CrossMark

"Exclusion Zone" Formation in Mixtures of Ethanol and Water

S. A. Skopinov[1] · M. V. Bodrova[2] · M. P. R. Jablon[3] ·
G. H. Pollack[3] · F. A. Blyakhman[1,2]

Abstract Brownian particles suspended in water or other polar liquids are pushed out of the region next to hydrophilic polymers, leaving a microsphere-free region known as the "exclusion zone" (EZ). This study aimed to test the hypothesis that the dilution of ethanol in water may influence EZ formation. EZs were created in aqueous media using Nafion tubes as EZ-nucleating surfaces. To define the outer edge of the EZ, carboxylate microspheres, 1 μm diameter, were used. Dynamic movement of microspheres away from Nafion surface was registered in mixtures of ethanol and water, the ethanol concentration varying from 0 to 95%. We found that mixtures with the highest concentrations of ethanol generally produced the smallest EZs and the slowest EZ buildup. However, an unexpected result was the presence of an extremum corresponding to ∼10% ethanol. At this concentration, the EZ is larger than in either pure water or almost pure ethanol.

Keywords Hydrophilic surfaces · Water · Ethanol · Brownian particle motion · Exclusion zone (EZ)

1 Introduction

Brownian particles (polymeric microspheres, 1.0 μm diameter) suspended in water are pushed out of the region next to hydrophilic polymers [1]. This region, often reaching as much as several hundred micrometers, has been dubbed the "exclusion zone" (EZ). Three main explanations of the effect have been put forth—electrostatic interactions, chemical

✉ F. A. Blyakhman
  Feliks.Blyakhman@urfu.ru

1   Biomedical Physics Department, Ural State Medical University, Yekaterinburg, Russia 620028

2   Physics Department, Ural Federal University Named After the First President of Russia B.N. Yeltsin, Yekaterinburg, Russia 620083

3   Department of Bioengineering, University of Washington, Seattle, WA 98195, USA

gradients, and the structuring of water induced by interaction with the polymer surface. Although the first two mechanisms may influence the formation of the exclusion zone, the water-structure mechanism has the most comprehensive supporting evidence [2]. The structure of water is defined by a crystal-like ordering, the crystals bearing some resemblance to ice [3].

Subsequent research has shown that exclusion zones are observed not only in water, but also in other polar liquids (methanol, ethanol, isopropanol, acetic acid) and in liquids with strong dipolar interactions (DMSO, acetone, diethyl ether) [4]. All of these substances form EZs with smaller size relative to those in distilled, deionized water. In pure ethanol, the width of EZ at the end of the observation period (10 min) was reported as approximately five times smaller than that in pure water [4].

Both water and ethanol are known to participate in hydrogen bonding, but their molecular structures differ. In water, molecules have been suggested to form a volume net similar to a hexagonal crystal system [5], whereas ethanol molecules form one-dimensional chains [6]. In spite of such notable differences, both pure water and pure ethanol form qualitatively similar zones next to hydrophilic surfaces [4]. This similarity implies that the EZ structures may differ altogether from the respective bulk features, but are related more closely to the hydrophilic surfaces next to which they form. As water is a good solvent for ethanol, we may assume that adding ethanol should have some influence on the EZ formation. To test this hypothesis, we investigated the formation of exclusion zones in various mixtures of ethanol and water.

Here we present the results of EZ width measurements at different concentrations of ethanol, ranging from 0 to 95%. We show an unexpected result, that in mixtures of 90% water and 10% ethanol the EZ size is significantly larger than that in either pure water or almost pure (95%) ethanol.

2 Materials and Methods

2.1 Materials

Hydrophilic polymer tubes of Nafion [7] with 1.8 mm outside diameter (Perma Pure LLC) were used. The tubes were initially kept in the respective solvents for 20 min to exclude any influence of transient tube swelling. Bi-distilled water, prepared using a water distillation unit J-WDR (JISICO CO, Ltd, Korea), and a solution of 95% ethanol and 5% water (chemically pure, "Rosbio Ltd", St. Petersburg, RF) were used for the preparation of mixtures, to an accuracy of approximately 1.0 vol%. All concentrations of the ethanol aqueous mixtures were corrected for the water content present in the source ethanol. For each mixture, the total volume was 20 mL.

To define the edge of the exclusion zone, carboxylate microspheres with a mean diameter of 1.0 μm (coefficient of variance: 3%; "Polysciences", Warrington, PA) were used. Microsphere suspensions with initial concentration about 5×10^7 mL^{-1} were added to the solution in the proportion 20 μL per 20 mL so that the final concentrations were $5 \times 10^4 \cdot$microspheres$\cdot$mL^{-1}. The microsphere suspensions were placed in a Petri dish together with the pre-soaked Nafion tube, which remained at the bottom of the chamber because of natural adherence to the hydrophobic plastic.

2.2 Measurements

Visualization of exclusion-zone formation was carried out using an inverted microscope ("Micromed-I" (Micromed Ltd., St. Petersburg, R.F.) equipped with a 10× objective and a digital camera (ToupTek Photonics, China). The microscope was focused at the mid-height of the external surface of the horizontal Nafion tube. Figure 1 presents typical images of

Fig. 1 Exclusion of microspheres close to the Nafion surface in pure water (**a**) and 95% ethanol by volume (**b**). *Arrows* indicate the EZ width as the distance between the edge of the polymer (*bottom*) and the edge of the microsphere zone
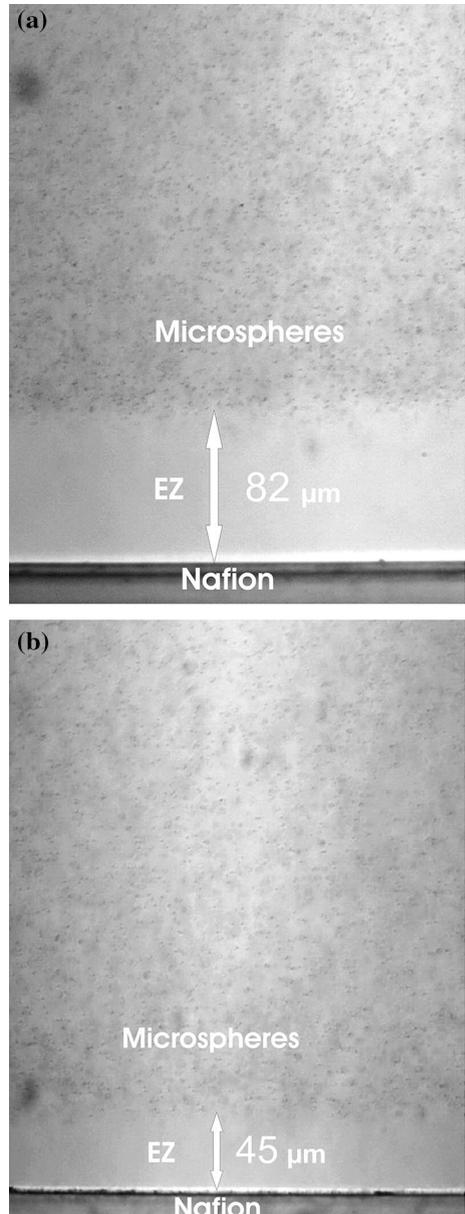
the microsphere distribution in pure water (A) and 95% ethanol (B) obtained to the end of the EZ observation period (10 min).

The dynamic movement of microspheres away from the Nafion surface was registered over 10 min, by which time the EZ had grown approximately to its full extent. Video images of EZ growth were recorded at 4.7 frames per second, and processed using the "ToupView" software package. To estimate the EZ width quantitatively, the distances between Nafion's external surface and 30 different border microspheres were measured for every frame. Typically, the standard deviation of mean distance (EZ size) in each frame was equal to $\pm$ (3–5) µm ($n = 30$).

2.3 Experimental Protocol

All experiments were carried out at room temperature, 24 ± 2 °C. The dynamics of EZ formation were examined in mixtures of ethanol and water, where the ethanol concentration was varied from 0 to 95% by volume. To properly track the initial stage of EZ formation, just before the video recording was to be made, the Petri dish was shaken to disrupt the EZ, so that regrowth dynamics could be tracked. For every ethanol concentration, three such runs were carried out to estimate the reproducibility. Thus, at any given time (frame) during EZ formation, the EZ size was determined three times, and the mean value served as the final EZ width.

To determine the effect of ethanol concentration on EZ size, EZ widths were measured at the end of the observation period (10 min) at each of 18 different solvent concentrations.

The statistic software package "SPSS Statistics 17.0" (IBM) was used for statistical analysis.
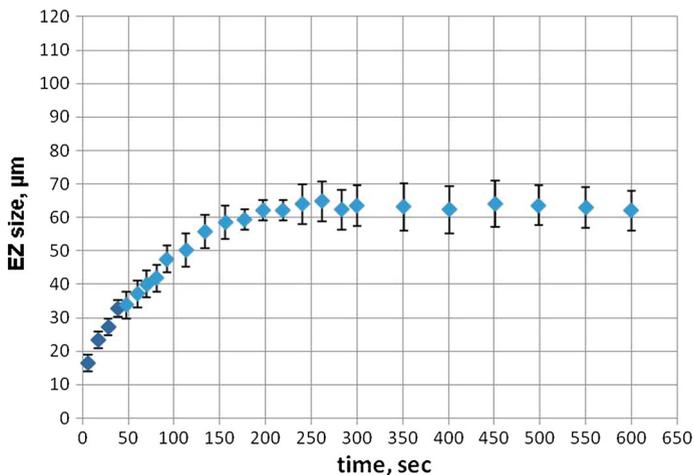
Fig. 2 EZ formation in aqueous solution of 25% ethanol by volume obtained in three runs with the same sample. *Bars* indicate maximum and minimum values

3 Results

Measurements of the dynamics of EZ formation showed good reproducibility. Figure 2 shows the results of three independent measurements of EZ size in one representative sample. Mean values are shown, with bars corresponding to the measured maximal and minimal values. The deviations of EZ size in all experiments never exceeded 15 μm.

Figure 3a–d present a gallery of graphs demonstrating the dependence of EZ size on time, for different concentrations of ethanol in water. One can see that the dynamics of EZ formation depend on the ethanol concentration. In all mixtures, the EZ width increased for up to 5 min and then remained stable to the end of observation (10 min). In the 10% ethanol mixture, the EZ is largest in comparison to the other solutions.

In the interval of 10–300 s, the dynamics of EZ formation are well fitted by the logarithmic equation $y = a\ln(x) + b$, where a is the characteristic EZ formation rate. The specific equations describing the dynamics of EZ development are indicated on the graphs of Fig. 3. One can see that, in the mixture of 90% water and 10% ethanol, the EZ grows at a higher rate than in pure water or in almost pure ethanol.

Figure 4 shows the dependence of EZ width on the ethanol concentration, obtained at the end of observation period (10 min). To be sure of the presence of an apparent maximum in the 10% ethanol concentration range, we tested the concentration effects in greater detail close to this ratio. Our data show that the increase of ethanol concentration from
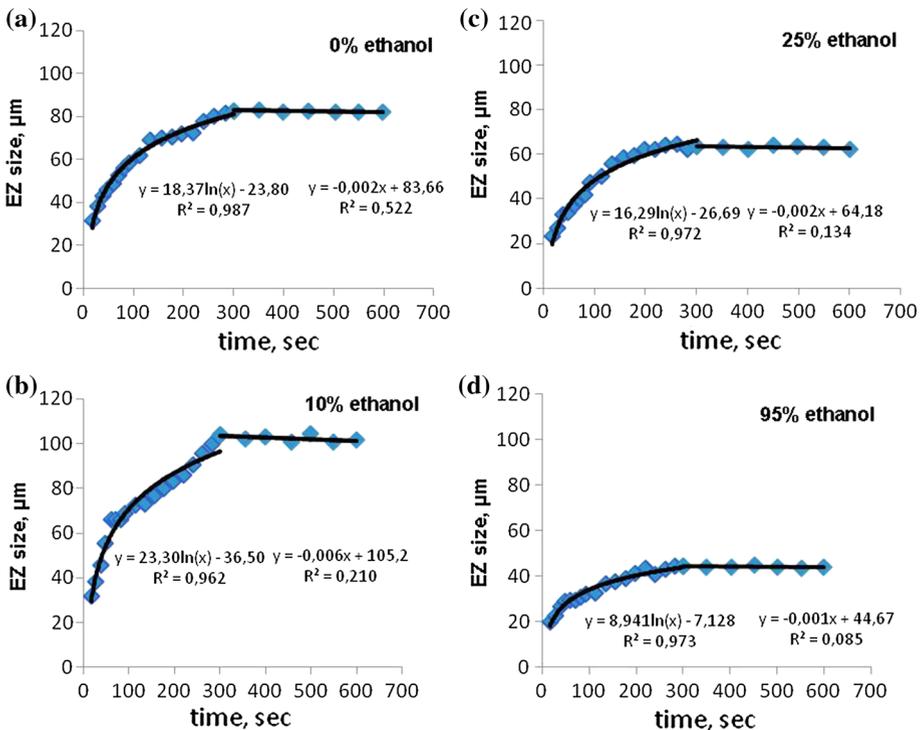
Fig. 3 Dependence of the EZ size on time, obtained at different concentrations of ethanol and water. Mean values of EZ width for three runs on the same tube are plotted: **a** 0% ethanol; **b** 10% ethanol; **c** 25% ethanol; **d** 95% ethanol (by volume)
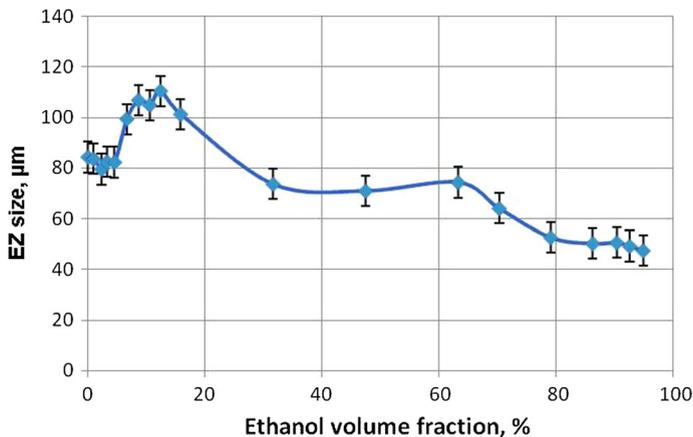
Fig. 4 Dependence of the EZ width on the ethanol concentration. Mean and SD values are plotted ($n = 3$–5 different Nafion samples)

(3–5) to (10–12) % results in an increase of the EZ size, while higher concentrations of ethanol diminish the size. Meanwhile, the EZ size reduction at higher ethanol concentrations shows a more complex curve. To some extent the results appear similar to those of an earlier investigation [4], where the EZ width in 100% ethanol was equal to 38 μm, close to the value of 45 μm reported here for the highest ethanol concentration, 95%.

4 Discussion

To explain the EZ phenomenon, it has been suggested that water molecules near hydrophilic surfaces form a layered honeycomb-sheet structure, called the "fourth phase" of water [2]. Under the influence of ambient infrared energy, water molecules split into negative and positive moieties, the negative OH components assembling next to the hydrophilic surface template to create the fourth phase, leaving the positive components, protons, in the bulk water. This separation forms a battery, from which extraction of electrical energy has been demonstrated [2].

The main point is the profound structural change that occurs during EZ formation. Solvent molecules re-organize into structures with qualitatively different physical properties [2]. Originally thought to occur in water alone, it was something of a surprise to find that the structural transformation was more general and involves other polar molecules [4].

Generally, the local structure of any liquid can be considered as "quasi-crystalline", characterized by a certain radius of local order and specific lifetime [8]. The proposed honeycomb structure of EZ water resembles a typical smectic-B liquid crystal [9]. The liquid-crystalline nature of EZ water is demonstrated by the presence of optical anisotropy (birefringence) in this region [2].

Unlike molecules of water, the ethanol molecules ordinarily participate only in two hydrogen bonds and consequently cannot form layered structures. However, ethanol molecules may form hexagonal cells containing six molecules, whose structure resembles that of disk-like (diskotic) liquid crystals [8]. The packing of ethanol molecules in the EZ has not yet been investigated.

The results obtained in this study show that the minimal EZ width corresponds to the solution with highest concentration (95%) of ethanol (Fig. 4). EZ buildup at this concentration took place much more slowly than in pure water (Fig. 3). Moreover, the results show a rather complex dependence of EZ size and formation dynamics on the ethanol concentration. Possibly, the different concentrations generate differences in the way that the ethanol EZ and the water EZ interact with one another.

The most unexpected result was the presence of an extremum corresponding to $\sim 10\%$ ethanol by volume (Fig. 4). At this ethanol concentration the EZ width is larger than in either pure water or almost pure ethanol. A possible explanation is that the hydroxyl groups of ethanol might favorably insinuate themselves into the hexagonal EZ lattice, thereby expanding said lattice. Alternatively, ethanol in the bulk water might sequester free protons, which ordinarily limit the EZ growth (unpublished observations); at some ethanol concentration that sequestration could then expand the EZ lattice. Why any such mechanisms would manifest at the ethanol concentration observed remains to be explored.

In conclusion, we find that mixtures of ethanol and water form exclusion zones in a concentration-dependent manner. Generally, the higher the volume fraction of water, the larger the exclusion zone, except for an anomaly at approximately 10% ethanol where the EZ was largest.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Zheng, J.M., Pollack, G.H.: Long-range forces extending from polymer–gel surfaces. Phys. Rev. E **68**, 031408 (2003)
2. Pollack, G.H.: The Fourth Phase of Water. Ebner and Sons Publisher, Seattle (2013)
3. Bernal, J.D., Fowler, R.H.: A theory of water and ionic solutions, with particular reference to hydrogen and hydroxyl ions. J. Chem. Phys. **1**, 515–548 (1933)
4. Chai, B., Pollack, G.: Solute-free interfacial zones in polar liquids. J. Phys. Chem. B **114**, 5371–5375 (2010)
5. Fletcher, N.H.: The Chemical Physics of Ice. Cambridge University Press, Cambridge (1970)
6. Allan, D.R., Clark, S.J.: Comparison of the high-pressure and low-temperature structures of ethanol and acetic acid. Phys. Rev. B **60**, 6328–6334 (1999)
7. Heitner-Wirguin, C.: Recent advances in perfluorinated ionomer membranes: structure, properties and applications. J. Memb. Sci. **120**, 1–33 (1998)
8. Pershin, V.K.: Pershin, Vl.K., Skopinov, S.A.: concept of the superpositional state in a problem of molecular mesomorphism: analytical review. Mol. Cryst. Liq. Cryst. **84**, 213–229 (1982)
9. de Gennes, P.J.: The Physics of Liquid Crystals. Clarendon Press, Oxford (1974)