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Redox reactions and fluorescence spectroscopic behaviour of trifluoperazine at the surface of colloidal silica

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Abstract

Fluorescence spectroscopic behaviour and redox reactions of trifluoperazine (TFP) were studied in aqueous solutions in the presence of silica (SiO_2) particles. The effect of surface heterogeneity on the secondary reactions of the transients was determined using optical absorption and fluorescence techniques. It appears that electrostatic interaction is the driving force for adsorption of TFP over SiO_2 particle. Contrary to the change in fluorescence intensity with time observed in an aqueous solution, fluorescence intensity of TFP did not change significantly with time over the surface of SiO_2. Fluorescence microscopic observations of spleen cells treated with TFP over SiO_2 showed that the drug got distributed in cells similar to that observed in homogeneous aqueous solution.

Keywords: Trifluoperazine; SiO_2, electrostatic interactions; Redox reactions; Fluorescence microscopy; Pulse radiolysis

1. Introduction

Phenothiazines are good electron donors and can be photo-oxidised by near UV light. It has been reported that photo-induced charge separation in porous inorganic materials can achieve long lifetimes of photo-induced radical ions at room temperature [1]. This is because the back electron transfer rates are retarded in heterogeneous systems compared to that in the homogeneous solution.

Phenothiazine derivatives are widely used in the formulation of drugs and as photosensitizers. Therefore, phenothiazine drugs reactions with free radicals are well studied [2–5]. However, serious photo-toxic reactions (mainly diseases of epidermis and dermis) as well as photo-allergic and photo-mutagenic effects can be induced in patients subjected to irradiation with sunlight while treated with pharmacologically important chemicals such as tranquilizers. Such noxious effects are correlated to the drug photochemical activity and are substantially reduced in the presence of cyclodextrins with phenothiazines [6].

It is known that adsorption of a drug or protein to a surface generally produces a change in its physicochemical properties. This may affect the biological functioning of the molecule. Thus, in

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biomedicinal, biotechnological applications, food processing, etc., the occurrence of interfaces is of significant importance. Owing to the above-mentioned reasons, it has become very important to study the effect of adsorption on the stability of drugs to develop new bio-compatible materials. Recently, we have studied redox reactions of trifluoperazine (TFP) in aqueous solutions [7]. In this study we have extended our work by embedding TFP onto silica surface.

There were three main aims of this study. First, redox reactions of TFP were investigated and compared with that of dissolved TFP. Second, stability of TFP was investigated over SiO_2 since it is known that phenothiazine drugs photo-degrade in the dissolved state. Third, whether the cells treated with TFP show similar uptake of the drug both when it is adsorbed and in the dissolved state.

2. Experimental

2.1. Materials

TFP was obtained from Sigma and used as received. Silica (SM-30, 30 wt.%) was obtained from Aldrich. All other chemicals and reagents were HPLC, AR or GR grade. IOLAR grade (purity >99.99%) gases (N_2 or N_2O) used for bubbling the solutions were obtained from Indian Oxygen Limited. The pH was adjusted with Na_2HPO_4 to the required value. Nanopure water (conductivity 0.1 $\mu S\,cm^{-1}$) from a Barnstead (USA) nanopure water unit was used for making solutions. Centrifuge tubes were obtained from Nalgene (10 kD).

2.2. Methods

Ground-state absorption spectra were recorded with a JASCO V-530 UV–visible spectrophotometer. Steady-state fluorescence studies have been carried out with a Hitachi F-4010 fluorescence spectrophotometer. Fluorescence microscopy was carried out on a Karlzen fluorescence microscope.

Pulse radiolysis studies were carried out by irradiating solutions in rectangular quartz cells of 1-cm optical path length. Pulses of 50 ns of 7-MeV electrons from a linear electron accelerator were employed. The details of the LINAC are given elsewhere [8]. An aerated 5×10^{-2} $mol\,dm^{-3}$ KSCN solution was used for dosimetry and $(SCN)_2^{\bullet-}$ radical was monitored at 475 nm. The absorbed dose per pulse was calculated assuming $G\varepsilon$ for $(SCN)_2^{\bullet-}$ radical to be 2.6×10^{-4} $mol\,J^{-1}$ [9], where G is the radiation chemical yield expressed as the number of molecules formed or destroyed per 100 eV of energy absorbed and ε is the molar absorptivity. The dose employed in the present study was typically 14 Gy [1 Gy = 1 $J\,kg^{-1}$] per pulse. The radiation chemical yields of e_{aq}^- and $OH^\bullet$ radicals for 1 Gy are 0.28 and 0.29 μmol, respectively.

On irradiation of H_2O the following primary radicals are produced.

$$H_2O \rightsquigarrow e_{aq}^-,\ H^\bullet,\ OH^\bullet \tag{1}$$

Reactions of e_{aq}^- with TFP adsorbed over SiO_2 particles were studied in N_2-bubbled aqueous solution containing *tert*-butanol to scavenge $OH^\bullet$ radicals. Secondary one-electron oxidants were produced by the reaction of $OH^\bullet$ radicals with inorganic anions [10] via the following reactions in N_2O-saturated solutions.

$$e_{aq}^- + N_2O \xrightarrow{H_2O} N_2 + OH^- + OH^\bullet \tag{2}$$

$$OH^\bullet + N_3^- \rightarrow N_3^\bullet + OH^- \tag{3}$$

Subsequent reaction of the secondary oxidant, $N_3^\bullet$, with TFP adsorbed over SiO_2 particles resulted in the generation of one-electron oxidised species of TFP.The concentration of SiO_2 particles was calculated from Eq. (4).

$$[SiO_2]_p = \frac{[SiO_2]_M}{\eta} \tag{4}$$

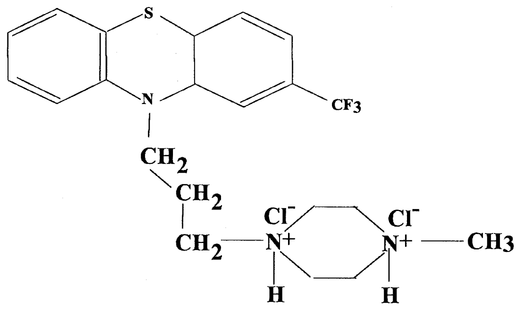
The aggregation number, η, was calculated from the radius, r, of the silica particles (Eq. (5))

$$\eta = \frac{4\pi r^3}{3}\rho\frac{N_o}{M_w} \tag{5}$$

where N_o is Avogadro's number, ρ is the density of silica (2.2 $g\,cm^{-3}$) and M_w is the formula weight of silica particles. The concentration of the colloidal silica particles in the stock solution was calculated to be 1.5×10^{-3} $mol\,dm^{-3}$.

3. Results and discussion

TFP is known to exist in aqueous solution in different states of protonation, depending on pH of the solution. Two pK_a values for TFP are reported to be 4 and 8.1 [11]. Therefore, most of our work was carried out at pH 6.



Trifluoperazine

3.1. Partition of TFP in homogeneous solution and at the surface of SiO_2

To carry out the work on TFP at the surface of SiO_2, it is important to know the partitioning of TFP in homogeneous solution and on the surface of SiO_2. For this, concentration of SiO_2 was kept constant at 1×10^{-4} $mol\,dm^{-3}$ and TFP concentration was varied from 1×10^{-5} to 5×10^{-4} $mol\,dm^{-3}$. The amount of TFP not adsorbed to the silica was determined by ultra-filtering the solution through centrifugating tube. The filtrate was then analysed for TFP by absorption spectroscopic method. However, no absorption of free TFP was observed in the filtrate. This showed that either TFP got completely adsorbed on SiO_2 or its concentration in aqueous solution was less than the detectable limit of the instrument.

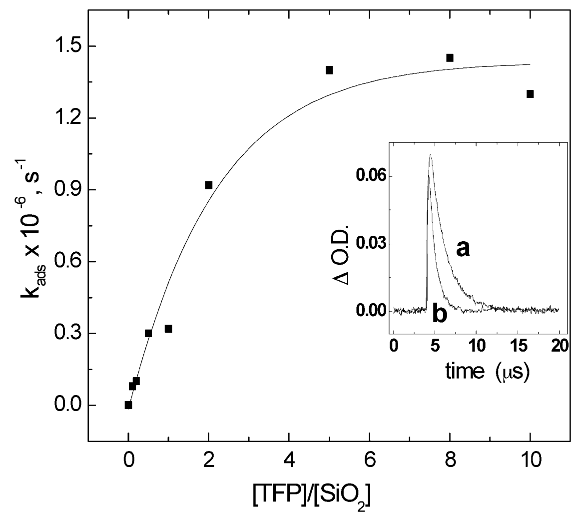


Fig. 1. Variation of rate constant for the reaction of e_{aq}^- with adsorbed TFP measured at 700 nm as a function of $[TFP]/[SiO_2]$ ratio. $[SiO_2]=10^{-4}$ $mol\,dm^{-3}$ and [TFP] varied from 10^{-5} to 10^{-3} $mol\,dm^{-3}$. Dose 14 Gy. Insert: decay at 700 nm in a solution containing (a) 1×10^{-4} $mol\,dm^{-3}$ SiO_2 without TFP; (b) 1×10^{-4} $mol\,dm^{-3}$ TFP over 1×10^{-4} $mol\,dm^{-3}$ SiO_2.

3.2. Redox reaction of TFP at the surface of SiO_2

The broad band gap (~10.5 eV) of the non-conducting SiO_2 precludes an electron transfer reaction from e_{aq}^- to SiO_2. It was observed that the decay rate of the solvated electrons, followed by monitoring its absorption at 700 nm, was independent of SiO_2 concentration.

The reaction of e_{aq}^- with TFP may be conveniently followed at 700 nm. Insert of Fig. 1 shows the decay of e_{aq}^- in the presence of TFP over SiO_2. Fig. 1 also shows the decay of e_{aq}^- in a solution containing only SiO_2. Assuming complete adsorption of TFP over SiO_2 and from the knowledge of the natural lifetime of the electrons under experimental conditions employed, the rate of the reaction of e_{aq}^- with TFP can be given as

$$k_{ads}=k_{obs}-1/\tau_0 \tag{6}$$

where k_{obs} is the measured pseudo-first order rate constant and τ_0 is the lifetime of $e_{aq}^-(=4.0\times10^5$ $s^{-1})$ in the absence of TFP in the experiments.

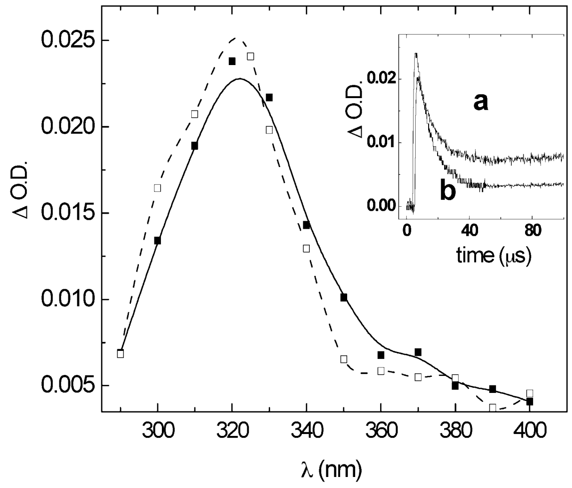


Fig. 2. Transient absorption spectrum obtained at 3 μs in N_2-bubbled aqueous solution of 1×10^{-4} mol dm^{-3} TFP, 2 mol dm^{-3} *tert*-butanol —■— without SiO_2 and – –□– – with 1×10^{-4} mol dm^{-3} SiO_2. Dose 14 Gy. Insert: decay at 330 nm (a) 1×10^{-4} mol dm^{-3} TFP; (b) 1×10^{-4} mol dm^{-3} TFP over 1×10^{-4} mol dm^{-3} SiO_2.

Fig. 1 shows the dependence of k_{ads} on the concentration of the adsorbed TFP. The concentration of SiO_2 used was 10^{-4} mol dm^{-3}. It can be seen that when [TFP]/[SiO_2] ratio is 5:1 the rate becomes independent of TFP concentration at the silica surface. It is important to mention that at TFP concentration $\geqslant 7\times10^{-4}$ mol dm^{-3} some turbidity starts appearing in the solution. This could be due to the aggregation of SiO_2 particles. Therefore, most of the work was carried out at [TFP]/[SiO_2] ratio = 1 (i.e. both at 10^{-4} mol dm^{-3}).

Fig. 2 shows the transient absorption spectrum of a solution containing 1×10^{-4} mol dm^{-3} TFP, at an average ratio of 1:1 TFP/SiO_2 following irradiation with 50-ns electron pulse (4×10^{-6} mol dm^{-3} of e^-_{aq} per pulse). An absorption band centered at approximately 325 nm forms following the pulse. The formation is complete within 2.5 μs. For comparison, reaction of e^-_{aq} with TFP is also shown in the same figure under identical conditions but without SiO_2. It can be seen that the absorption features remain the same in both the cases. An interesting phenomenon was observed on comparing the decay of TFP radical anion with and without the presence of SiO_2. It can be seen from the insert of Fig. 2 that TFP radical anion decays almost completely over the surface of SiO_2. This shows that over SiO_2 surface the formation of radiolytic products of TFP are less as compared to that in its absence. This point has been further confirmed by fluorescence technique also (see later).

One-electron oxidation of TFP was also studied with $N_3^{\bullet}$ radical. Fig. 3 shows the transient absorption spectrum of a N_2O-saturated solution containing 1×10^{-4} mol dm^{-3} TFP over 1×10^{-4} mol dm^{-3} SiO_2 and 5×10^{-2} mol dm^{-3} NaN_3. It can be seen that the transient exhibits an absorption maximum at approximately 500 nm. The concentration of N_3^- was kept high as compared to that of TFP to ensure that $OH^{\bullet}$ radicals react predominantly with N_3^-. As $N_3^{\bullet}$ is a specific one-electron oxidant, the observed transient spectrum is assigned to the radical cation of $TFP^{\bullet+}$. Insert of Fig. 3 shows formation of TFP cation radical with and without the presence of SiO_2 in solution. It can be seen that the formation of the radical cation is slower when TFP is adsorbed on SiO_2 surface as compared to its formation in an aqueous solution. Also, the radical cation yields are the same

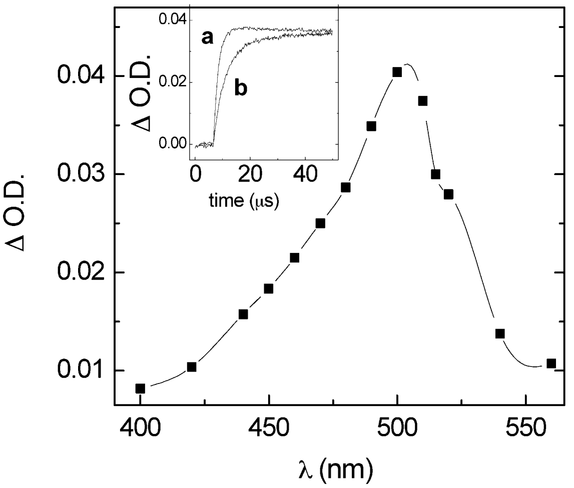


Fig. 3. Transient absorption spectrum of N_2O-saturated solution of 1×10^{-4} mol dm^{-3} TFP, 1×10^{-4} mol dm^{-3} SiO_2 and 5×10^{-2} mol dm^{-3} NaN_3 —■— at 40 μs. Dose 14 Gy. Insert: formation of the transient at 500 nm (a) without SiO_2; (b) with 1×10^{-4} mol dm^{-3} SiO_2.

for both the cases. Thus, it can be inferred that complete oxidation of TFP takes place over the surface of SiO_2. The rate constants for the formation of TFP radical cation are 2.7×10^5 and 1.0×10^5 s^{-1} for TFP without SiO_2 and TFP over SiO_2 surface, respectively.

To explain the above results the following mechanism can be invoked. That is, initially the diffusion of e_{aq}^- or $N_3^\bullet$ to the silica particle takes place followed by diffusion of e_{aq}^- or $N_3^\bullet$ along the silica particle till it encounters an adsorbed TFP. Under such conditions the rate-determining step is the diffusion of e_{aq}^- or $N_3^\bullet$ to the particle; therefore, we expect a decrease in the rate of the reaction. Indeed, the rates of reactions of e_{aq}^- or $N_3^\bullet$ with TFP over SiO_2 decreased. The bimolecular rate constants of the reactions of e_{aq}^- and $N_3^\bullet$ with TFP over SiO_2 at pH 6 were calculated to be 5.4×10^9 and 9.9×10^8 $dm^3\,mol^{-1}\,s^{-1}$. Corresponding rate constants without SiO_2 was found to be 1.5×10^{10} and 2.7×10^9 $dm^3\,mol^{-1}\,s^{-1}$. It was observed that once the primary/secondary radicals diffused to the particles they reacted with TFP completely. This is evident from the absorbance of TFP radical anion and cation formed on reaction with e_{aq}^- or N_3 with and without SiO_2 particles.

3.3. Fluorescence properties of TFP in aqueous solution and over SiO_2 surface

In this section we describe photophysical studies of TFP on surrogate surface (SiO_2) that is likely to be energetically incapable of functioning as an electron acceptor. Fig. 4a shows the fluorescence spectrum of TFP at pH 6. The corresponding excitation spectrum for 475-nm fluorescence band is shown in the insert of Fig. 4a. It can be seen that the fluorescence spectrum exhibits the bands at 410 and 475 nm. It is reported that TFP exhibits fluorescence with only one maximum at approximately 465 nm [12]. However, on UV irradiation another band appears at 410 nm in the fluorescence spectrum. This band has been assigned to the formation of photo-products. The excitation spectrum agrees roughly with the reported excitation spectrum for TFP and its photo-products. The fluorescence spectrum of TFP shows a peak at approximately 310 nm in its excitation spectrum, while the photo-products that show emission at 410 nm has a peak in the excitation spectrum at 350 nm. It is known that TFP undergoes photooxidation on UV irradiation [12]. Therefore, an attempt has been made to see whether TFP stability can be increased over the surface of SiO_2. Fig. 4b shows the fluorescence spectrum of TFP over the surface of SiO_2. It can be seen that only one fluorescence maximum at ~475 nm was observed under identical conditions as in Fig. 4a. The excitation spectrum showed a maximum at 310 nm. This showed that the formation of the photoproducts gets inhibited over the surface of SiO_2. To further substantiate this observation we have recorded the fluorescence spectra of TFP with and without SiO_2 after 24 h. It can be seen from Fig.

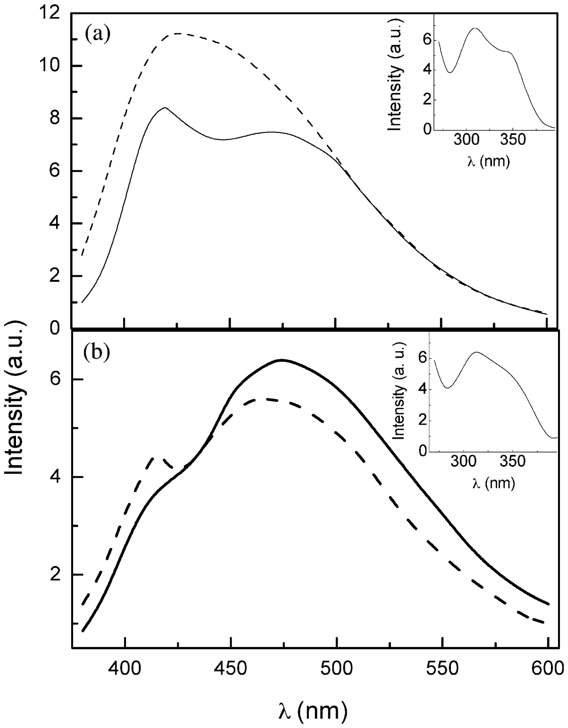


Fig. 4. (a) Fluorescence spectrum of 1×10^{-4} mol dm^{-3} TFP, $\lambda_{exc}=310$ nm, at pH 6, — 5 min and – – – – 24 h after preparation of the solution. Insert: excitation spectrum ($\lambda_{emm}=475$ nm). (b) Fluorescence spectrum of 1×10^{-4} mol dm^{-3} TFP over 1×10^{-4} mol dm^{-3} SiO_2; $\lambda_{exc}=310$ nm, at pH 6, — 5 min and – – – – 24 h after preparation of the solution. Insert: excitation spectrum ($\lambda_{emm}=475$ nm).

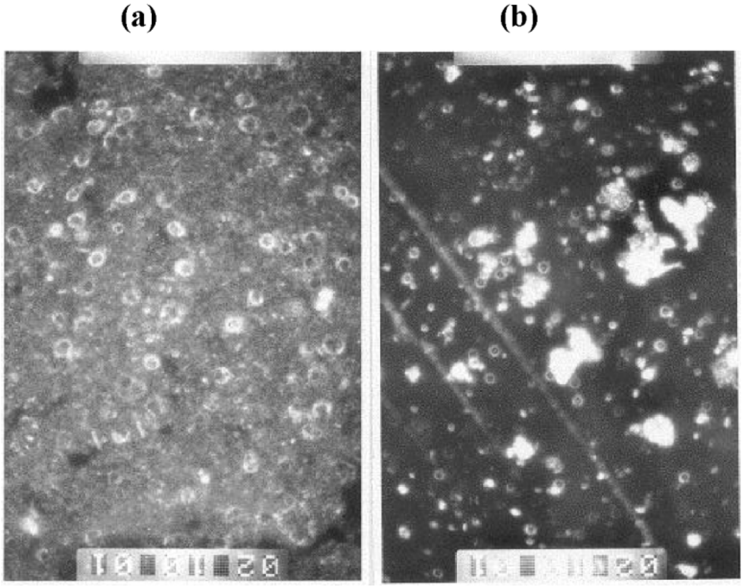


Fig. 5. Fluorescence microscopic photographs. (a) Spleen cells treated with 1×10^{-4} mol dm^{-3} TFP over 1×10^{-4} mol dm^{-3} SiO_2 in physiological saline solution. (b) Spleen cells treated with 1×10^{-4} mol dm^{-3} TFP in physiological saline solution.

4a that TFP dispersed in aqueous solution shows a maximum at 410 nm that is of its photo-products only. Contrary to this, only a slight hump was observed for TFP solution having SiO_2 (Fig. 4b). This experiment clearly showed that the formation of the photo-products of TFP (or its photo-degradation) gets inhibited over the surface of SiO_2. It is important to mention here that under radiolytic conditions (Fig. 2) we have also observed that radiolytic products get inhibited over the surface of SiO_2.

Colloidal silica is an inorganic colloid and the particles are spherical, possessing a negative charge consisting of SiO^- groups and adsorbed OH^- ions. The silanol groups of the surface of SiO_2 particles are ionized at $pH \geqslant 5.5$ and produce a diffuse double layer. The above observation can be explained as on oxide support, the nature and extent of the surface reactive groups drastically affect the mode of distribution and the local organization of TFP, which gets reflected in the observed photophysics of the adsorbates due to the surface interactions. It is important to mention here that the majority of electron–hole pairs created in SiO_2 rapidly recombine. Recent ultrafast measurements have estimated the mean lifetime of photo-generated electrons to be on the order of 150 fs [13]. The remaining charge carriers move to the conduction and valence bands, eventually forming excitons or localizing in trap levels that are non-interfering in nature [14–18].

3.4. Fluorescence microscopy

It is known that the drug-induced photosensitivity of the skin is caused by exposure to sunlight [1]. Photo-degradation of drug, which also results from exposure to sunlight, sometimes leads to side effects. The results obtained above show that photo-degradation of TFP decreases when bound to the surface of SiO_2. To substantiate the results obtained we have made an attempt to examine whether TFP on SiO_2 enters the cells. Three different cells from three different tissues of mice—spleen, liver and fibrosarcoma tumour—were incubated separately with TFP and TFP–SiO_2 in physiological saline for 1 h at 28 °C. After incubation the cells were observed under a Karlzen fluorescence microscope. In all the cells it was observed that the drug with and without SiO_2

enters into the cells. A representative case of spleen cells treated with TFP and TFP/SiO_2 is shown in Fig. 5a. It can be seen that the drug gets distributed homogeneously inside the cells. Similar result was obtained with TFP that is without SiO_2 (Fig. 5b). Thus, it can be inferred that the presence of SiO_2 not only provides stability to the drug but also does not interfere with the mobility of the drug into the cells.

4. Conclusion

The radiolytic and photolytic degradation of TFP gets inhibited when it is adsorbed over the surface of SiO_2. Also, the adsorbed TFP over the surface is able to penetrate into the cells. The work presented may have important biological implications.

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