



Effect of buffers on aqueous solute-exclusion zones around ion-exchange resins

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ABSTRACT

Interaction between charged surfaces in aqueous solution is a fundamental feature of colloid science. Theoretically, surface potential falls to half its value at a distance equal to a Debye length, which is typically on the order of tens to hundreds of nanometers. This potential prevents colloids from aggregating. On the other hand, long-range surface effects have been frequently reported. Here we report additional long-range effects. We find that charged latex particles in buffer solutions are uniformly excluded from several-hundred-micron-thick shells surrounding ion-exchange beads. Exclusion is observed whether the beads are charged similarly or oppositely to the particles. Hence, electrostatic interactions between bead and microsphere do not cause particle exclusion. Rather, exclusion may be the consequence of water molecules re-orienting to produce a more ordered structure, which then excludes the particles.

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1. Introduction

Previous work from this laboratory has revealed a feature of aqueous solutions that is unexpected. Adjacent to hydrophilic surfaces, an aqueous zone several hundred micrometers wide excludes colloidal particles and various solutes [1,2]. Because this zone exhibits broad exclusionary features, it has been labeled the “exclusion zone” or EZ.

One feature of the EZ is its sensitivity to pH. Studies have shown that the size of the exclusion zone is very much dependent on pH [1]. In pure water the EZ is smallest near neutral pH, while a five-fold increase in size is observed as the pH is adjusted downward to 2 or upward to 12. In aqueous systems a shift in pH is analogous to an oxidative state change, which impacts the bulk charge distribution [3,4]. Change of pH also brings about change of structural and thermodynamic properties [5,6] that can be augmented by the addition of a polyelectrolyte surface [7].

From these previous studies, it appeared as though a critical variable in establishing EZ size might be charge. Hence it seemed worthwhile to check this expectation by using buffers to attenuate charge [8,9]. We found indeed that buffers exerted a stabilizing influence on the EZ, and also that in the presence of buffers, EZs could be seen adjacent to both negatively charged and positively charged surfaces.

2. Methods

Colloidal particle exclusion from both cation (H^+ form) and anion (OH^- form) exchange-bead surfaces was observed under low magnification ($50\times$) on a Zeiss Axiovert 35 microscope. Ion exchange resin beads (Bio-rad, Hercules, CA, Cat. # 142-6425 & Cat. #142-7425), composed of cross-linked polystyrene divinylbenzene backbones functionalized with either sulfonic groups (H^+ form) or quaternary ammonium groups (OH^- form), were first rinsed with spectroscopy-grade methanol and then flushed with deionized water prior to use.

Sulfate-functionalized, latex microspheres, 0.5 μm diameter (Interfacial Dynamics, Inc., Portland, OR) were used exclusively in the experiments. Zeta-potential measurements throughout the relevant pH range confirmed that sulfate functional groups retained a negative charge (inset, Fig. 3).

All reagents used in this work were obtained from Sigma-Aldrich unless otherwise noted.

Aqueous buffer solutions of imidazole (pK_a 7.1), MOPS (pK_a 7.2), and TES (pK_a 7.4) were prepared in freshly deionized water (18.2 M Ω Barnstead Nanopure Diamond). These stock buffer solutions were adjusted to various pH values with either 1 N HCl or 1 M NaOH.

Electrical potentials were recorded with an Electro 705 Electrometer (WPI) using pulled glass microelectrodes filled with 3 M KCl. Tip diameters were less than 1 μm . A flexible reference electrode was placed in the same sample chamber many centimeters distant from the location where measurement was being made. Electromotive observations, i.e., measurements of microsphere dy-

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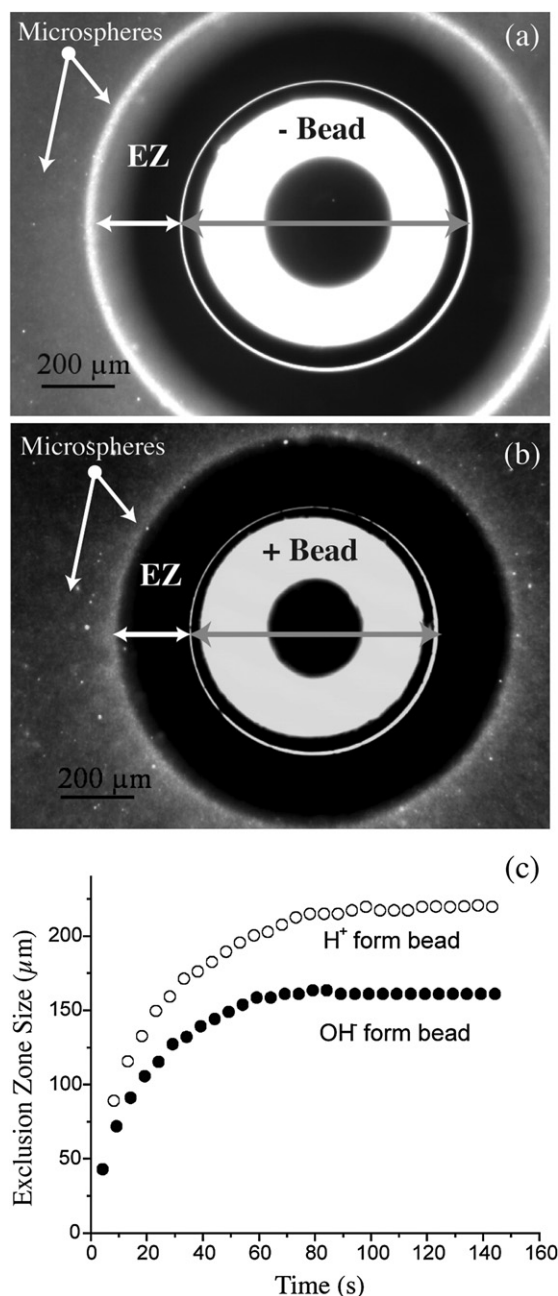


Fig. 1. Representative optical images of colloidal particle exclusion zones formed around (a) negative and (b) positive ion-exchange beads. Images captured 90 s after introducing the bead into the microsphere suspension (pH 7.0, 10 mM imidazole, 0.5 μm sulfate-functionalized latex microspheres). A shell-like EZ is observed to form around the bead, the timecourse of this process is plotted in (c). During the active expansion of this EZ shell, displaced microspheres accumulate at the boundary. After 10–15 min the microspheres diffuse away into the bulk solution beyond, but do not enter the exclusion zone.

namics under the influence of a pure electric field, were made using 99.99% Pt wire electrodes (0.25 mm diameter) connected to a regulated DC power supply (Ambitrol TW-4005). Voltage values were confirmed with a hand held digital voltmeter (Tenma 72-7750).

3. Results

Representative results obtained using an imidazole buffer are presented in Fig. 1. Panel (a) shows the results obtained with a negatively charged cation exchange bead, while panel (b) was ob-

tained using a positively charged anion exchange bead. In both situations, negatively charged sulfate microspheres were used.

Substantial EZs developed over several minutes in both cases (Fig. 1(c)). The EZs formed in the shape of an enveloping shell. Their thickness was approximately 200 μm. Microspheres were fully excluded from these zones and only rarely was the occasional particle trapped within. The concentrated ring of particles at the boundary (Fig. 1(a)) eventually diffused away after 10–15 min, leaving the exclusion zone clear and the surrounding suspension filled with a uniform distribution of microspheres. At low buffer concentration the boundary ring was less well defined and constituent microspheres typically diffused away more quickly than at higher buffer concentrations.

Dynamics of EZ formation are plotted in Fig. 1(c). Initially, the rate of formation was very high (10 μm/s); then, it slowed considerably, the EZ reaching a plateau after approximately 10 min. The rate of EZ development for the other two buffers was considerably lower. Once formed, the exclusion zone tended to persist for many hours, and long-term observations showed that the region was stable for days.

Noteworthy is the observation that whether the nucleating surface was anionic or cationic, the EZ was seen, and was in fact approximately the same size. That is, negatively charged microspheres were excluded from a 200-μm zone around the bead, whether the bead's polarity was negative or positive.

Several potential artifacts attendant with the formation of exclusion zones have previously been considered and tested [1]. No reason could be found to suggest that the EZ was anything but a genuine feature. An additional control was carried out here, using exhausted beads, i.e., beads that had effectively lost their ion-exchange properties. This loss is detectable through an indicator dye incorporated by the manufacturer into the beads, which fades with diminishing exchange capacity. Initially, it was noted that when one of the colorless (exhausted) beads was placed in the experimental chamber, no exclusion could be seen. To confirm this connection, beads that showed exclusion were soaked for several hours in either 1 M NaOH (H⁺ form bead) or 1 N HCl (OH⁻ form bead) to completely neutralize the free counter ions from the exchange resin. Again, the beads lost their color. When placed into the microsphere suspension, the negatively charged beads produced only a small EZ, while the positive beads produced no EZ at all – confirming that the presence of exclusion depends on the physical chemical state of the nucleator, and is not the product of some secondary effect such as microsphere settling [10–12], colloidal void formation [13,14], or convective flow [12,15].

In addition to imidazole, two other buffers were explored. We used TES and MOPS, buffers commonly employed in biological preparations, and tested them over physiologically relevant concentrations and broad pH range [8]. Both buffers exhibited EZs; however, exclusion zones were smaller and developed more slowly than with imidazole. Therefore, the imidazole buffer was chosen for use in all subsequent studies.

The reason that imidazole was more successful may lie in that molecule's unique electronic properties. TES and MOPS are both aliphatic zwitterionic molecules; imidazole on the other hand is aromatic. Furthermore, one nitrogen in imidazole is part of the π -conjugate aryl ring, while the second nitrogen projects beyond this conjugate system and presents sigma-unpaired electrons. How this may play a role in the improved pH stability of the EZ is not entirely clear, but may indicate that the region of water adjacent to these surfaces has a novel electronic structure that may include conjugated or solvated electrons. Imidazole is also a weak chelating agent, and in previous work [16] it was found that salt contaminants could diminish the EZ size. Therefore, experiments were conducted replacing buffer with EDTA, an effective chelator; an exclusion zone could be found around negative beads only, and

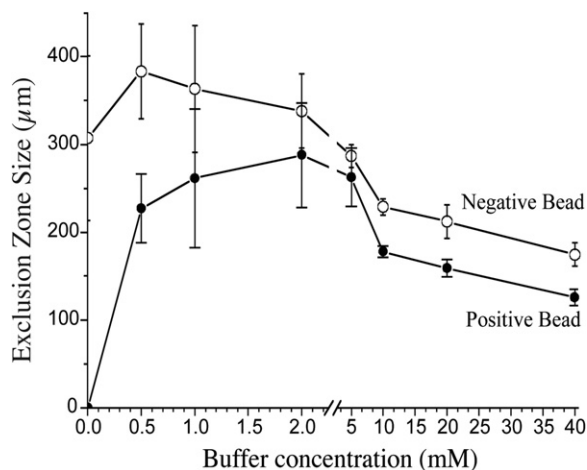


Fig. 2. Effect of imidazole concentration on exclusion-zone size at pH 7.0. Error bars indicate degree of spherical symmetry within the EZ, i.e., shorter bars represent near perfect symmetry.

the shape of the EZ was easily distorted. Thus, it is apparently not Imidazole's chelating capacity that accounts for its stabilizing influence.

To explore the role of buffering capacity the concentration of imidazole was varied from zero up to 50 mM. Results are shown in Fig. 2. EZ width increased sharply with increasing buffer concentration, reaching a maximum of 280 μm at 2.0 mM and 380 μm at 0.5 mM, respectively, for positively and negatively charged beads. Higher buffer concentrations diminished EZ width to stable values near 200 μm , the negative bead consistently exhibiting slightly larger exclusion zones than the positive one. Hence, for both types of bead, addition of buffer tended to stabilize EZ width to relatively large values.

The EZ was not always spherical. At low buffer concentration (e.g. <5 mM) the EZ was typically distorted into elliptical or even teardrop shape, whereas at higher buffer concentrations (>10 mM) the exclusion region retained near-perfect spherical geometry. In Fig. 2, distortion is shown by the size of the error bars – smaller bars indicating more spherical symmetry. It was common to observe streaming behavior of microspheres in the surrounding bulk suspension, most likely driven by thermal convection. If the EZ distortion occurs as a result of streaming-induced shear, then the results imply that addition of buffer stabilizes the EZ, allowing it to retain its spherical shape.

Preparing imidazole-buffered microsphere suspensions with pH values ranging between 3 and 9 tested the stabilizing influence of buffer on the exclusion zone. Solutions outside of this pH range quickly exhausted the ion-exchange capacity of the beads, which resulted in a collapse of the EZ or no EZ formation at all. In the absence of buffer the EZ is profoundly sensitive to solution pH, showing up to a 5-fold size variation [2]. Addition of 10 mM imidazole limited the mean size variation to $\pm 30\%$.

To determine whether electrical features of the EZ are similar to those seen in the absence of buffer, potential measurements were carried out using glass microelectrodes. A measuring electrode was translated vertically downward towards the bead surface; the reference electrode was immersed within the same chamber many centimeters away from where the measurement was made. Electrical potential measured as a function of distance from the bead surface is plotted in Fig. 3. For both types of bead, the highest magnitude was found at the bead surface (~ 150 mV), cationic beads showing positive potential, anionic showing negative. The potential decayed steadily with distance from the bead surface, reaching zero at the outer edge of the EZ. This potential distribution is consistent with

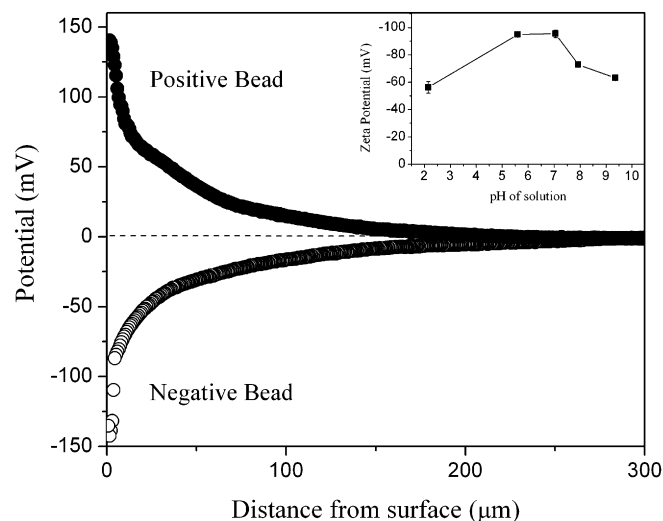


Fig. 3. Potential profiles extending from ion-exchange beads. Measurements taken in 10 mM imidazole at pH 7.0, with KCl glass microelectrode. Inset: Zeta-potential of microspheres in 10 mM imidazole at varying pH values.

observations from earlier work with negatively charged surfaces in pure water only [2,16].

To check for possible aberrant microsphere behavior, microsphere dynamics were observed under the influence of a purely coulombic force. Platinum electrodes were immersed into the buffered suspension and DC voltages between 250 mV and 5 V were applied. As anticipated, the negatively charged microspheres translated away from the cathode and accumulated around the anode. Switching polarity *in situ* resulted in immediate reversal of microsphere movement. Varying the applied voltage changed the microsphere migration speed proportionally; i.e., at higher voltages the microspheres moved more rapidly. Thus, microspheres situated in applied electric fields behaved entirely as expected. Nothing anomalous that might have contributed to the generation of exclusion zones could be found.

4. Discussion

The results show that exclusion zones similar to those found in pure water or in water with salt are also found in buffered solutions. Previous work had indicated that pH was an important determinant of EZ size [1], and the present work was carried out with a series of buffers designed to stabilize pH. We found that the buffers did stabilize the EZ, making it more robust. In cases where pure water showed little or no EZ, addition of buffer in even modest concentrations elicited large EZs, which were then maintained at a size relatively independent of additional buffer (Fig. 1).

EZ stability was also manifested physically, in terms of averting any shear-induced shape change. That is, convective flow was present to some extent in most experiments, and almost certainly exerted shear on the EZ, causing EZ distortion. Distortion was most prominent at low buffer concentrations (Fig. 2, large error bars), but decreased as buffer concentration increased, implying once again the stabilizing influence of the buffer.

A second point of significance is the similarity of results obtained with the two types of bead, anionic and cationic. This similarity was evident not only in terms of EZ size, but also in the potential distributions, which were largely mirror images of one another (Fig. 3). Hence, the exclusionary behaviors of the two bead types were quite similar.

Further to this point, in zeta-potential measurements the microspheres translated away from the cathode and accumulated around the anode. Switching polarity *in situ* resulted in immedi-

ate reversal of microsphere movement. Varying the applied voltage changed the microsphere migration speed proportionally; i.e., at higher voltages the microspheres moved more rapidly. Thus, microspheres situated in applied electric fields behaved entirely as expected. Nothing anomalous that might have contributed to the generation of exclusion zones could be found. It appears that exclusion of microspheres of either polarity is genuine.

The similarity of behaviors of positive and negative beads is significant in that the excluded microspheres always had the same negative polarity. A lingering suspicion has been that the EZ arises because a charged nucleating surface repels like-charged microspheres electrostatically, creating a zone of exclusion in between. In other words, exclusion arises from electrostatic repulsion, which, for some unexplained reason might extend much farther than anticipated by standard DLVO theory [17–19]. The fact that an exclusion zone remains in evidence when the negatively charged microspheres are exposed to a positively charged bead rebuffs any such mechanism: Oppositely charged entities should attract, not repel.

This finding adds strength to earlier findings showing the complementary result: Adjacent to negatively charged nucleating surfaces, EZs could be seen whether the excluded microspheres were positive or negative [1]. Thus, the evidence is now more comprehensive: EZs can be seen in situations in which polarity switch from same to opposite occurs in the nucleating surface or in the excluded particles. Simple electrostatic repulsion does not fit.

More likely, the exclusion zone forms when bulk water condenses to a more ordered near-surface array. This may arise in response to features of the bead's polyelectrolyte surface. As the ordered EZ grows, solutes are excluded in the same manner as they would be excluded from advancing ice [20,21]. This nongentropic response of water is known [22–24] and further implies that the EZ is not merely an electrostatic repulsive effect. Retarded T2 relaxation in 2D NMR studies, as well as reduced infrared emission imply ordering [2], as do the results of recent studies showing high EZ viscosity and EZ birefringence [25]. Hence, the exclusion zone is likely the result of ordering of water molecules.

On the other hand, the EZ is evidently spatially coincident with an electric field (Fig. 3), and a question that arises is the genesis of such field. Experiments have shown that the EZ region can sequester electrical charge [25]. The charge contained within the EZ would then be the source the measured potential distribution. The latter would not be a manifestation of nucleator-surface charge extending somehow over an unexpectedly long range, but would instead be intimately associated with the ordered water's charge. Indeed, it has been possible to draw current from electrodes placed in the EZ and the region beyond [25,26].

Another point is the relevance of the EZ phenomenon for biology. Biological tissues are usually well buffered at pH values near neutral. One lingering question has been whether the exclusion zones initially observed in unbuffered water have relevance for biology. The finding that EZs are especially robust in the presence of buffers, and observed (albeit smaller) in the presence of physiological salt levels [1], implies that they are indeed pertinent to biology [27–29].

Finally, it goes without saying that the results have implication for the function of ion exchange beads, commonly used in biolog-

ical and chemical separations. From the results reported here, it appears that the environment surrounding the beads may be far different from the environment presumed to exist near polyelectrolyte surfaces, and that further study is warranted to see how this difference may impact the interpretation of results.

In conclusion, adding buffer to water has a salutary effect on exclusion zones – they are more stable and more robust in the presence of buffer. The exclusion zones are evident whether the nucleating surface has charge polarity opposite to, or the same as, the polarity of the excluded microspheres, implying that the exclusion phenomenon cannot arise from simple electrostatic repulsion. More likely, it arises from some physical change in the water surrounding the bead, which implies an environment very different from what is generally assumed.

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