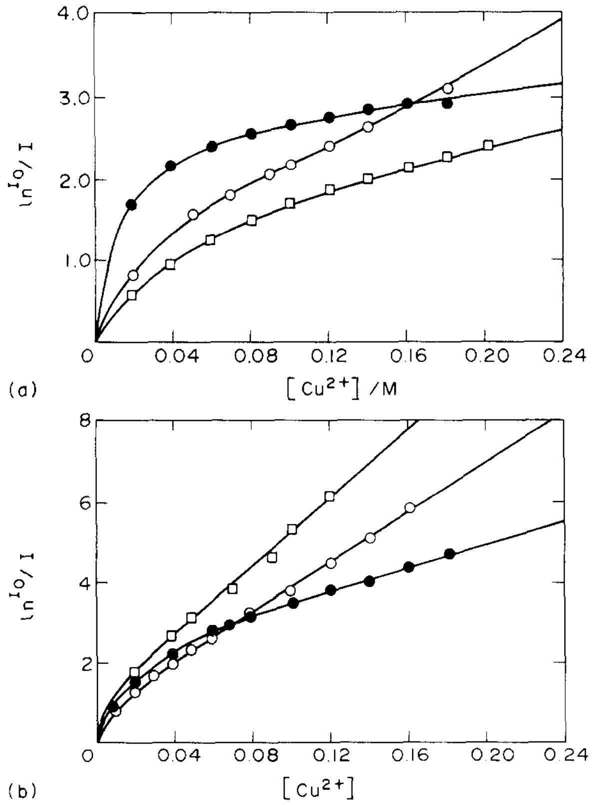


Fig. 3. (a) Plots of $\ln(I_o/I)$ vs. [Cu^{2+}] for 1- and 2-ROH* in H_2O and O/W ME: ○, 1-ROH* in ME; □, 2-ROH* in H_2O; ●, 2-ROH* in ME. (b) Plots of $\ln(I_o/I)$ vs. [Cu^{2+}] for 1- and 2-RO^-* in H_2O and O/W ME: (○, 1-RO^-* in H_2O; □, 1-RO^-* in ME; 2-RO^-* in H_2O).

earlier, below this limiting concentration of Cu^{2+} the quenching process obeys the SV equation, suggesting a dynamic process. This infers that quenching is purely dynamic up to a limiting concentration of Cu^{2+}, beyond which both dynamic and transient quenching processes are taking place.

Hence a model involving both dynamic and static quenching can be proposed for the present system [14]:

$$\frac{I_0}{I} = (1+K_{SV}[Q])\exp(K_T[Q]) \qquad (4)$$

The K_{SV} and K_T values are calculated using Eqs. (1) and (2) respectively and are given in Table 1.

The radii of the sphere of action are calculated from the values of $K_T (= VN_a)$ and are also compiled in Table 1.

The values of R are found to be in the order 1-RO^- (ME) > 1-RO^- (H_2O) > 2-RO^- (H_2O) > 1-ROH (ME) > 2-ROH (H_2O) > 2-ROH (ME). The radii of the sphere of action calculated for Cu^{2+} and $RO^{-}*$ (Table 1) are larger than the values for neutral molecules.

In microemulsion media the Cu^{2+} is partitioned between the ME surface and water so that the effective concentration of Cu^{2+} in the bulk water pool decreases as compared with that in aqueous medium. Boaz and Rollefson [15] have proposed a hydration model for the fluorescence quenching of acridones with I^-. In this model the fluorophore, quencher and water molecules are represented as spheres. In the present work the anions ($RO^{-}*$) are solvated, as a result of which ${Cu_{aq}}^{2+}$ ions remain at a greater distance than in the case of naphthols (Fig. 4(a)). Nigam et al. [16] have made a similar observation for the iodide ion–benzimidazole system. The fluorophores and quencher ions can be represented as spheres which may be in a solvent cage. If a solvent molecule comes between them, their separation increases. In a polar solvent such as water the ions form solvent-separated species and the entire system remains in a solvent cage. The naphthol–Cu^{2+} system may be covered by a solvent sheath without a solvent molecule between species (Fig. 4(b)). Thus the quenching process can be explained by a biphasic model consisting of two phases, i.e. (a) quenching spheres containing the excited fluorophores and the quencher, resulting in a transient component, and (b) the bulk phase where quenching is dynamic or diffusional. Such a situation exists in both aqueous and ME media.

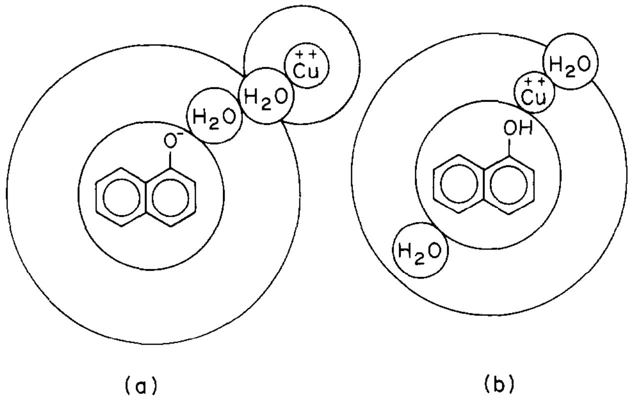
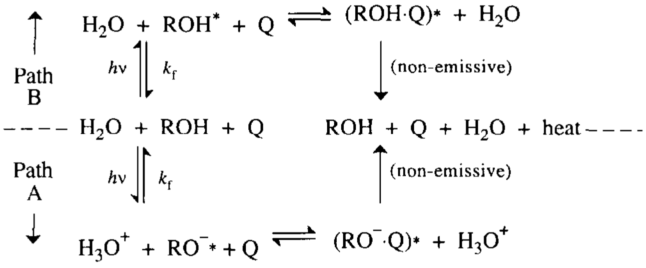


Fig. 4. Models for approximating the fraction of naphthols and naphtholates (represented as spheres) that are nearest neighbours to a Cu^{2+} ion in aqueous solution: (a) naphtholates are surrounded by a sheath of water which separates the quencher from the fluorophore; (b) naphthols may contain quencher for immediate contact with the fluorophore.

3.4. Mechanism of fluorescence quenching

From the absorption spectra and quenching behaviour of the system the biphasic model of fluorescence quenching of 1-ROH and 2-ROH can therefore be represented by Scheme 1.



Scheme 1. ROH in the scheme refers to both 1-ROH and 2-ROH.

We have reported previously that the average environment of 1-ROH and 2-ROH in O/W ME is hexane–isobutanol [17]. On excitation 1-ROH dissociates in ME partially to 1-RO^-, which resides only in aqueous environment. Therefore the model proposed in Scheme 1 holds good in both aqueous and O/W ME systems.

From the absorption spectra and quenching behaviour it is found that 1-ROH follows path A only in aqueous medium but both path A (48%) and path B (52%) in O/W ME. 2-

Table 1
Values of Stern–Volmer quenching constant (K_{SV}), radius of quenching sphere (R) and transient quenching constant (K_T) for ROH–Cu^{2+} systems in various environments at 27 °C

	R (nm)	K_{SV} ($mol^{-1}\ dm^3$)	K_T ($mol^{-1}\ dm^3$)
1. 1-RO^- (H_2O)	2.325	146.8 (0–0.02 M)	31.72 (0.03–0.12 M)
2. 1-ROH (ME)	1.671	75.0 (0–0.038 M)	11.77 (0.038–0.12 M)
3. 1-RO^- (ME)	2.575	234.0 (0–0.019 M)	43.10 (0.049–0.12 M)
4. 2-ROH (H_2O)	1.430	43.0 (0–0.1 M)	7.38 (0.1–0.2 M)
5. 2-RO^- (H_2O)	1.833	182.0 (0–0.03 M)	15.55 (0.06–0.2 M)
6. 2-ROH (ME)	1.252	200.7 (0–0.035 M)	4.95 (0.054–0.18 M)

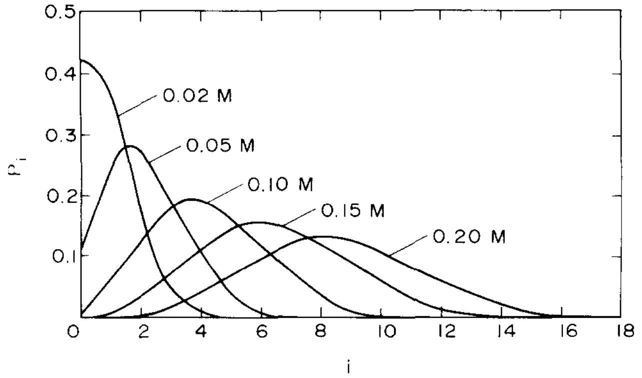


Fig. 5. Probability distribution for Cu^{2+} incorporation into the quenching sphere of 1-RO^{-}* present in O/W ME ($[1\text{-}RO^{-}] = 1\times10^{-4}$ M).

ROH, however, follows both paths (33% path A, 67% path B) in aqueous medium but path B only in O/W ME.

3.5. Probability distribution of Cu^{2+} in quenching sphere

Assuming that the association of Cu^{2+} in forming the quenching sphere obeys Poisson statistics, the probability distribution (P_i) that a fluorophore is bound with i copper ions is given by

$$P_i = \frac{m^i}{i!}\exp(-m) \qquad (5)$$

Where $m(\text{ROH}) = [Cu^{2+}]_b/[\text{ROH}]_t$ and $m(\text{RO}^-) = [Cu^{2+}]_b/[\text{RO}^-]_t$, where $[\text{ROH}]_t$ and $[\text{RO}^-]_t$ denote the total concentrations of naphthol and naphtholate respectively and $[Cu^{2+}]_b$ is the concentration of Cu^{2+} bound to the fluorophore.

The values of m have been calculated from the equation $m = K_T[Q]$, where K_T is the transient quenching constant [18]. The values of P_i are obtained for different i values (1, 2, 3, ...) of Cu^{2+} at any $[Cu^{2+}]$ and then theoretical plots of P_i vs. the number of Cu^{2+} ions incorporated in the sphere at various quencher concentrations are made. Representative plots for 1-RO^{-}* in O/W ME are shown in Fig. 5. The maximum of each curve is taken as the number of Cu^{2+} ions incorporated in the sphere at a specific $[Cu^{2+}]_t$. It is observed that the anion fluorophores incorporate a greater number of Cu^{2+} ions than do the un-ionized species at any $[Cu^{2+}]_t$ in all media, because of a greater degree of solvation of the former. At the concentration 1.2 M of Cu^{2+} the i values are found to be in the order 1-RO^- (ME, $i=8$) > 1-RO^- (H_2O, $i=6$) > 2-RO^- (H_2O, $i=3$) > 1-ROH (ME, $i=2$) ≈ 2-ROH (H_2O, $i=2$) ≫ 2-ROH (ME, $i\approx1$), which is the same order as for the change in radii of the sphere.

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