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Correlations between the self-organization, physicochemical properties and biological activity of Mebicar in dilute aqueous solutions

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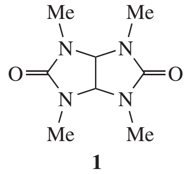
The nanoassociates formed in the aqueous solutions of the tranquilizer Mebicar were found to affect the physicochemical properties of the solutions and hence to result in its unusual concentration pharmacological profile.

The physicochemical mechanisms of the effects of dilute solutions on living organisms are of considerable current interest. Previously, we found that nanoassociates are formed in the highly dilute aqueous solutions of bioactive compounds (1×10^{-20}– 1×10^{-6} mol dm^{-3}) with the participation of external electromagnetic fields (EMFs). These nanoassociates are responsible for the unusual physicochemical properties and bioeffects of solutions.[1–8] If the EMF level is reduced (hypoelectromagnetic conditions), nanoassociates are not formed in highly dilute solutions; hence, the properties of low-concentration solutions are lost or weakened considerably.

This study was aimed at establishing a relationship between the self-organization, physicochemical properties and pharmacological profile of dilute (1×10^{-18}–0.1 mol dm^{-3}) aqueous solutions of 2,4,6,8-tetramethyl-2,4,6,8-tetraazabicyclo[3.3.0]octane-3,7-dione **1** upon exposure to natural *vs.* hypoelectromagnetic conditions.



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Compound **1** is the active ingredient of the tranquilizer Mebicar.[9] The intermolecular interactions and specific features of solvation in the solutions of compound **1** were previously studied in the ranges of 0.15–1.5 and $5.0\text{–}10^{-4}$–5.0×10^{-3} mol dm^{-3}.[10–12] These values are close to the recommended therapeutic doses of Mebicar.[9] We studied the self-organization and properties of **1** in solutions under natural and hypoelectromagnetic conditions in a broad range of concentrations using physicochemical methods [dynamic light scattering (DLS), microelectrophoresis, pH measurement, conductometry, polarimetry, *etc.*].[†]

The effect of the reduced levels of external EMFs on the formation of nanoassociates and the physicochemical properties of the solutions of compound **1** with various concentrations were examined using a three-layer shielding permalloy container that protected its contents from exposure to external EMFs. The

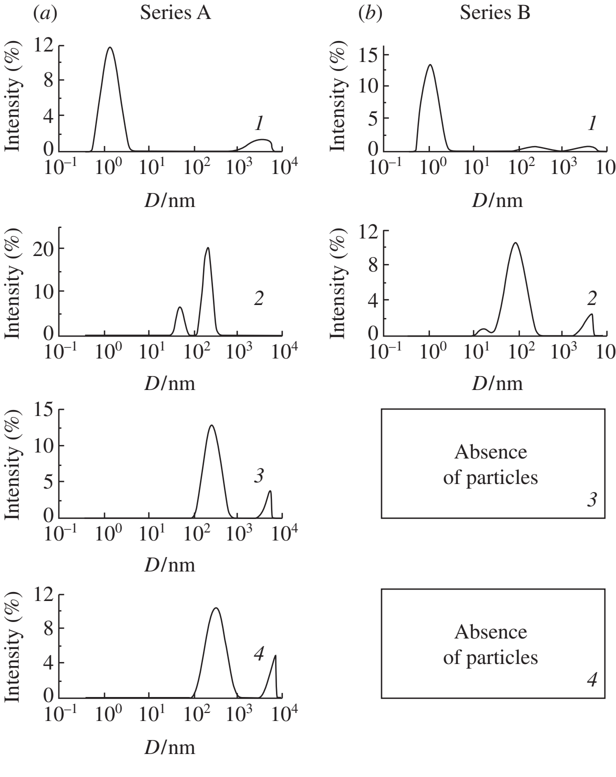

Figure 1 Size distribution of particles in an aqueous solution of **1** kept for 24 h on the laboratory bench (series A) and in the shielding permalloy container (series B). Concentration of the aqueous solution of **1** is (*1*) 0.1, (*2*) 10^{-3}, (*3*) 10^{-6} and (*4*)10^{-16} mol dm^{-3}.

[†] The test solutions of compound **1** were prepared using twice-distilled water with electric conductivity of no higher than 1.5 μS cm^{-1}. The specific electric conductivity (χ), pH, dielectric constant (ε) and optical activity of the solutions (α, angle of rotation of the polarization plane of light with a sodium D-line wavelength of 589 nm) were measured with an inoLab Cond Level 1 conductivity meter, a BI-870 dielcometer (Brookhaven Instruments), an inoLab pH meter and a Perkin-Elmer-341 polarimeter using a thermally controlled cell (25±0.1 °C) 5.6 cm long; the instrument accuracy was ±0.002°. The particle size (*D*, effective hydrodynamic diameter of kinetically mobile particles at a maximum of the distribution curve) was found by dynamic light scattering (DLS) using a Zetasizer Nano ZS high-sensitivity analyzer (Malvern Instruments). The ζ-potential was determined by microelectrophoresis using the same instrument. The samples for size and ζ-potential measurements were prepared in accordance with published procedures.[1–8] The measurements were carried out at 25 °C. The errors in the measurements of particle sizes and physicochemical properties of solutions did not exceed 15%.

working solutions were kept for 24 h on a laboratory bench (natural conditions, series A, Figure 1) and in a shielding container (hypoelectromagnetic conditions, series B, Figure 1). The parameters of particles and physicochemical properties were compared for series A and B using the same solution. Each experiment was performed in triplicate similarly to the previous studies.[7,8]

Figure 1(*a*) presents the size distribution of particles in solutions of compound **1**, series A, kept under natural conditions. At concentrations of 0.1 and 0.01 mol dm^{-3}, the solution contains hydrated molecules of **1** about 1.5 nm in size [Figure 1(*a*), *1*], in agreement with microwave spectroscopy data.[10,11]

In a concentration range from 1x10^{-3} to 1x10^{-5} mol dm^{-3}, particles with sizes from tens to hundreds of nanometers are detected in the solutions [Figure 1(*a*), *2*]. These particles can be attributed to supramolecular domains.[13–15] At concentrations of 1x10^{-6} to 1x10^{-18} mol dm^{-3} [Figure 1(*a*), *3* and *4*], molecular assemblies of size from hundreds to thousands nanometers (nanoassociates) are formed in the solutions of series A.

In the solutions of **1**, series B, which were kept in the shielding container, particles were formed only in an ordinary concentration range from 1x10^{-5} to 0.1 mol dm^{-3} [Figure 1(*b*), *1* and *2*] but not in highly diluted solutions of 1x10^{-6} mol dm^{-3} or lower.

As can be seen in Figures 1(*a*),(*b*), the formation of supramolecular domains in a concentration range from 1x10^{-3} to 1x10^{-5} mol dm^{-3} occurs under both natural and hypoelectromagnetic conditions regardless of the presence of external EMFs, whereas nanoassociates can be formed at low concentrations only in the presence of such fields. Figure 2 shows the concentration dependence of the size (*D*) and ζ-potential of particles in the solutions of compound **1** (standard deviations are indicated); the threshold concentration (C_{thr}) is 1x10^{-5} mol dm^{-3} below which nanoassociates are formed in solutions of **1**.

Thus, a comparative study of the self-organization of solutions of compound **1** kept under natural and hypoelectromagnetic conditions has shown that particles varying in nature and size are formed in different concentration ranges: nanoassociates (200–400 nm), supramolecular domains (170–270 nm) and hydrated molecules (1.5 nm) are formed at 1x10^{-18}–1x10^{-6}, 1x10^{-5}–1x10^{-3} and 0.01–0.1 mol dm^{-3}, respectively.

A study of the concentration plots of *D* and ζ-potential of nanoassociates in solutions of **1** and specific conductivity (χ) of these solutions revealed that changes in these parameters are interrelated and nonlinear (Figure 2). Three concentration regions of 1x10^{-8}–1x10^{-6}, 1x10^{-11}–1x10^{-9} and 1x10^{-17}–1x10^{-12} mol dm^{-3} can be distinguished, within which a relationship between these parameters is most pronounced. For example, the maximum values of χ (~7 μS cm^{-1}) at Mebicar concentrations of 1x10^{-10} and 1x10^{-15} mol dm^{-3} are primarily due to the formation of nanoassociates with the most negative ζ-potentials (–12 and –6 mV, respectively) in these concentration ranges.

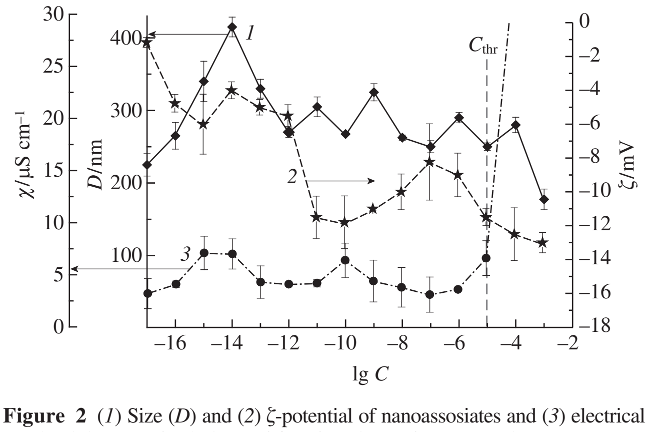

Figure 2 (*1*) Size (*D*) and (*2*) ζ-potential of nanoassosiates and (*3*) electrical conductivity (χ) of solutions of **1** as functions of concentration.

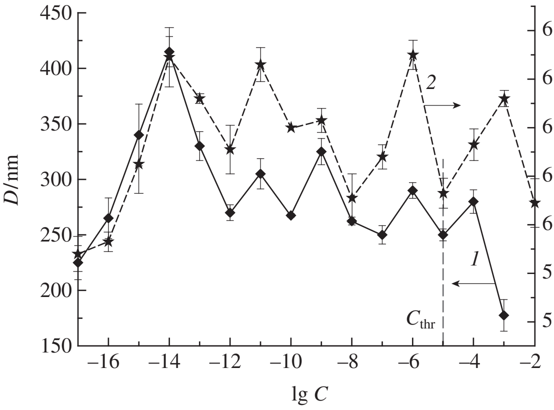

Figure 3 (*1*) Size (*D*) of nanoassociates and (*2*) pH of solutions of **1** as functions of concentration.

The relationships between the concentration plots of the *D* of nanoassociates and the pH of solutions of **1** (Figure 3) and between the ζ-potential of nanoassociates and the dielectric constant (ε) of the solutions (Figure 4) are similar.

Figure 3 shows a correlation between the concentration plots of the D of nanoassociates and the solution pH (the shapes of the curves are nearly the same). In a concentration range of 1x10^{-16}–1x10^{-10} mol dm^{-3}, correlation analysis revealed a relation (r = = 0.845, p = 0.04) between the nanoassociate size and the solution pH, which suggests that the reorganization of nanoassociates involves hydroxonium and hydroxy ions in the water structures that form the nanoassociates, as well as bulk water molecules.

A comparison of the concentration plots of the ζ-potential of nanoassociates and $\Delta\varepsilon$ of solutions of compound **1**, where $\Delta\varepsilon$ is the difference of ε values of the solution and solvent (twice-distilled water, ε = 80.1), also suggests that the concentration plots of nanoassociate parameters (*D*, ζ-potential) and dielectric constant $\Delta\varepsilon$ of solutions correlate: the extremums of both values occur at nearly the same concentrations as for χ, namely, 1x10^{-7}, 1x10^{-10} and 1x10^{-14} mol dm^{-3} (see Figures 2 and 4).

It is evident from Figure 4 that, as the concentration of **1** decreases, the sign of $\Delta\varepsilon$ changes: $\Delta\varepsilon$ is negative at 1x10^{-7} and 1x10^{-14} mol dm^{-3} and positive at 1x10^{-10} mol dm^{-3}. According to the dielcometry theory,[16] it may be due to changes in the way in which the structures self-assemble as they are formed in solution. In the concentration range where the $\Delta\varepsilon$ values of the solution are negative, nanoassociates are likely to line up to low-polar multipole structures, thus decreasing the dielectric constant ε of the solution in comparison with that of the solvent. In the region of positive $\Delta\varepsilon$ values with a maximum at 1x10^{-10} mol dm^{-3}, nanoassociates can form chain (band) structures where the dipole moments are arranged unidirectionally. It is at this concentration

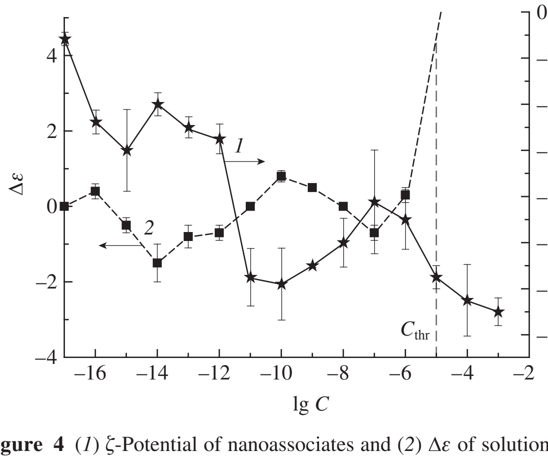

Figure 4 (*1*) ζ-Potential of nanoassociates and (*2*) $\Delta\varepsilon$ of solutions of **1** as functions of concentration.

that the maximum ζ-potential (–12 mV) is recorded in a highly diluted solution of **1**.

The capability of nanoassociates to form structures with diverse spatial organization may become the origin of optical activity in highly diluted solutions.[8] The molecules of chiral compounds can assemble surrounding achiral solvent molecules into chiral aggregates that possess optical activity; in this case, the contribution of the chiral compound itself to the total optical activity is insignificant.[17,18] In order to check whether optical activity can appear in the highly diluted solutions of **1**, we measured the optical rotation (rotation angle of light polarization plane α) of solutions in a concentration range of 1×10^{-18}–1×10^{-3} mol dm^{-3}. Only at 1×10^{-10} mol dm^{-3}, α has been found to reliably exceed the experimental error; it was (–)0.007±0.002° based on a triplicate experiment. This result confirms that the nanoassociates formed in a 1×10^{-10} mol dm^{-3} solution of **1** differ in their ordering from the nanoassociates formed at the other concentrations.

The solutions of **1**, series B, kept in the shielding container, have the following properties below C_{thr} (1×10^{-5} mol dm^{-3}): the mean pH values of the solutions (pH 6) do not change; the $\Delta\varepsilon$ values of the solutions approach zero; and α does not reliably exceed the measurement error. Hence, in the absence of nanoassociates, many physicochemical properties of low-concentration solutions of **1** nearly match those of distilled water. These findings serve as additional evidence that, in spite of the possibility of the influence of such contaminations as CO_2 from the atmosphere or Ca^{2+} and silicate ions from glass, the true cause of the nonlinear concentration dependences of physicochemical properties is the formation and rearrangement of nanoassociates. If there are no nanoassociates, the properties of low-concentration Mebicar solutions correspond to the properties of distilled water.

The occurrence of bioeffects in the solutions of bioactive compounds at low (1×10^{-11}–1×10^{-7} mol dm^{-3}) and ultra-low concentrations (1×10^{-20}–1×10^{-12} mol dm^{-3}) is well known.[19–21]

We were the first to discover the property of highly diluted Mebicar solutions to affect the behavioural characteristics of animals by changing the pharmacological profile to an opposite one. A 1×10^{-3} mol dm^{-3} solution of compound **1** exhibits an anxiolytic activity, *i.e.* it provides a sedative effect combined with a decrease in exploratory activity while retaining the motion activity. As the Mebicar concentration is reduced, the solutions of **1** start to show a psychostimulating effect. It has a maximum at 1×10^{-7} mol dm^{-3}, increasing the motion and exploratory activities of mice by factors of 3 and 1.5, respectively. At concentrations of 1×10^{-10} and 1×10^{-14} mol dm^{-3}, the psychostimulating effect decreased. However, at 1×10^{-14} mol dm^{-3}, the motion activity, which is a component of the psychostimulating effect, increased again. This behavioural reaction of mice upon treatment with highly diluted Mebicar solutions is a typical example of a complex concentration–bioeffect correlation that reflects the existence of hormesis.[19–21]

A hypothesis based on experimental data was proposed[2,5,8] to explain the paradoxical phenomenon of the complex concentration–bioeffect relationships by differences in the structures formed at regular and low concentrations of bioactive compounds in solutions . The results obtained for Mebicar solutions confirm this hypothesis. The solutions exhibited a psychostimulating effect at concentrations below 1×10^{-5} mol dm^{-3}, where nanoassociates were formed, or an anxiolytic effect in the region above the threshold concentration where supramolecular domains are formed.

Thus, the study of aqueous Mebicar solutions allowed us to establish that nonlinear concentration plots of the size and ζ-potential of nanoassociates and the physicochemical properties of highly diluted solutions of **1** in a concentration range 1×10^{-18}–1×10^{-6} mol dm^{-3} correlate. Furthermore, the extremal parameters of the nanoassociates occur at nearly the same concentrations as those of the physicochemical properties and bioeffects of solutions. Nanoassociates are not formed in Mebicar solutions kept under hypoelectromagnetic conditions, and the properties of such solutions match those of distilled water.

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