Counting ions and other nucleophiles at surfaces by chemical trapping

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Abstract The interfaces of membranes and other aggregates are determined by the polarity, electrical charge, molecular volume, degrees of motional freedom and packing density of the head groups of the amphiphiles. These properties also determine the type of bound ion (ion selectivity) and its local density, i.e. concentration defined by choosing an appropriate volume element at the aggregate interface. Bulk and local ion concentrations can differ by orders of magnitude. The relationships between ion (or other compound) concentrations in the bulk solvent and in the interface are complex but, in some cases, well established. As the local ion concentration, rather than that in the bulk, controls a variety of properties of membranes, micelles, vesicles and other objects of theoretical and applied interests, measurement of local (interfacial, bound) ion concentrations is of relevance for understanding and characterizing such aggregates. Many experimental methods for estimating ion distributions between the bulk solution and the interface provide indirect estimates because they are based on concentration-dependent properties, rather than concentration measurements. Dediazonation, i.e. the loss of N₂, of a substituted diazophenyl derivative provides a tool for determining the number of nucleophiles (including neutral or negatively charged ions) surrounding the diazophenyl derivative prior to the dediazonation event. This reaction, defined as *chemical trapping*, and the appropriate

reference points obtained in bulk solution allow direct measurements of local concentrations of a variety of nucleophiles at the surface of membranes and other aggregates. Here we review our contributions of our research group to the use, and understanding, of this method and applications of chemical trapping to the description of local concentrations of ions and other nucleophiles in micelles, reverse micelles, vesicles and solvent mixtures. Among other results, we have shown that interfacial water determines micellar shape, zwitterionic vesicle-forming amphiphiles display ion selectivity and urea does not accumulate at micellar interfaces. We have also shown that reaction products can be predicted from the composition of the initial state, even in non-ideal solvent mixtures, supporting the usefulness of chemical trapping as a method to determine local concentrations. In addition, we have analysed the mechanism of dediazonation, both on theoretical and experimental basis, and concluded that the formation of a free phenyl cation is not a necessary part of the reaction pathway.

Keywords Membrane interface · Local ion concentration · Bulk solution · Chemical trapping · Dediazonation

Introduction

Substantial attention has been paid to Hofmeister (Kunz et al. 2004) phenomena because of their relevance to a broad range of fields. A few examples of physical behaviour obeying the Hofmeister series include enzyme activity (Pinna et al. 2005), protein stability (Broering and Bommarius 2007; Zhao 2016), protein–protein interactions (Yeh et al. 2010), protein crystallization (dos Santos et al. 2017) and bacterial growth (Lo Nostro et al. 2005), as well as solvent properties in non-aqueous systems (Mazzini and Craig 2016). Although Hofmeister effects on macromolecules in aqueous solution

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are ubiquitous, the molecular-level mechanisms by which ions operate are only just beginning to be unravelled (Schwierz et al. 2016; Zavitsas 2016). Understanding the molecular nature of ion macromolecule or interface interactions is also important for membrane protein function, bilayer dynamics and cellular biophysics (Valley et al. 2011; Woolf 2013). Detailed molecular dynamics simulations have begun to illustrate that the behaviour of cations and anions may, or may not, vary within different regions of the membrane bilayer (Berkowitz and Vacha 2012). This, in turn, could lead to significant changes in bilayer shape and function with variations in the concentration and types of anions or cations.

Understanding the specific effects of ions in biology requires further development of the capacity to theoretically reproduce force fields and analytical descriptions of charge distributions not only of the hydrated ions but also of the potential binding regions or sites (Berkowitz and Vacha 2012; Leontidis 2016). The effort to advance in these directions is clearly increasing. However, as in many other challenging scientific areas, the theoretical descriptions should be accompanied with increasingly accurate measurements of ion distribution between the bulk solution and the interface affected by the specific ion binding in order to validate the theoretical descriptions.

Supramolecular structures, such as biological membranes, micelles, liposomes and bilayers, can bind ions and organic molecules at the surface or at the organic core, leading to different concentrations (higher or lower) of these molecules at these sites when compared with the analytical concentration in the continuous solvent. Several experimental methods are available to estimate the distributions of ions between these interfaces and the bulk phase, including solubility, conductivity, potentiometric studies and nuclear magnetic resonance, UV/visible and fluorescence spectroscopy. Most of these methods rely on adjustable parameters to describe the measured property of the ion when bound to the interface, monitoring only one component at a time, and some are limited to narrow ranges of solution compositions (Fabre et al. 1980; Tanford 1980; Zana 1985).

Ideally, the determination of ion composition at an interface should yield data independent of adjustable parameters, in particular those that depend critically on some property of the bound ion. In general, these parameters are not directly determined, but rather they are deduced indirectly. Romsted and coworkers first used diazonium salts as probes for directly determining the concentration of anions and organic compounds at the interface of different colloids (Chaudhuri et al. 1993a, b). Since then several groups, including ours, have used the spontaneous N_2 loss from diazonium salts and resulting reaction of the phenyl carbocation with several nucleophiles to estimate interfacial concentrations (Fernandez-Alonso and Bravo-Diaz 2007; Pazo-Llorente et al. 2003; Romsted and Bravo-Diaz 2013).

The decomposition of aromatic diazonium salts in aqueous acid was thought to occur exclusively by a slow spontaneous elimination of N_2 , yielding a very reactive carbocation, followed by a fast step where any nucleophile present in the vicinity would react at a similar rate (Fig. 1). The rate differences of the reaction of the phenyl carbocation with neutral molecules, such as water (or alcohols), and anions like Cl^- and Br^- are within a factor of two. This selectivity is relatively small, taking into account the big differences between structures and charges of all the nucleophiles. The striking feature of this reaction is that, at least for nucleophile-defined concentration ranges, there is a linear relationship between the percentages of product yields, percentage yield (%Yield) and the molar fraction of the nucleophiles in the solution. This linear relationship allows the use of a standard curve to determine both the concentrations and nucleophile ratios at an interface as a function of the concentration of the nucleophiles in the bulk. In special cases, such as at membranes or micelle interfaces, the direct determination of the concentration of such molecules is difficult to assess directly by other methods.

A great variety of aromatic diazonium salts can, in principle, be used to determine the concentration of ions or neutral molecules at interfaces. Important features for choosing a specific aryldiazonium salt are: (1) it must react with the target molecules to yield a relatively high amount of product with the desired nucleophile, sufficient to be detected with high accuracy and (2) absence, or very low formation, of secondary products. In some cases, however, depending on the solvent the formation of secondary products cannot be avoided. However, the interference can be minimized by choosing the adequate substituted aromatic diazonium salt and the pH (Chaudhuri et al. 1993a; Pazo-Llorente et al. 2004). In addition, the rate constant for the spontaneous N_2 loss from the diazonium salts must be insensitive to the nature of the solvents and nucleophile concentrations. In general, the surfactant to probe (i.e. diazonium ion) ratio is at least 100:1. In an equilibrium system and given the slow rate of dediazonation, the probe is not expected to have an important effect on the aggregate structure. These are essential conditions for a direct correlation between the final %Yield of products and the nucleophile concentration.

All reactions at interfaces are affected by the local composition. Added salts, counterion type, possible reactions with the monomer headgroups and added solvents can lead to complex product mixtures. Other problems can arise with this method. The first is the complete identification of all products resulting from the dediazonation. In all of the results and reactions presented in the following sections, we obtained or synthesized all reaction products. This step is particularly important when analysing reactions of phenyl derivatives (Soldi et al. 2000), urea (Romsted et al. 2003), mixed solvents (Cruz et al. 2015) or reactive monomers, such as sulphates, sulfonates (Cuccovia et al. 2000a, b) or choline surfactants (de

Fig. 1 R-ArN₂⁺(diazonium compound/salt) reactions in water in the presence of halide salts, where Ar represents an aryl group.

Souza et al. 2012). Very accurate analytical procedures need to be maintained, since unstable products may be undetected on occasion, and a quantitative comparison between the initial diazo derivative and the sum of all products may provide data suggesting that one reaction path is not being considered. When all these conditions are met, the validity of the method relies on kinetics and an analytical correct production of a standard curve using the solvent-soluble diazo probe (1-ArN₂⁺). The decomposition rates of 1-ArN₂⁺ in the reference solution need to be essentially equal to that of the long chain derivative (16-ArN₂⁺) incorporated into the interface of interest. In addition, the solution used to obtain the standard curves has to contain adequate concentrations of all components expected to be present at the interface of interest. As an example, the standard curve for probing the interfacial concentration of a halide in a zwitterionic micelle of dodecylphosphocholine contains 1.6 M methyl-phosphocholine and variable concentrations of the halide of interest. Another practical problem is that some of the reaction products of 1-ArN₂⁺ may evaporate from the aqueous solution; therefore, it is essential that the reaction be carried out in stoppered vials and with a layer of an organic solvent immiscible with water (e.g. cyclohexane). Good analytical results are obtained by mixing the upper layer at the end of the reaction, by adding methanol or another suitable soluble solvent.

Determining interfacial compositions of micelles and vesicles using chemical trapping

Ion dissociation from charged micelles

Using the dediazonation reaction we determined the degree of ion dissociation (α) and the ion selectivity exchange constant (Quina and Chaimovich 1979) between Cl[−] and Br[−] ($K_{\text{Br/Cl}}$) of cationic micelles. The α of cetyltrimethylammonium

chloride (CTAC) and bromide (CTAB) micelles was determined by chemical trapping (Cuccovia et al. 1997a) using 2,4,6-trimethylbenzenediazonium tetrafluoroborate (1-ArN₂BF₄) (Fig. 1). 1-ArN₂⁺ is very soluble in water and does not interact with the positively charged micelles. Consequently, the phenyl cation produced after N₂ loss only reacted with the available nucleophiles, i.e. H₂O, Cl[−] and/or Br[−], in the bulk solution yielding, respectively, the phenol (1-ArOH) and halobenzene products (1-ArCl and 1-ArBr).

The calibration curves of %Yield versus [NaCl] (or [NaBr]) of each product (%1-ArOH, %1-ArCl and %1-ArBr) of the reaction of 1-ArN₂⁺ and the concentrations of NaCl and NaBr in water were obtained by determining the products by high-performance liquid chromatography (HPLC). The %Yield of each product was calculated using Eq. 1:

$$\%1\text{-ArX} = \frac{[1\text{-ArX}]}{[1\text{-ArX}] + [1\text{-ArOH}]} * 100 \quad (1)$$

The free chloride concentration, [Cl]_f in CTAC and the free bromide concentration, [Br]_f in CTAB was obtained by determining the %Yield of products in the presence of micelles and by using the standard curves. Knowing the concentrations of [Cl]_f and [Br]_f, it was possible to calculate the degree of ion dissociation, α , of these micelles. The α 's obtained for CTAC ($\alpha = 0.29$) and CTAB ($\alpha = 0.25$) using this method are consistent with published values, proving that chemical trapping can be used to determine both the interfacial and bulk ion concentrations (Romsted 1975).

Adding NaBr to CTAC or NaCl to CTAB micellar solutions, we obtained $K_{\text{Br/Cl}}$. The value for $K_{\text{Br/Cl}}$ was 2.65 ± 0.4 , which falls within the range of the results obtained using other methods. The agreement of this value of $K_{\text{Br/Cl}}$ with values reported in the literature also indicates that 1-ArN₂⁺ resides at a sufficient distance from the micellar surface such that we can

consider the probe to be sensing only the “free” anions. This was confirmed by determining the Stern–Volmer constant for the fluorescence quenching of Tris(2,2'-bipyridyl)ruthenium(II) $[\text{Ru}(\text{bpy})_3^{2+}]$ by 1-ArN_2^+ in CTAC/NaCl solutions.

Ion concentration at micellar interfaces

The salt concentration of ionic micelles is higher at the interface than in the bulk solution and, consequently, the local water concentration is lower at the former. Measuring these parameters in different systems is therefore relevant, particularly because the interface of ionic micelles mimics those in diverse biological environments. A hydrophobic, micelle-bound arenediazonium salt is needed when the aim is to probe exclusively the micellar interface as this ensures that the probe is monitoring only the micellar interface and not the aqueous bulk media. The 4-hexadecyl-2,6-dimethyl-benzenediazonium cation (16-ArN_2^+) was used to estimate the concentration of Br^- and H_2O at the micelle $\text{Br}_m/\text{H}_2\text{O}_m$ interface in derivatives of cetyltrialkylammonium bromide $[(\text{CTRA})\text{Br}]$ where R was methyl $[(\text{CTMA})\text{Br}]$, i.e. CTAB, ethyl $[(\text{CTAE})\text{Br}]$, *n*-propyl $[(\text{CTPA})\text{Br}]$ and *n*-butyl $[(\text{CTBA})\text{Br}]$. The standard curves of %1-ArOH, 1-ArX and H_2O versus [salt] were obtained in aqueous solutions of tetralkylammonium halides $[(\text{TRA})\text{X}]$ with variable head group sizes, where R = methyl, ethyl, *n*-propyl and *n*-butyl, respectively.

The local concentration of Br^- (Br_m) and water (H_2O_m) at the micelle interface of $(\text{CTRA})\text{Br}$ was determined at different concentrations of micelles and salt (Soldi et al. 2000). The critical micelle concentrations (CMCs) and α 's of $(\text{CTRA})\text{Br}$ are shown in Table 1. The CMCs decrease with increases in the size of the R group, as could be predicted, considering the increase in the hydrophobicity of the R groups. The small increase in α 's with larger R can be ascribed to the steric hindrance of the bulky groups and ensuing decrease in the repulsion between the positive charges of the monomers. These data agree with those obtained using other techniques (Bacaloglu et al. 1989). The values of Br_m and H_2O_m change with detergent concentration, reaching an

almost constant value near 0.1 M of detergent (Soldi et al. 2000). The concentration of the Br_m decreases with increasing R from 1.53 M in $(\text{CTMA})\text{Br}$ to 0.68 in $(\text{CTBA})\text{Br}$ (Table 1). This decrease in the Br_m concentration with increasing R chain length is compatible with a greater distance between the monomers in the micelles and higher α values, which diminishes ion condensation at the surface to decrease the electrostatic repulsion between the monomers head groups.

The H_2O_m , at 0.1 M $(\text{CTRA})\text{Br}$, without added salt, is 45.16 M in $(\text{CTMA})\text{Br}$ and increases with increasing R chain length, reaching a value near 54 M with $(\text{CTBA})\text{Br}$, near the bulk water concentration. This increase in H_2O_m can be due to the greater distance between the head groups in a $(\text{CTBA})\text{Br}$ micelle, resulting in a higher interfacial hydration compared with micelles of $(\text{CTMA})\text{Br}$. The addition of 0.1 M tetramethylammonium bromide $[(\text{TMABr})]$ at constant $(\text{CTRA})\text{Br}$ concentration increases Br_m and decreases H_2O_m , as expected (Table 1).

Shape changes and interface composition

The properties of the interface of cationic amphiphiles, which contain a quaternary ammonium head group, can be significantly modified by changing the counterion (Lima et al. 2013). Anions containing a more hydrophobic group, like triflates, alkyl carboxylates, aromatic carboxylic acids and aromatic sulfonic acids, can lead to viscoelastic solutions with different aggregates structures, such as rodlike, threadlike, wormlike or polymer-like micelles. The sphere-to-rod transition is strongly dependent on the structure and concentration of the counterion and, in some cases, such as for dichlorobenzoate anions and CTA^+ , the transition depends strongly upon the type and location of substituents on the aromatic ring (Gamboa and Sepulveda 1986).

The sphere-to-rod transitions of micelles of CTA^+ containing, as counterions, 3,5-dichlorobenzoate (3,5OBz), or 2,6-dichlorobenzoate (2,6OBz), have been studied in mixtures with CTAC (Geng et al. 2005). The micelles were prepared with different molar ratios of CTAC and $\text{CTA}2,5\text{OBz}$, or

Table 1 Values of critical micelle concentrations, degree of ion dissociation, Br_m and H_2O_m in derivatives of cetyltrialkylammonium bromide and HCl or HBr

$(\text{CTRA})\text{Br}^a$	CMC ($\times 10^3 \text{M}$)	α	Br_m (no salt)	H_2O_m (no salt)	Br_m (0.1 M TMABr)	H_2O_m (0.1 M TMABr)
$(\text{CTMA})\text{Br}$	0.85	0.25	1.53	45.16	2.10	40.19
$(\text{CTEA})\text{Br}$	0.80	0.26	1.28	47.90	1.52	45.22
$(\text{CTPA})\text{Br}$	0.55	0.28	0.87	52.16	1.14	49.07
$(\text{CTBA})\text{Br}$	0.30	0.32	0.68	53.57	0.96	51.01

From (Soldi, Keiper et al. 2000) and Supplementary Material from the same reference

CMC, Critical micelle concentration; α , degree of ion dissociation; Br_m , local concentration of Br^- at the micellar interface; H_2O_m , local concentration of water at the micellar interface; TMABr, tetramethylammonium bromide

^a Derivatives of cetyltrialkylammonium bromide $(\text{CTRA})\text{Br}$ where R is methyl $(\text{CTMA})\text{Br}$ (i.e. CTAB), ethyl $(\text{CTAE})\text{Br}$, *n*-propyl, $(\text{CTPA})\text{Br}$ or *n*-butyl $(\text{CTBA})\text{Br}$

CTA2,6OBz, and the interface of the micelles was probed by chemical trapping using micelle-bound 16-ArN₂⁺. Standard curves relating product yields of 1-ArN₂⁺ with H₂O, 2,6OBz and 3,5OBz in the aqueous phase were obtained. The products with both 1-ArN₂⁺ and 16-ArN₂⁺ are the biphenyl derivative (z-ArBzOH, where z = 1 or 16) and the ester, z-ArOBz, resulting from the reaction with the carboxylate group (Fig. 2).

Using the appropriate standard curves, we obtained the concentration of H₂O_m, Cl_m and OBz_m at several mixtures of CTAC and CTA2,6OBz, at a fixed total detergent concentration (0.01 M). The increase in the CTA2,6OBz mole fraction, in the CTAC/CTA2,6OBz mixtures, produces a linear increase in the interfacial concentration of 2,6-OBz and a simultaneous decrease in Cl_m. No abrupt changes in H₂O_m and 2,6OBz_m are observed, indicating no change in the micellar structure, i.e. no phase transition. The H₂O_m decreases from 45 M in pure CTAC to 30.5 M in CTA2,6OBz and the concentrations of the counterions in CTAC and CTA2,6OBz pure micelles are Cl_m = 1.24 M and 2,6OBz_m = 1.33 M. These micelles seem surprisingly similar, despite the huge structural difference between the counterions.

In pure CTA3,5OBz micelles, H₂O_m is 19.8 M, which significantly lower than in CTAC (and in CTA2,6OBz micelles). 3,5OBz_m is 2.83 M, more than double the concentration of 2,6OBz_m (see above). In contrast with CTA2,6OBz, the concentration of H₂O_m, Cl_m and 3,5OBz_m, at different ratios of CTA3,5OBz/CTAC and at 0.01 M total detergent, changes dramatically at a 0.4 M fraction of CTA3,5OBz. This abrupt change indicates that the mixed micelle suffers a shape transition. A sphere-to-rod transition under these conditions has been demonstrated by cryogenic transmission electron microscopy (Kreke et al. 1996; Magid et al. 1990). The

agreement between the data obtained by measuring interfacial ion concentrations and interfacial water and the electron microscopy increases confidence on the validity of the chemical trapping results. The changes in the interfacial water concentration and the strong interaction of the quaternary ammonium group with 3,5OBz are a major contribution to the forces governing micellar morphology. It is attractive to speculate that morphology changes in subcellular structures are governed by similar changes in interfacial ion and water concentrations (Scott and Youle 2010).

Urea at micellar interfaces

The versatility of the dediazotization reaction allows another important question to be addressed: the effect of urea on the stability of macromolecules in aqueous solution. Whether the effect of urea is due to a direct interaction with surfaces or due to the properties of the solvent is still a matter of debate (Politi et al. 2017). The basic intermolecular and ionic forces controlling surfactant aggregate and protein stabilities are similar. The addition of urea, up to its solubility limit of approximately 10 M, to aqueous solutions of anionic, cationic, zwitterionic and nonionic surfactant increases the CMC of the detergents, reduces aggregation numbers of ionic and nonionic micelles and increases the α of ionic micelles. Urea also reduces the differences in counterion affinities toward cationic monolayers and micelles (Florenzano et al. 1996; Souza et al. 1995).

Using chemical trapping we determined the concentration of urea at the interface of micelles of CTAX, (X = Cl, Br), sodium dodecylsulfate, sodium dodecyl sulphate (SDS), sodium dodecylsulfonate (SDSu) and the zwitterionic *N*-hexadecyl-*N,N*-dimethylammonium propanesulfonate (HPS)

Fig. 2 R-ArN₂⁺ reactions with the dichlorobenzoates 2,5OBz and 3,5OBz

(Romsted et al. 2003). Urea reacts with 1-ArN₂⁺ (and 16-ArN₂⁺) to yield two stable products (Fig. 3), *N*-arylurea (*z*-ArU) and *O*-arylurea (*z*-ArisoU).

The effect of adding urea to micellar solutions of CTAC, CTAB, SDS, SDSu and HPS was analysed using 16-ArN₂⁺. The standard curve of [urea] versus product(s) %Yields was constructed with 1-ArN₂⁺ using a fixed salt concentration representing the ion concentration at the micellar interface, in the presence of 0.001 M HCl. The salts used were TMACl (1.5 M), TMABr (1.5 M), methylsulfate (2.18 M) and methanesulfonate (1.37 M) in HCl 0.001 M. For all these salts, the slope of the standard curves of %1-ArU versus urea are almost identical, indicating that the presence of salts does not affect the %Yields of the reaction of urea with 1-ArN₂⁺ (Romsted et al. 2003). A standard curve relating the [urea] and [H₂O] was also obtained (the water concentration was obtained by weight). The water concentration of a solution of TMAX 1.5 M is 46.9 M, and in the presence of 6 M urea and 1.5 M TMAX the [H₂O] decreases to 30.8 M due to both the high molar volume of TMAX and high urea concentration.

The interfacial H₂O_m was measured using 16-ArN₂⁺. In the absence of urea, the H₂O_m at the micellar interface is approximately 46.4 M in CTAC (0.05 M), 45.3 in CTAB (0.05 M) and 39.7 in SDSu (0.04 M) micelles. At a fixed detergent concentration (0.05 M), the addition of 6 M urea decreases H₂O_m to 29.8 M in CTAB, 31.0 M in CTAC and 32.0 M in SDSu. This result indicates that H₂O_m corresponds to the concentration of water in a concentrated 6 M urea solution containing 1.5 M TMAX. At the micellar interface, the ratio H₂O_m/U_m for all micelles, with 6 M urea, is approximately 6.2 in CTAB 0.05 M, 5.4 in CTAC and 6.0 in SDSu 0.04 M (Romsted et al. 2003). In a solution containing 6 M urea and 1.5 M TMAX, the ratio H₂O/urea is 30.8/6 = 5.1, indicating that the ratio H₂O/U_{urea} is somewhat higher at the micellar interface than in a similar aqueous solution containing TMAX and urea. We proved, therefore, that urea is not concentrated at the micellar interface. Interestingly, the counterion concentration (Br_m, Cl_m) of the micelles in the presence of 6 M urea shows decreases between 41 and 35%, depending on the detergent concentration; this change is consistent with the known effect of urea of increasing the α and the CMC of the detergents.

Taken together, these results provide substantial evidence that urea destabilization of interfacial head group/counterion ion pair

is a major contributor to urea's effects on micellar properties, such as increasing the critical micelle concentration and the degree of ionization and decreasing aggregation number.

Determining co-ions at the micellar surfaces and the dediazonation mechanism

Another question addressed using chemical trapping was the presence of co-ions, i.e. ions with the same charge as the monomer, at the micellar interface. It is frequent, in the study of reactions involving the hydroxide ion, that positively charged micelles increase the reaction rate while negatively charged ones inhibit the reaction. The alkaline hydrolysis of *p*-nitrophenyl-dodecanoate (pNPD) is inhibited by SDS micelles (Quina et al. 1982). This effect was attributed to the lower concentration of hydroxide ion ([OH⁻]) at the negatively charged SDS interface. We later demonstrated that the addition of NaCl decreases the reaction inhibition and attributed this effect to the increase of Na⁺ at the micellar surface and the simultaneous displacement of H⁺ at the SDS surface (Quina et al. 1982). The decrease in H⁺, because of the water ionic equilibrium, resulted in an increase of OH⁻ which catalysed the hydrolysis of pNPD. At the same time, the question arose as to whether this effect occurs also with co-ions other than OH⁻.

16-ArN₂⁺ was used to estimate the local concentration of Cl⁻ and Br⁻ (co-ions) at the interface of SDS micelles (Cuccovia et al. 1997c). 16-ArN₂⁺ in SDS micelles and in the presence of Cl⁻ or Br⁻ should react to yield the phenol and halo-derivatives (Fig. 1). We noted, however, that the sum of total products did not correspond to the initial 16-ArN₂⁺ concentration: about 20% of the diazonium salt reacted with SDS, but no other product was identified in the chromatogram. To estimate the local concentration of Cl⁻ and Br⁻ at the SDS micellar surface, we obtained standard curves using 1-ArN₂⁺ in water of %1-ArX versus [NaX] in 1.5 M of methylsulfate [CH₃OSO₃⁻ or methanesulfonate (CH₃SO₃⁻)] solutions. This salt concentration is near that of the calculated interfacial ionic strength of SDS and SDSu micelles. Under these conditions the total product yield was, as described earlier, 80%, indicating that sulphate and sulfonate groups react with the phenyl carbocations both in water and in the micellar interface.

The concentrations of co-ions (Br⁻ or Cl⁻) at the micellar interface ([X_b]) were determined with 16-ArN₂⁺, at a fixed

Fig. 3 R-ArN₂⁺ reactions with urea

0.04 M [SDS] and different [NaX]. The increase in [NaBr] or [NaCl] led to a smooth increase in the concentration of the co-ions ($[\text{Br}]_b$ or $[\text{Cl}]_b$) at the interface of SDS micelles (Cuccovia et al. 1997b). $[\text{X}]_b$ at the SDS interface was, as expected, much smaller than that in the bulk solution, but perfectly measurable by chemical trapping. For example, the addition of 1 M NaX to SDS, $[\text{X}]_b$ is approximately 0.15 M, ($\text{X} = \text{Cl}^-$ or Br^-), as expected with the observed inhibition of reactions of alkaline hydrolysis of esters by SDS (Quina et al. 1982).

The Br/Cl ion selectivity exchange constant at the SDS interface ($K_{\text{Br/Cl}}$; Quina and Chaimovich 1979) was measured to determine the concentrations of the products, i.e. 16-ArOH, 16-ArCl and 16-ArBr, at fixed [SDS] and [NaBr] and varying NaCl concentration. The value of $K_{\text{Br/Cl}}$ in SDS is 0.9 (± 0.1), demonstrating under a large range of conditions the absence of co-ion selectivity, as expected from a purely Coulombic exclusion.

The local concentrations of co-ions of SDS and SDSu were calculated for a spherical micelle, using a cell model, by numerical integration of the Poisson–Boltzmann equation considering salt-induced micellar growth. The correlation between the experimental data and theoretical calculations of the concentration of bound ions was excellent (Cuccovia et al. 1997c).

The dediazonation of 16-ArN₂⁺ in SDS and SDSu was further investigated to identify the products formed when the probe reacted with the detergent monomers (Cuccovia et al. 2000a). Instead of determining the product after complete dediazonation, we carried out a kinetic study. Surprisingly, we observed that, in SDS, in addition to 16-ArOH, another product appeared as a new peak in the chromatogram. The relative concentration of this new compound, proven to be the dodecylsulfate ester 16-ArO-SO₃Do (Fig. 4), increased over time; however, after reaching a maximum, 16-ArO-SO₃Do decreased with time (Cuccovia et al. 2000b). However, although 16-ArO-SO₃Do ultimately decreased over time, the concentration of 16-ArOH remained constant. We concluded that the ester was not hydrolysed to the corresponding phenol, but to 16-ArO-SO₃⁻, which did not appear in the chromatogram. These experimental results explain the apparent loss of 20% of the initial probe. To confirm the formation of 16-ArO-SO₃⁻, we added an aliquot of concentrated HCl at the end of the reaction in SDS and observed that the 16-ArO-SO₃⁻ was hydrolysed, giving 16-ArOH (Fig. 4).

The same phenomena occurred with the reaction done in SDSu, where one of the products was shown to be 4-hexadecyl, 2,6 trimethylbenzenedodecylsulfonate (16-ArO₃SDo) which is stable under these conditions. The addition of concentrated HCl did not change the area of the 16-ArO₃SDo. All esters were isolated and the kinetics of hydrolysis of 1-ArO-SO₃Me and 16-ArO-SO₃Do were studied in acid, confirming the kinetics of hydrolysis observed in SDS.

Standard curves to calculate the local head group concentrations with SDS or SDSu micelles were obtained with 1-ArN₂⁺ reacting with either CH₃OSO₃⁻ or CH₃SO₃⁻, respectively. Using the reaction with 16-ArN₂⁺, the %Yields of 16-ArOH, 16-ArOSO₃Do (calculated from the difference between the initial diazonium and the %16-ArOH), and 16-ArO₃SDo (calculated directly from the product area) were determined in SDS and SDSu, respectively. With these data it was possible to calculate the local concentrations of SDS and SDSu at the micellar interface (SDS_m and SDSu_m), which were 2.27 M and 2.14 M respectively. These values are in excellent agreement with previous estimates of local head group concentrations obtained by a large variety of both experimental and theoretical methods for micelles of the same surfactants.

Ab initio calculations of the dediazonation of phenyldiazonium ion in the gas phase and in aqueous solution were performed with or without MeSO₃⁻, yielding the potential energy surfaces for the reactions (Cuccovia et al. 2000a). The results were similar to the experimental values for the reaction with 1-ArN₂⁺ and indicate that the diazonium ion and the nucleophile must form a complex before N₂ loss in order to compete with water. It was also suggested from our calculations that, in the presence of nucleophiles, the phenyl cation is not an obligatory intermediate in aqueous phase due to the absence of a saddle point in the potential energy surface. This contribution was the first experimental and theoretical analysis disputing the long-held view that only a SN1 N₂ loss from aryldiazocations could explain the experimental results obtained up to that date. Later work by others substantiates our hypothesis (Martinez et al. 2013).

Ion binding at the interface of zwitterionic micelles

The biological membrane interface is the target for the binding of anions and cations and thereby responsible for several biological phenomena, such as receptor binding of peptides, proteins and small molecules, which work in the signalling process of the cell. In the presence of salts zwitterionic micelles bind cations and anions with differential affinity, with the affinity defined not only by the dipole of the head group of the detergent but also by both the anion and cation of the added salt. Two zwitterionic detergents (Fig. 5) whose polar groups have opposite dipoles, namely HPS and hexadecylphosphoryl choline (HDPC) (Cuccovia et al. 1999) were studied (de Souza et al. 2012).

Using 16-ArN₂⁺, the average interfacial anion concentration of Cl_b and Br_b in HPS micelles, where the negative sulfonate is distal to the hydrophobic surface, was underestimated while the average interfacial anion concentration in HDPC micelles, where the negative charge is near the hydrophobic core, was overestimated. This occurred because we were using the same probe to estimate anion concentration in micelles with differently oriented dipoles. In the case of

Fig. 4 Reaction products of $R\text{-ArN}_2^+$ with sulphate and sulfonate esters

HDPC, as the dipole extends to bulk water the average anion concentration must reflect more the bulk anion concentration. The 16-ArN_2^+ also reacts with the phosphate and sulfonate groups of the HDPC and HPS, but we did not consider this reaction in this review.

The addition of NaCl or NaBr to HPS and HDPC micelles leads to the binding of both anions (Table 2). As these detergents have two binding sites with opposite charges, both anions and cations can bind to the interface. We investigated the binding of Cl^- and Br^- to HPS and HDPC using salts with different cations (Cuccovia et al. 1999). Cl_b and Br_b were determined in HPS micelles using 16-ArN_2^+ and, as can be seen in Table 2, both anions concentrated at the interface, $[\text{X}]_b$, at the order of three to five times higher than the concentration of the added salt. $[\text{Br}]_b$ is higher than $[\text{Cl}]_b$ at any added salt concentration, and the Br_b/Cl_b ratio ranges from approximately 1.9 at the low salt concentration to 1.2 at the high salt concentration. This ratio is slightly higher only when calcium salts are used, when it reaches 2.5. In HPS, the ratio Br_b/Cl_b is

always higher than 1.5, showing that bromide binds better than chloride.

In HDPC micelles, which is a simpler model of biological membranes, anion concentration is either lower or higher than the bulk concentration, depending both on salt concentration and the nature and charge of the cation. With Ca^{2+} and Mg^{2+} , which are known to strongly interact with phospholipid assemblies (Melcrova et al. 2016), $[\text{Br}]_b$ and $[\text{Cl}]_b$ reach values that are comparable to, but somewhat lower than, those determined with HPS micelles. However, TMA does not favour Cl^- and Br^- binding at the HDPC interface and, as a result, $[\text{Cl}]_b$ is about half that in the bulk solution (Cuccovia et al. 1999).

As TMA is a large molecule, it can increase the distance between the phosphate groups of HDPC, thereby decreasing the positive charge density at the surface near the trimethylammonium ammonium groups of choline and likely decreasing the binding of the anions. The $[\text{Br}]_b/[\text{Cl}]_b$ ratio in HDPC micelles, with all salts, is >1 under all conditions

Fig. 5 Cartoon of 3-(*N*-hexadecyl-*N,N*-dimethylammonium) propane sulfonate (HPS) and hexadecylphosphoryl choline (HDPC) micelles. R_i Internal radius, R_e external radius, R_c cell radius. ^aFrom de Souza et al. (2012)

Table 2 Relative affinities of Br^- and Cl^- (Na^+ salts) for 3-(*N*-hexadecyl-*N,N*-dimethylammonium) propane sulfonate and hexadecylphosphoryl choline micelles

Added [X] (in moles)	$[\text{Br}]_b$	$[\text{Cl}]_b$	$[\text{Br}]_b/[\text{Cl}]_b$
HPS			
0.05	0.30	0.16	1.88
0.10	0.51	0.35	1.46
0.20	0.79	0.61	1.25
0.40	1.31	0.97	1.35
0.60	1.62	1.34	1.21
0.75	2.05	1.69	1.21
0.95	2.28	1.96	1.16
HDPC			
0.05	0.05	0.03	1.67
0.10	0.09	0.06	1.50
0.20	0.25	0.12	2.08
0.38	0.48	0.32	1.50
0.56	0.71	0.49	1.45
0.75	1.07	0.64	1.67

[X], Concentration of added anion; HPS, 3-(*N*-hexadecyl-*N,N*-dimethylammonium) propane sulfonate; HDPC, hexadecylphosphoryl choline (HDPC); $[\text{Br}]_b$, $[\text{Cl}]_b$, binding of Br^- and Cl^- , respectively

(Table 2), showing that Br^- , as in HPS, binds better than Cl^- to HDPC micelles. The sensitivity of the value of the $[\text{Br}]_b/[\text{Cl}]_b$ ratio to ionic strength, however, is lower than that in HPS micelles, suggesting that in HDPC micelles cation binding to the phosphate group maintains anion selectivity.

The effect of the cation on anion binding on HPS and HDPC micelles was quantified by dividing the value of $[\text{Cl}]_b$ obtained with a particular cation by the value of $[\text{Cl}]_b$ obtained with Na^+ at the same [X] (Table 3). The selectivity of HPS and HDPC micelles for the halides followed the series: $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$, which correlates with the size of the naked cation. This shows that for the smaller cation, the interaction is stronger. This effect was associated with a high field generated by surface charges partially or completely dominating the hydration of the cation. The data in Table 3 suggest that divalent cations, for HDPC micelles, interact strongly with phosphate groups closer to the micellar core (Cuccovia et al. 1999).

The study of ion binding to zwitterionic micelles was advanced taking into account all of the products formed in the reaction of diazonium salts with the sulfonate group of HPS and the phosphate of HDPC (de Souza et al. 2012). The reaction of 1-ArN_2^+ was studied in the presence of the short analogs *N,N,N*-trimethyl-3-ammonio-1-propanesulfonate (MPS) and methyl 2-(*N,N,N*-trimethylammonio)ethyl-phosphate (MPC), both of which react given the respective esters (Fig. 6). The products do not appear in the HPLC chromatogram, but the concentration of the diazonium salt, which reacts with MPS

Table 3 Selectivity of 3-(*N*-hexadecyl-*N,N*-dimethylammonium) propane sulfonate and hexadecylphosphoryl choline micelles for cations relative to Na^+

Cation	HPS ^a	HDPC ^a
Li^+	1.01	1.25
Na^+	1.00	1.00
K^+	0.94	0.83
Rb^+	0.88	0.74
Cs^+	0.92	0.75
TMA^+	0.78	0.53
Mg^{2+}	0.96	1.75
Ca^{2+}	1.00	1.83

^a [Salt] = 0.5 M; [Detergent] = 0.04 M

and MPC, was evaluated taking in account the initial concentration of the diazonium salt and subtracting the concentration of 1-ArOH obtained at different concentrations of MPS and MPC. In the presence of 1.5 M MPS and 1.5 M MPC, the %1-Ar-MPS and %1-Ar-PC was about 15 and 5%, respectively, of the total diazonium salt. The standard curves of %1-ArX versus $[\text{NaX}]$ were then obtained in the presence of 1.5 M MPS and 1.5 M MPC and used to calculate the Cl_b and Br_b in the micellar interface.

The experimental results were analysed using a cell model and nonlinear Poisson Boltzman (PB) equations. Ion specificity was built into the model using the difference in the standard chemical potential between the ions adsorbed onto the surface and the ions free in the bulk solution as a (the only) variable parameter necessary to fit the data with the PB analysis.

The values of the ratios $[\text{Cl}]_b/[\text{Cl}]_T$ in HPS and HDPC micelles, obtained at a fixed detergent concentration of 0.04 M and a total added chloride concentration $[\text{Cl}]_T$ of 0.5 M for all salts, are shown in Table 4. $[\text{Cl}]_b$ at the micellar interface of HDPS increased with added salt and was cation dependent. $[\text{Cl}]_b$ was similar to the concentration of the added salt with Na^+ , K^+ , Rb^+ , and Cs^+ . With TMACl, the $[\text{Cl}]_b$ was significantly lower than that in the bulk solution, while the salts of divalent cations and Li^+ resulted in an interfacial concentration higher than that in the bulk.

It is evident that the anion concentration at the interface of HDPC micelles is cation dependent. For MgCl_2 and CaCl_2 , the doubly charged cations tightly bind to phosphate at the internal layer of HDPC, decreasing the negative charge of this layer. Ca^{2+} (or Mg^{2+}) binding may also decrease the distance between the trimethylammonium groups, thereby increasing the positive charge density and as a consequence the capacity of binding anions (Manning 1979). Li^+ must bind better than the other cations, probably due to its small size, leading to higher chloride binding. For TMA^+ , the data suggest that this cation either binds very weakly to the zwitterionic micelle or separates the head groups, and chloride is effectively excluded from the interface. Qualitatively, the effects of cations on Br^- binding to HDPC micelles were similar to those observed with

Fig. 6 Products of the reactions of diazonium salts with zwitterionic compounds (de Souza et al. 2012)

Cl^- (Table 4). $[\text{Br}]_b$ was of the same order of the added salt, with the exception of CaBr_2 , MgBr_2 and LiBr (Table 4).

Values of $[\text{Cl}]_b$ and $[\text{Br}]_b$ are higher (~1.5 times) in HPS micelles, than in HDPC micelles, but cation dependence is less evident (Table 4). The values of $[\text{Cl}]_b$, with TMA as an exception, are similar for all salts, and Cl_b/Cl_T is generally twice that of added external salt. Bromide binding to HPS micelles shows the same trends as those observed with Cl^- but the binding of $[\text{Br}]_b$ is threefold higher than that of the added salt, with the exception of Li^+ and TMA^+ .

The inner positive layer of the HPS micelle attracts the anions, with the access of the anion to the inner layer hindered by the negatively charged outer layer (Fig. 5). This barrier is reduced by the cations in the proximity of the external layer which partially screen the outer layer repulsive interaction (Iso

and Okada 2000; Lukac et al. 2010). The small differences in the local anion concentrations in the HPS micelle with different added salts suggest that the interfacial anion concentration is almost independent of the cation.

Our analysis is consistent with cation and anion distribution in the external zwitterionic region increasing with the concentration of the added salt. Ion adsorption at the zwitterionic region was accompanied with lateral mobility, and the fit of the experimental data with theory was not adequate upon the supposed fixed binding sites. In fact, experimental data obtained using dielectric relaxation spectroscopy show the importance of surface diffusion of the bound ions (Buchner et al. 2005).

Langmuir isotherms that are necessarily implied in fixed binding sites can also be used to fit anion binding to zwitterionic micelles (Iso and Okada 2000). Ion-induced perturbations to head group tilt, the extent and direction of which is sensitive to ion charge and size for all ion types, including Cl^- , have been observed (Aroti et al. 2007). These data, however, do not preclude a local salt concentration effect, rather than a localized, site-related description. It has been shown here that ion concentration and specificity in zwitterionic micelles can be modelled by assuming that ions can diffuse on the interfacial plane while bound to the micelle.

Modelling the adsorption of ions into the micellar surface

The interface of the micelle of a defined surface, A , contains M ion sites with a unit area b (de Souza et al. 2012). The total number of ions, N_i , can be fixed at a site or diffuse freely on the surface, depending on the magnitude of N_i and the nature of the ions. The superscript a, (adsorbed) refers to ions that can freely move in the micellar. The fraction of occupied sites, θ_i , called degree of coverage of Langmuir, is given by: $\theta_i = \frac{N_i^a}{M}$ where M is the number of adsorption sites. The equilibrium ion distribution was calculated using a Poisson–Boltzmann

Table 4 Br_b/Br_T and Cl_b/Cl_T ratios for 3-(*N*-hexadecyl-*N,N*-dimethylammonium) propane sulfonate and hexadecylphosphoryl choline at $[\text{Detergent}] = 0.04 \text{ M}$ and 0.5 M salt

Salts [0.5 M]	$\text{Cl}_b/\text{Cl}_T^a$		Salts [0.5 M]	$\text{Br}_b/\text{Br}_T^a$	
	HDPC	HPS		HDPC	HPS
TMACl	0.80	1.80	TMABr	1.01	2.37
LiCl	1.43	2.23	LiBr	1.32	2.73
NaCl	1.02	2.20	NaBr	1.04	3.10
KCl	0.96	2.36	KBr	1.02	3.15
RbCl	1.01	2.24			
CsCl	1.00	2.38			
CaCl_2	1.58	2.35	CaBr_2	2.02	3.49
MgCl_2	1.61	2.49	MgBr_2	1.84	3.32

^a In the original paper, the number corresponds to the ratio Cl_b/Cl_T and Br_b/Br_T and not to Cl_T/Cl_b and Br_T/Br_b . The numbers are correct but the ratio was reversed, i.e. as can be confirmed by the data in figures 1 and 2 of de Souza et al. (2012). Br_T and Cl_T are the total concentrations of added bromide and chloride salt, respectively

distribution (PBE), allowing the estimation of θ_i for cations and anions for HDPC and HPS micelles. The screened charge in the inner layer of HDPC micelles by bound cations reached approximately 90% for divalent cations and was in the 70–80% range for univalent ions such as chloride and bromide. The calculated concentrations of bound anions were much lower, i.e., less than 5% for 1 M of added salt. These calculated ion coverages indicate a decrease in the number of negative charge at the inner surface, as well as a weak attenuation of the positively charged outer layer. The zwitterionic micelles will attract and bind more chloride or bromide (Iso and Okada 2000; Tondo et al. 2007).

The degree of Cl^- coverage, θ_i , in the inner, positively charged layer of the HPS micelle reached 35% with TMA^+ at high salt concentration, while with Ca^{2+} and Mg^{2+} and the alkaline cations, $\theta_i < 10\%$. The values corresponding to the Br^- are $< 10\%$. The negatively charged outer layer of the HPS micelle has a maximum of 20% of the charges neutralized by the cations when these are associated with chloride. A maximum of 60% attenuation is reached by cations from bromide salts, implying that most of the negative charge of the micelle is neutralized, leading to a change in the electrostatic potential in its neighbourhood.

The results suggest that the lower percentage of bound ions, taking electrostatic interaction into consideration, results in higher absolute values for the mean electrostatic potential nearby. Consequently, it leads to higher values for the probability for the presence of counter ions and thus higher values of anion local concentration in the micelle HPS compared with the values of HDPC micelles.

Ion composition at the surface of membrane models: vesicles

The binding of ions to zwitterionic phospholipids in liposomes, which are more complex than micelles but more alike to cell membranes, was evaluated by chemical trapping. We studied the binding of salts to liposomes prepared with pure or mixtures of zwitterionic phospholipids [dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylcholine (DMPC)] and the cationic dioctadecyldimethylammonium chloride (DODAC) and bromide (DODAB) (Scarpa et al. 2002). The values of Cl_b and Br_b in DODAC and DODAB vesicles, using 16-ArN_2^+ , were determined for vesicles of different sizes. For sonicated DODC vesicles, with a hydrodynamic diameter (D_h) of 25 nm, Cl_b was of the order of 1.2 M with or without 5 mM NaCl and was 1.8 M for vesicles prepared by the injection method (using chloroform or dichloromethane) in the presence of 5 mM NaCl. The relative extent of ion dissociation (α) of small sonicated DODAB vesicles, without added salt, is 0.13, while the value of α for large, HCCl_3 -injected vesicles is

0.04. These results are in agreement with previous data showing that counterion dissociation decreases with vesicle size.

The selectivity between Br and Cl at the vesicle surfaces was measured by the addition of TMACl to DODAB vesicles during preparation of the vesicles. The value for the $K_{\text{Br/Cl}}$ at the DODAB vesicular interface was $\cong 2.0$, well within the values obtained for CTAB or CTAC micelles.

Anion binding to vesicles prepared at different molar ratios between DPPC (or DMPC) and DODAC was also determined. Cl_b increased sigmoidally with increasing molar ratio of DODAC in the DODAC/lipid mixtures. The binding of Cl^- at the interface of pure DPPC vesicles is low and, with increasing DODAC, $[\text{Cl}_b]$ remains almost constant until 30% of DODAC in the vesicle mixture. At this molar ratio there is an abrupt increase in Cl_b , indicating that a minimum amount of charge at the interface is necessary to induce ion condensation at the interface. In sonicated vesicles prepared with DODAC and DMPC (or DPPC), the values of Cl_b reach local concentrations measured for the pure amphiphile at 80% DODAC.

In vesicles of DPPC the values of Cl_b are also very sensitive to the nature of the cation and decrease in the order $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Li}^+ > \text{Na}^+ > \text{K}^+ = \text{Cs}^+ = \text{Rb}^+ \geq \text{TMA}^+$. The effect of the cation becomes more important as temperature increases above the phase transition temperature (T_m) of the lipid. These results represent the first extensive study of local concentrations of ions determined directly by chemical trapping in vesicles prepared with lipids, synthetic amphiphiles and their mixtures.

Ion distribution in reverse micelles

Reverse micelles (RM) can be prepared with the same amphiphiles that form micelles in water if the adequate solvent mixtures are used. RM are more like liposomes, in the sense that there is an aqueous compartment inside of the aggregate, separated from the external environment by the hydrophobic alkyl chain of the surfactant, but the external media is composed of an organic solvent. The properties of the internal aqueous compartment of RM and of the interface water/oil of these aggregates were investigated by chemical trapping (Cuccovia et al. 2001).

Reverse micelles were prepared with CTAB in two different solvent mixtures: *n*-dodecane/ CHCl_3 and isooctane/*n*-hexanol. The aqueous compartment was evaluated with 1-ArN_2^+ and the interface with 16-ArN_2^+ . Quantitative analysis of the reaction products of 1-ArN_2^+ and 16-ArN_2^+ with water and bromide ion allowed us to obtain the local concentrations of Br^- in the water pool ($[\text{Br}]_f$) and micellar interface ($[\text{Br}]_b$) in CTAB RM.

With 1-ArN_2^+ , the expected product of the reaction between the 1-ArN_2^+ and *n*-hexanol was not detected, which is in agreement with previous results. Since *n*-hexanol in RM resides preferentially in the interface, this result strongly

suggests that, in these RM, 1-ArN_2^+ resides exclusively in the water compartment.

The values obtained for Br_f using 1-ArN_2^+ depend on the water/surfactant ratio (W/S). At a high water content, W/S = 44, in isooctane/*n*-hexanol 6:4 and 0.05 M CTAB, Br_f at the RM aqueous pool reaches 0.32 M. Br_f increases with decreasing W/S, reaching 1.15 M at W/S = 20. This increase in Br_f can be attributed to the insertion of the probe in the interface (surfactant/organic), leading to the increase in the %1-ArBr.

In RM of CTAB prepared in *n*-dodecane/ CHCl_3 , Br_f is much smaller than in isooctane/*n*-hexanol, reaching 0.15 M at W/S = 40.7. This can be attributed to a lower dissociation of the surfactant head groups at the water/organic interface in this solvent mixture, leading to a smaller Br_f in the water pool. At W/S = 20.7, Br_f is 0.5 M, but as W/S decreases to below 10, Br_f increases to 16 M, indicating that at low water content 1-ArN_2^+ binds to the bromide ion and migrates to the organic phase and that the reaction product is almost exclusively 1-ArBr.

The interface of CTAB/isooctane/*n*-hexanol RM was probed with 16-ArN_2^+ . The values of $[\text{Br}]_b$ range from 3.7 M to 2.9 M when W/S changes from 20 to 44 in HBr 1 mM, CTAB 0.05 M. Almost 5% of 16-ArN_2^+ reacted with hexanol to form 16-ArOH_{hex}, in excellent agreement with published data. These results strongly suggest that 16-ArN_2^+ is located at the micellar interface.

Using 16-ArN_2^+ in CTAB/ CHCl_3 /*n*-dodecane RM, at several W/S ratios %16-ArBr decreases sharply with increasing W/S, reaching 85% at W/S = 40.7. This product percentage is much higher than that obtained in isooctane/*n*-hexanol and exceeds the values at the highest $[\text{NaBr}]$ in the standard curve. The $[\text{Br}]_b$ calculated at the interface from extrapolation using the standard curve was close to 18 M at W/S 40 and 20,. Therefore, in CTAB RM, with CHCl_3 /*n*-dodecane as the organic phase, 16-ArN_2^+ reacts in the bulk organic phase to yield almost exclusively 16-ArBr and 16-ArOH is formed from the reaction of 16-ArN_2^+ with water solely at the micellar interface.

Determination of product yields of the reactions of 1-ArN_2^+ and 16-ArN_2^+ in a two-phase system prepared with CHCl_3 /*n*-dodecane/water and isooctane/*n*-hexanol/water, without CTAB, at different NaBr concentrations helped us to rationalize the results obtained with CTAB RM. The calculated $[\text{Br}]$ obtained from %1-ArBr corresponds, within 10% error, to the concentration of NaBr in the aqueous phase. Therefore, 1-ArN_2^+ reacts only in the water pool and the estimation of Br_f in CTAB RM is correct. However, 16-ArN_2^+ reacts in wet *n*-dodecane/ CHCl_3 to yield exclusively the corresponding 16-ArBr, while in isooctane/*n*-hexanol the %Yield of the 16-ArN_2^+ reaction changes with NaBr concentration, becoming %16-ArOH = 60%, %16-ArHex = 19.8 and 16-ArBr = 60.6% in 1 mM HBr. These results indicate that Br^- forms a complex with 16-ArN_2^+ and that at the organic phase, the complex yields exclusively 16-ArBr. As the organic phase

is water-saturated, the presumably hydrated ion complex ($16\text{-ArN}_2^+\text{Br}^-$) reacts preferentially with Br^- . Therefore *n*-hexanol decreases the binding of Br^- to 16-ArN_2^+ and increases the reaction of water with the diazonium ion in the organic phase or, alternatively, increases the solubility of the free diazonium ion in the aqueous phase.

A PB equation above a W/S of 14 appropriately describes the values of $[\text{Br}]_f$. Comparison of the experimental values of $[\text{Br}]_b$ with those predicted by PB with changing W/S suggest that 16-ArN_2^+ extends from the interface 0.6–1.2 nm with increasing W/S. Both the PB calculations and experimental data indicate that the degree of counterion dissociation from CTAB RM in *n*-dodecane/ CHCl_3 reaches a value of approximately 0.2 above a W/S of 15.

The large effect of organic solvents in the product yields of the dediazonation of 1-ArN_2^+ and 16-Ar_2^+ led us to investigate the effect of other different organic solvents, water miscible, in the mechanism of this reaction (Cruz et al. 2015). The rate of dediazonation of 1-ArN_2^+ was studied in pure solvents (methanol, ethanol, propanol and acetonitrile) and in their mixtures with water containing 0.001 M HCl. The reaction rates were similar in all of the mixtures, but the product yields were dependent on the nature of the solvents in the mixture and not only on their molar ratios.

The increase in the water mole fraction in water/methanol mixtures produces a linear increase of the %1-ArOH and an equivalent decrease in the molar concentration of 2,4,6-trimethylanisole (1-ArOMe). At a water mole fraction of 0.5, the %1-ArOH and %1-ArOMe are 50% each.

Dediazonation in mixed solvents: experimental results and theoretical predictions of product distributions

In mixtures of water/ethanol and water/*n*-propanol, the ethers 2,4,6-trimethyl-1-ethoxy-benzene (1-ArOEt) and 2,4,6-trimethyl-1-propoxy-benzene (1-ArOPr), are formed, respectively. The results with those alcohols are very different from those obtained with water/methanol. The formation of 50% 1-ArOEt and 1-ArOPr occurs at 0.36 and 0.32 water/ethanol and water/*n*-propanol molar ratio, respectively. This effect was observed previously with water/butanol mixtures upon butanolysis of 4-methylbenzenediazonium ions in binary *n*-BuOH/ H_2O mixtures and in *n*-BuOH/SDS/ H_2O reverse micelles by Bravo and co-workers in monosubstituted methylbenzenediazonium salts (Fernandez-Alonso and Bravo-Diaz 2010). In water/acetonitrile mixtures, 1-ArN_2^+ reacts with acetonitrile producing the acetamide 1-ArNHAc. The %1-ArOH and %1-ArNHAc reaches 50% of each product water/acetonitrile molar ratio of about 0.7 (Fig. 7).

The question to be answered is whether there is a relationship between preferential solvation and product ratios. We calculated the local solvent distribution around the diazonium ion from molecular dynamics (MD) simulations. The local solvent

Fig. 7 Reactions of diazonium salts with solvents

composition depends linearly on the fraction of water for water/methanol binary mixtures, but it varies nonlinearly for the other solvent mixtures. The oxygen atom (or nitrogen) of the solvent interacts preferentially with the ipso carbon, or the surrounding $-\text{N}_2^+$ group, and there was no preference to interact with any other groups of the diazonium ion. Using classical simulations and assuming an equal probability of reaction, the calculated local number of solvent molecules around the aryl diazonium cation predicts the product ratios, but only when the preferential solvation shell on the ipso carbon site is considered. Our simulations also considered two different atomic charge distributions, i.e. an atomic charge distribution considering electrons delocalized over all the molecule (M2 model) and an atomic charge distribution considering electrons delocalized over an aryl cation and a neutral N_2 moiety (M1 model). We demonstrated that in mixed solvents the reaction can proceed by a $\text{S}_{\text{N}}1$ or $\text{S}_{\text{N}}2$ pathway and that product distribution is only proportional to the composition of the solvation shell of the carbon attached to the $-\text{N}_2$ group (ipso carbon). Solvent distribution in the first solvation shell of the ipso carbon is equal to the measured product distribution, calculated from classical molecular dynamics simulations.

Conclusion

Chemical trapping has proven to be a unique method to determine interfacial concentrations in a wide variety of colloidal aggregates ranging from micelles to membranes. In addition, the same method can determine multiple nucleophiles and probe bulk solution properties. The basis of the method relies on a reaction of nucleophiles with an aryl carbocation in a time scale shorter than that for nucleophile diffusion. By fixing the initial aryl diazo derivative either at the interface

or in the bulk solution, products from known positions can be determined. Reference to standard curves, obtained in bulk solutions with compositions similar to the site being probed, enable a direct estimation of local concentrations. As in all methods, the limitation of the reaction imposes that the interpretations of the results must be preceded by careful experimental controls. The assumption that comparison of the product yield of micelle fixed probes with a standard curve obtained in the bulk has resisted attempts to undermine the directness of the chemical trapping method. Our results with mixed solvents and our proposal for the mechanism of dediazonization further support the validity of the method. We determined ion dissociation from ionic micelles and co-ion association to similarly charged micelles, establishing the first direct demonstration of the previous theoretical Poisson–Boltzmann co-ion distributions. We have extended the application of this method, probed zwitterionic ion selectivity in aqueous and reverse micelles and vesicles and demonstrated that urea does not concentrate on micellar surfaces. Our results have contributed to shed light on the causality of shape changes in micelles, which are clearly related to surface dehydration. Several characteristics of these systems have been extremely difficult to obtain with other techniques.

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Compliance with ethical standards

Conflicts of interest Iolanda Midea Cuccovia declares that she has no conflicts of interest. Filipe da Silva Lima declares that he has no conflicts of interest. Hernan Chaimovich declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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