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Electrochemistry of Non-Redox-Active Poly(propylenimine) and Poly(amidoamine) Dendrimers at Liquid–Liquid Interfaces

Alfonso Berduque, Micheál D. Scanlon, Courtney J. Collins, and Damien W. M. Arrigan*

Tyndall National Institute, Lee Maltings, University College, Cork, Ireland

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The electrochemistry of a series of dendrimers was examined at the interface between two immiscible electrolyte solutions (ITIES), enabling study of non-redox-active dendrimers. Different generations of poly(propylenimine) (DAB-AM-*n*) and poly(amidoamine) (PAMAM) dendrimers were studied. In their protonated states, the dendrimers were transferred across the ITIES, with the electrochemical behavior observed depending on the dendrimer family, the generation number, and the experimental pH. The electrochemistry of the lower generations studied was characterized by well-defined peaks for both dendrimer families and with small peak–peak separations in the case of the PAMAM family. The voltammetry of the higher generations was more complex, showing distorted voltammograms and instability of the interface. The charges of the transferring dendrimers were calculated by convolution of the voltammetric data and were similar to the theoretical charges for DAB-AM-*n*. For PAMAM, only the lowest generation exhibited reversible behavior, with higher generations having irreversible behavior. Using cyclic voltammetry, low micromolar concentrations of the dendrimers were detected. The results show that electrochemistry at the ITIES can be a useful method for characterization of ionizable dendrimers and that voltammetry can be a simple method for detection of low concentrations of these multicharged species.

Introduction

Dendrimers are a new class of polymeric materials^{1,2} possessing a well-defined structure, consisting of a central core, emanating branched units ending in terminal groups,^{2–5} and internal cavities within the branched structure. Dendrimers have highly symmetric⁶ and globular structure, are highly branched, and, unlike linear polymers, are monodisperse macromolecules.^{2,3,7} The cyclic manner in which they are built results in a globular shape and a large number of end groups (surface or terminal groups). The more layers of branched units added, the higher the generation of the dendrimer obtained.² Moreover, the dendritic molecules can be functionalized with specific functional groups to impart specific chemical features.^{7,8} Due to their structural characteristics, dendrimers offer improved physical and chemical properties when compared to linear polymers.^{7,8} In solution, dendrimers do not exist as flexible coils but as compact (if the generation is not too low) and globular structures,⁷ which influences their rheological properties. Dendrimers are generally synthesized using two different synthetic routes: divergent routes^{1,7,9,10} and convergent routes.^{7,9,11,12}

The highly structured characteristics make dendrimers very suitable molecules for a wide range of biomedical applications,^{2,4,5,7,13,14} including in vitro diagnostics,⁷ in drug delivery,^{4,5,7,13,15–19} as unimolecular micelles,^{2,20} and as the mimics of proteins.^{20,21} However, the biological properties of dendrimers need to be more deeply investigated to enable further therapeutic applications.² Dendrimers are also used as catalysts,^{3,7,20,22–28} combining the advantages of homogeneous and heterogeneous catalysts.^{7,23} Metallo dendrimers with interesting electronic, magnetic, and catalytic properties have been reported.^{23,24} The structure, size, shape, and solubility of dendrimers and metallo dendrimers can be tuned, and the catalytic sites of a metallo dendrimer can be located either at the periphery of the dendrimer or at the core.^{3,29,30} Other applications of dendrimers are their use as encapsulating agents for insoluble materials (e.g., metals),^{7,20} self-assembled monolayers (self-assembling dendrimers are considered as models for biological systems such as vesicles or micelles),²⁰ cast films, colloids and nanoclusters,²⁰

* To whom correspondence should be addressed. Phone: +353-21-4904079. Fax: +353-21-4270271. E-mail: damien.arrigan@tyndall.ie.

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liquid crystals,²⁰ biochemicals and pharmaceuticals²⁰ and as chemiresistors.³¹ Furthermore, electroactive dendrimers are of great relevance in understanding biological electron-transfer processes²⁰ as well as finding applications as ion sensors or electrooptical devices when they possess suitable receptors within their structure or as electron-transfer mediators.²⁰

There has been quite a lot of interest in the electrochemistry of different types of dendrimers.^{32–44} However, all these studies are limited to redox processes,^{32–44} using dendrimers functionalized with redox-active functionalities. One study⁴⁵ on the potentiometric response of polycation-sensitive membrane electrodes to poly(propyleneimine) (DAB-AM-*n*) and poly(amidoamine) (PAMAM) dendrimers did not rely on the redox properties of dendrimers. In this paper we report on the possibility of studying non-redox-active dendrimers, such as PAMAM and DAB-AM-*n* of different generations, by electrochemistry at the interface between two immiscible electrolyte solutions (ITIES),^{46–51} where charged species are moved across the interface as a function of the applied potential difference between the two immiscible phases.⁵² This mode of electrochemical study, at which dynamic electroanalytical methods such as cyclic voltammetry are routinely applied to the study of small ionic molecules, offers scope for the study of electrochemical properties of dendrimers at the ITIES if the dendrimers contain ionized or ionizable functional groups. There has been much interest in the study of macromolecules at the ITIES recently. For example, the transfer of the polyionic polypeptide protamine, using micrometer-sized ITIES,^{53,54} and the detection of heparin (a sulfonated polysaccharide) at polarized blood plasma/1,2-dichloroethane interfaces⁵⁵ or at aqueous/PVC plasticized liquid membranes^{56,57} have been reported. The aim of the work reported herein was to examine the electrochemical properties of dendrimers as model macromolecules easily

obtainable in different molecular sizes with known charge properties and of known molecular changes as the mass increases. The behavior of this type of macromolecule at electrified liquid/liquid interfaces can provide a basis for the rational design of electrochemical methodologies for study and detection of ionized macromolecules while at the same time giving access to dendrimer electrochemical properties not amenable to study save for those functionalized with redox-active groups.

In this study, two families of dendrimers (and various generations of each) were examined: DAB-AM-*n* dendrimers (where DAB refers to the diaminobutane core and *n* is the number of amino surface groups) and PAMAM dendrimers. DAB-AM-*n* dendrimers have two types of amino groups: primary amino surface groups and tertiary amino groups located at the branching sites in the core of the dendrimer. The number of primary amino groups is 2^{y+1} , where *y* is the generation number, and the number of tertiary groups is $2^{y+1} - 2$.⁶ PAMAM dendrimers, also known as starburst dendrimers, are based on either an ethylenediamine (EDA) core or an ammonia core.⁷ The PAMAM dendrimers based on EDA cores have branched units built from both ethylenediamine and methyl acrylate.^{1,2} EDA-core PAMAM dendrimers were used in this work. Physically, the DAB-AM-*n* dendrimers behave more like linear polymers, having a more open structure, whereas the PAMAM dendrimers are more globular. These structural differences will influence the electrochemical behavior of the two families.

Dendrimers of low generations (i.e., 0, 1, and 2) have highly asymmetric and open structures in comparison to dendrimers of higher generations,⁷ which have more compact and globular structures. There is a critical branched state, known as the starburst effect, at which dendrimers cannot keep growing for spatial reasons. For starburst PAMAM dendrimers, this is observed after the 10th generation.⁷

The basis of this work is the acid–base properties of dendrimers and how this makes them amenable to direct electrochemical study at the ITIES. The primary amino surface groups of PAMAM dendrimers have a pK_a of 9.5.^{2,58,59} In the pH range 5.7–8, 97–100% of the amino groups are charged. Several papers have been published containing discussion of the charge degree of both DAB-AM-*n* and PAMAM dendrimers using the Ising model.^{8,62–64} Using that approach, the charge for any dendrimer in aqueous solution of known pH can be obtained. Electrochemistry at the ITIES is often undertaken at the aqueous/1,2-dichloroethane interface. We note that some redox-functionalized dendrimers have been studied in 1,2-dichloroethane and its mixtures (mixtures of acetonitrile and 1,2-dichloroethane), where these dendrimers are also soluble.^{65,66}

Experimental Section

Reagents. The aqueous-phase electrolytes were 10 mM LiCl (pH 5.5) or 0.1 M HCl (pH 1.0), prepared in purified water (18 MΩ cm

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Table 1. Dendrimers Studied and Their Properties (Dendrimer Family, Generation, Nature, and Number of Surface and Interior Ionizable Groups) and Net Charge Calculated from CV Calibration Curves (Using the Randles–Sevcik Equation, CV z_i) and the Convolution Technique (Convolution z_i), versus the Theoretical Net Charge (Theoretical z_i)

dendrimer	generation	no. of surface amino groups	no. of interior amino groups	theoretical z_i (pH 5.5)	CV z_i (pH 5.5)	convolution z_i (pH 5.5)
DAB-AM-4	1	4	2	6		5
DAB-AM-16	3	16	14	25		18
DAB-AM-32	4	32	32	50		42
DAB-AM-64	5	64	62	100		83
PAMAM-0	0	4	2	5	2	2
PAMAM-1.0	1	8	6	11	3	5
PAMAM-2.0	2	16	14	24	5	15
PAMAM-3	3	32	30	50	7	16

purity). The dendrimers investigated were generations 0, 1, 2, and 3 of the PAMAM dendrimers (PAMAM-0, PAMAM-1.0, PAMAM-2.0, and PAMAM-3.0) with an EDA core and the DAB-AM- n dendrimers poly(propylenimine) tetramine dendrimer (DAB-AM-4), poly(propylenimine) hexadecamine dendrimer (DAB-AM-16), poly(propylenimine) dotriacontamine dendrimer (DAB-AM-32), and poly(propylenimine) tetrahexacontamine dendrimer (DAB-AM-64). All the dendrimers were purchased from Sigma-Aldrich. Solutions of dendrimers were prepared in the appropriate aqueous-phase solution (10 mM LiCl or 0.1 M HCl), depending on the experimental pH desired. The pH of the solutions was checked with a calibrated glass combination electrode/pH meter (Orion pH meter, model 520A, Allometrics).

The organic phase was 1,2-dichloroethane (DCE) containing bis-(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)-borate BTTPATPBCl (10 mM) as an organic electrolyte salt. BTTPATPBCl was prepared by metathesis^{52,67,68} of bis(triphenylphosphoranylidene)ammonium chloride ((BTTPA)Cl) and potassium tetrakis(4-chlorophenyl)borate KTPBCl. Solutions of tetraethylammonium (TEA⁺) were prepared in the corresponding aqueous-phase solution (10 mM LiCl, 0.1 M HCl). TEA⁺, obtained as the chloride salt (Sigma-Aldrich), was used as a reference ion for calibration of the potential axis.

Apparatus. A CHI660B electrochemical analyzer (CH Instruments, Texas) was used for all electrochemical investigations. A customized four-electrode electrochemical cell was used, where the interfacial potential difference between the two immiscible electrolyte solutions was controlled via two Ag/AgCl reference electrodes (one in each phase) and the current was measured via two platinum-mesh counter electrodes (one in each phase).⁶⁹ The geometric interface area was ca. 1.005 cm² and was flat in appearance to the naked eye. In these experiments, a positive cell current, by convention,⁷⁰ signifies the transfer of a cationic species from the aqueous phase to the organic phase or the transfer of an anionic species from the organic phase to the aqueous phase, whereas a negative cell current indicates the transfer of an anionic species from the aqueous phase to the organic phase or the transfer of a cationic species from the organic phase to the aqueous phase.

Methodology. Investigations were initiated by cyclic voltammetry (CV) of the electrolyte solutions without any species under study added to the system (i.e., without dendrimers or reference ions). These CVs show the background response due to the aqueous and organic electrolyte salt dissolved in their respective solvents. These CVs were followed by additions of increasing concentrations of dendrimer to the aqueous phase by additions of a stock solution of dendrimers at known concentration using a micropipet or microsyringe, unless stated otherwise. All experiments were performed in triplicate.

Results and Discussion

Electrochemistry of DAB-AM- n Dendrimers. Table 1 summarizes the DAB-AM- n dendrimer generations investigated, indicating the number and nature of the surface groups, as well

as the number of interior tertiary amino groups. All the amino surface primary groups and the interior tertiary amino groups in the DAB-AM- n family can be protonated, but obviously the degree of protonation is a function of the experimental pH. Table 1 also summarizes the approximate net charge of these dendrimers at pH 5.5, which was calculated from the data of van Duijvenbode et al.⁶³

Electrochemistry at pH 5.5. Initially, the electrochemical behavior of the dendrimers was studied using 10 mM LiCl as the aqueous-phase electrolyte solution at pH 5.5. The addition of DAB-AM-4 dendrimer to the aqueous phase led to the emergence of a CV peak at very positive potentials (in the forward sweep), which may be due to the transfer of the dendrimer from the aqueous into the organic phase, and a negative CV peak in the reverse sweep due to a back-transfer into the aqueous phase (Figure 1A). At very low concentrations of dendrimer, such transfer peaks were difficult to visualize, as the current magnitudes were still quite low and overlapped with the positive (right-hand side) potential window limit in the cyclic voltammogram. However, the addition of increasing concentrations of DAB-AM-4 resulted in a linear increase of the peak current response (see calibration curve in Figure 1A, inset) and a slight shift of the transfer peak toward less positive potentials for the highest concentrations (not shown). This shift of potential was independent of any influence on the reference electrode potential by dendrimer adsorption on the electrode, as determined by control experiments with TEA⁺ in the aqueous phase during dendrimer experiments. It is believed that the interaction between the organic-phase electrolyte anions tetrakis(4-chlorophenyl)borate (TPBCl[−]) and the dendrimer is very important in the voltammetry of all the dendrimers studied. The shift of the transfer peaks toward lower potentials may be due to the formation of more stable dendrimer–TPBCl[−] complexes when the concentration of dendrimer in the solution is increased.

The electrochemistry of DAB-AM-16 dendrimer was similar to that of DAB-AM-4. Nevertheless, the electrochemistry of DAB-AM-16 showed a diffusion-controlled transfer peak (peak B in Figure 1B) at ca. 0.8 V (forward peak), preceded by a small current shoulder at ca. 0.68 V (shoulder A in Figure 1B) due to the adsorption of dendrimer at the interface, similar to that shown by Girault and co-workers in similar studies with poly(ethylenimine) (PEI), a positively charged polyelectrolyte.⁷¹ Figure 1B shows the effect of the scan rate in the electrochemical response of 2.5 μ M DAB-AM-16 dendrimer. A plot of the current versus the sweep rate for process A (peak A) was linear, which is characteristic of an adsorption process, whereas for process B (peak B), the peak current increased linearly with the square

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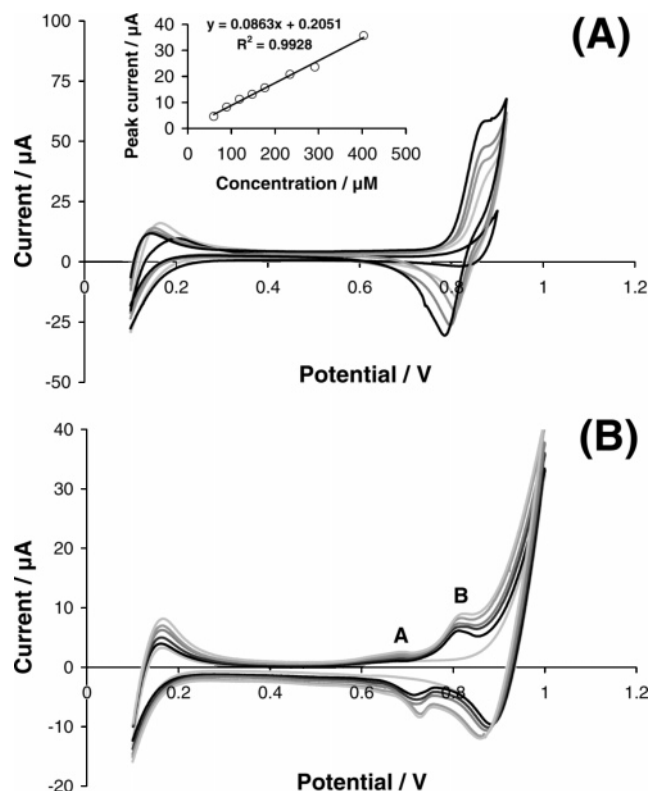


Figure 1. Electrochemistry of DAB-AM-*n* dendrimers at pH 5.5: (A) CV response of the blank (black) and of 60 (light gray), 90, 120, and 177 (black) μM DAB-AM-4 and the calibration curve (inset) (scan rate 5 mV s^{-1}); (B) CV response of the blank (gray) and of 2.5 μM DAB-AM-16 as a function of the scan rate (scan rates from 5 (black) to 15 (light gray) mV s^{-1}).

root of the sweep rate, characteristic of a diffusion-controlled process.

The voltammetry of DAB-AM-32 dendrimer (Figure 2A) was more complicated than the electrochemistry of DAB-AM-4 or DAB-AM-16: concentrations greater than 10 μM produced a multipeak CV response in the forward (positive-direction) scan. This might be due to both the size of DAB-AM-32, resulting in a greater adsorption at the interface, and the higher net charge. Furthermore, differences between the forward and reverse peaks were observed: the forward sweep was more complex, with multipeaks, whereas the reverse sweep generally displayed a single peak. As with DAB-AM-4 and DAB-AM-16, the increase in concentration of DAB-AM-32 caused not only the peak current to increase but also the transfer peaks to shift to less positive potentials.

The voltammetric distortion observed from the experiments with DAB-AM-32 was magnified when the dendrimer generation was increased to generation 5.0 (DAB-AM-64), as shown in Figure 2B. Initially, the addition of increasing concentrations of DAB-AM-64 caused very distorted voltammograms, with enormous current spikes at very positive potentials. Subsequent cyclic voltammograms showed a more stabilized signal, although they were still characterized by a multipeak response. Figure 2B shows the complex voltammetric responses after stabilization, illustrating the CV peak increase with concentration. Similar multipeak and distorted voltammetric behavior was observed by Kakiuchi and co-workers⁷² in the study of ion transfer of relatively large anionic and cationic surfactants across the ITIES. They refer to this as the electrochemical instability of the liquid/liquid interface during the transfer and ascribe it to the potential-dependent adsorption and partition of an ionic surfactant between the two immiscible phases. Both processes are dependent on the

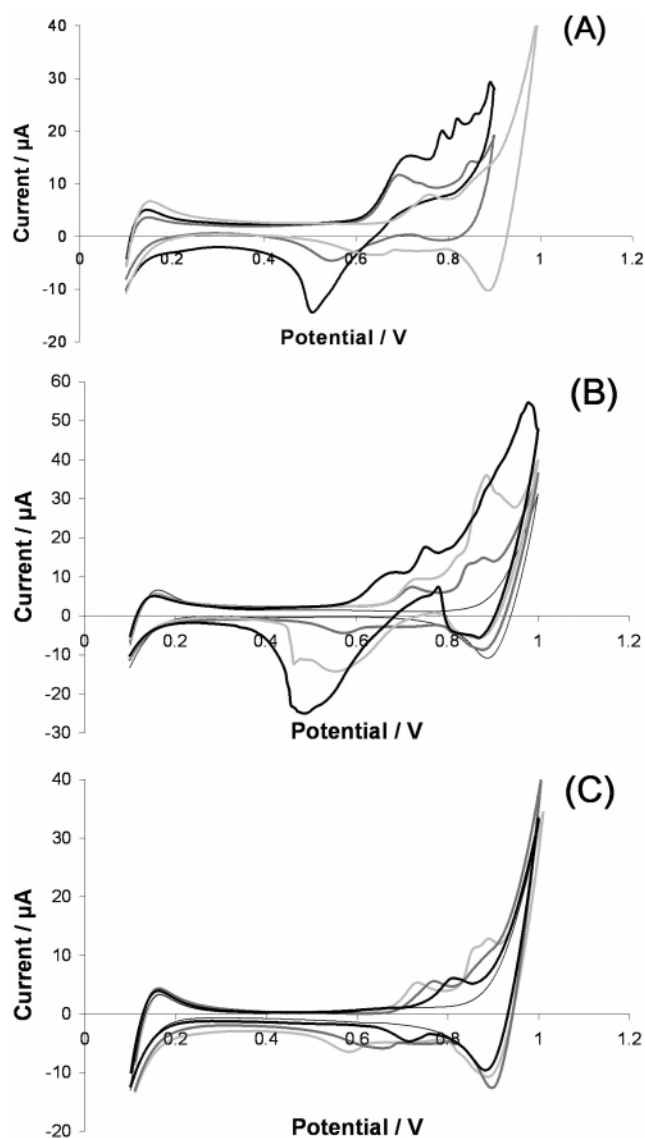


Figure 2. Electrochemistry of DAB-AM-*n* dendrimers at pH 5.5: (A) CV response of 2.5 μM (light gray), 10 μM (dark gray), and 12.5 μM (black) DAB-AM-32 dendrimer; (B) CV response of the blank (black thin line) and after additions of 2.5 μM (dark gray), 5 μM (light gray), and 7.5 μM (black) DAB-AM-64 dendrimer; (C) comparison among 2.5 μM DAB-AM-16 (black), 2.5 μM DAB-AM-32 (dark gray), and 2.5 μM DAB-AM-64 (light gray) (scan rate in all experiments 5 mV s^{-1} ; aqueous-phase electrolyte 10 mM LiCl).

interfacial potential. In addition, the adsorption of species produces a decrease in the interfacial tension, which may bring about a change in interfacial capacitance. In one paper,^{72d} very distorted voltammograms were reported, consisting of voltammograms with irregular pulslike current spikes in specific potential intervals. These chaotic currents are associated with Marangoni-type convection and emulsification at the interface. The current spikes are attributed to the ion transfer at the interface leading to formation of emulsions.⁷² Amemiya and co-workers⁵³ studied the voltammetry of protamines (positively charged polypeptides with molecular masses in the range 4000–4250 g mol^{-1}) at the water/nitrobenzene, water/1,2-dichloroethane, and water/1,6-

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dichlorohexane interfaces. The voltammograms showed forward (positive) prepeaks with a main wave due to the irreversible transfer of protamines. The dendrimer results presented here have some similarities with the transfer of protamines. We observed similar types of multipeak response at the high dendrimer generations (DAB-AM-16, DAB-AM-32, and DAB-AM-64).

Figure 2C compares the voltammetric response after interface stabilization of some DAB-AM-*n* dendrimers at the same concentration. The higher the generation number (from DAB-AM-4 to DAB-AM-64), the more complex the voltammetry. The transfer peaks shifted toward less positive potentials, meaning that with higher generations the transfer process became easier; i.e., DAB-AM-64 is the most hydrophobic as it has a much greater number and length of hydrocarbon chains, and the transfer peaks occur at lower potentials (lower energy required for ion transfer; see the light-gray cyclic voltammogram in Figure 2C). The second most hydrophobic is DAB-AM-32, and the transfer peaks occur at slightly more positive potentials than those of DAB-AM-64 (dark-gray cyclic voltammogram in Figure 2C). The voltammetric peaks for DAB-AM-16 (black cyclic voltammogram) appear at more positive potentials than DAB-AM-32, and finally, the transfer peaks using DAB-AM-4 are observed at higher potentials. The latter dendrimer is not shown in the comparison in Figure 2C as it cannot be detected at such low concentrations. Cyclic voltammograms of DAB-AM-4 are not compared to the other DAB-AM-*n* dendrimers at the same concentration as higher concentrations of the higher generations lead to very distorted voltammograms (not shown here). The peak–peak separations for the dendrimers in Figure 2C are 100, 120, and 150 mV for DAB-AM-16, DAB-AM-32, and DAB-AM-64, respectively.

Reversible electrochemistry means that diffusion-controlled reactions occur in accordance with the Nernst equation. Under such circumstances, the peak–peak separation (difference between forward and reverse peak potentials) is $59/z_i$ mV, and the peak currents depend on the concentration and square root of the voltammetric sweep rate, as described by the Randles–Sevcik equation

$$i_p = (2.69 \times 10^5) z_i^{3/2} A D^{1/2} C \nu^{1/2} \quad (1)$$

where z_i is the net charge of the species, A the interface area (cm^2), D the diffusion coefficient of the species studied, in the aqueous phase in this case ($\text{cm}^2 \text{s}^{-1}$), C the concentration of the species (mol cm^{-3}), and ν the voltammetric scan rate (V s^{-1}). From Figures 1 and 2, it is clear that the DAB-AM-*n* dendrimers do not obey the conditions for electrochemical reversibility, as the peak–peak separations are too large (i.e., $> 59/z_i$ mV). The transfer processes may therefore be said to be irreversible or quasi-reversible, with some adsorption occurring before transfer. The charge number (z_i) of the transferring species can be evaluated from the voltammetric data by use of the convolution technique.⁷³ Background-subtracted cyclic voltammograms of the DAB-AM-*n* dendrimers were used to obtain plots of the semi-integral of the current response against the applied potential.^{73d} As shown by Amemiya for ClO_4^- transfer,^{73d} the curves for the resulting forward and reverse scans were sigmoidal. In our experiments, the TEA^+ (reference ions) charge was calculated to confirm the suitability of the convolution technique to calculate the dendrimer charge, using the following equation:^{73d}

$$I_L = z_i F A D^{1/2} C \quad (2)$$

where I_L is the limiting value of the semi-integral and the other parameters have their previous definitions. The values of the diffusion coefficients of the DAB-AM-*n* dendrimers were calculated from the Stokes–Einstein equation^{74,75}

$$D = k_B T / 6\pi\eta R_h \quad (3)$$

where k_B is the Boltzmann constant, T the temperature, η the viscosity (which was taken as the viscosity of water, 0.89 cP, since the viscosity of 10 mM LiCl was measured to be similar to that of water), and R_h the hydrodynamic radius. The viscosity was taken as that of water since the final concentration of dendrimer (and methanol) in the ITIES aqueous phase is too low to change the liquid viscosity. The hydrodynamic radius of the DAB-AM-*n* dendrimers was obtained from published data, where these values were determined from small-angle neutron scattering (SANS), viscosimetry, and molecular dynamics calculations.^{76–78}

From eq 2, the charge calculated for TEA^+ was +1. The charge of the DAB-AM-*n* dendrimers was then calculated using this technique from the slopes obtained from the I_L versus C calibration curves, and the values obtained were very similar to the theoretical charges (Table 1). The agreement between the convolution values and the theoretical values suggests that the dendrimer transfer is irreversible.

The peak current versus concentration calibration curves of DAB-AM-4, DAB-AM-16, and DAB-AM-32 were linear over a wide concentration range (from 60 to 400 μM , from 2.5 to 70 μM , and from 2.5 to 15 μM for DAB-AM-4, DAB-AM-16, and DAB-AM-32, respectively). Conversely, the calibration curves for DAB-AM-64 did not extend over such a wide range, being restricted to 2.5–7.5 μM . This is probably a result of its complicated voltammetry described above. In addition, the organic phase became cloudy with increasing concentration of dendrimer. This reaction (the increase of organic-phase turbidity) started at the interface and progressed into the bulk organic phase until the entire organic phase volume was cloudy.

Electrochemistry at pH 1.0. At very acidic pH values (pH 1.0), all the amino sites in the DAB-AM-*n* dendrimers are protonated. The increase of the dendrimer net charge, using these acidic conditions, should lead to an increase of the hydrophilicity of the dendrimer.

The use of very acidic aqueous-phase conditions (0.1 M HCl) led to a shortening of the available potential window; i.e., the positive potential window (or right-hand-side potential window) was decreased from ca. 0.85–0.9 V to potentials under 0.7 V. This was due to the commencement of H^+ transfer at ca. 0.7 V. In these conditions, no dendrimer transfer was observed within the available potential window. Thus, DAB-AM-*n* dendrimers cannot be detected within the potential window under these experimental conditions. In these studies, both DAB-AM-4 and DAB-AM-64 (the extreme generations of DAB-AM-*n* available) were investigated. No significant change in the CV response could be observed at least below ca. 0.45 mM, which was the highest concentration investigated.

Electrochemistry of PAMAM Dendrimers. The PAMAM dendrimers examined are summarized in Table 1. As for the

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DAB-AM-*n* dendrimers, the molecular charge of the PAMAM dendrimers is a function of the experimental pH. The approximate charges of these dendrimers were obtained from published data based on potentiometric (acid–base) titrations⁶² and are summarized in Table 1. Protonation of PAMAM dendrimers first involves the protonation of primary amino surface groups at the outer rim of the dendrimer, at high pH, while the tertiary amino groups in the interior are protonated at lower pH. The last group to be protonated at low pH is the central tertiary amine.⁶²

Electrochemistry at pH 5.5. The cyclic voltammogram of PAMAM dendrimers was characterized by sharper peaks with smaller peak–peak separations than those observed for the DAB-AM-*n* dendrimers. The peak–peak separations for PAMAM were generally lower than the 59 mV which corresponds to the reversible transfer of singly charged species. Peak–peak separations of less than $59/z_i$ mV imply the transfer of multiply charged species. Figure 3A shows the voltammetric behavior and resultant calibration curve for PAMAM-0. The voltammograms also show the response to TEA⁺, which was used as a reference ion (peak at 0.55 V). The peak–peak separation for PAMAM-0 was ca. 30 mV for the lower concentrations studied, which suggests a net charge $z_i = 2$ for the transferring species. The theoretical value is $z_i = 5$. In contrast with DAB-AM-*n* dendrimers, the addition of relatively high concentrations of PAMAM dendrimers did not cause a shift of the transfer peaks toward less positive potentials.

Figure 3B shows the voltammetric response of PAMAM-1.0, in which sharp peaks were obtained. The peak–peak separation was ca. 28 mV (note that the TEA⁺ peak–peak separation was ca. 60 mV in the same cell). Such a low peak separation implies that $z_i = 2$. Increasing concentrations of dendrimer led to a similar response, but the forward peak became less sharp as the concentration of PAMAM-1.0 increased, as in Figure 3C, and the reverse TEA⁺ peak (back-transfer of TEA⁺ into the aqueous phase) shifted to a potential more positive than the forward peak potential. The voltammetric response of PAMAM-2.0 (not shown) was similar to that shown for PAMAM-1.0 (Figure 3B,C). The forward peak became less sharp as the concentration of dendrimer was increased, and the reverse TEA⁺ peak again shifted to a potential more positive than the forward peak potential. The voltammetric response observed for PAMAM-1.0 and PAMAM-2.0 is very similar to that presented by Osakai and co-workers⁷⁹ in the electrochemical extraction of proteins by reverse micelle formation using the anionic surfactant bis(2-ethylhexyl) sulfosuccinate (AOT). In the absence of AOT in the organic phase, cytochrome *c* (Cyt *c*) produced a voltammetric response very similar to that obtained from these high-generation dendrimers. However, they suggested⁷⁹ that the forward current was not due to Cyt *c* transfer but due to the transfer of the organic-phase supporting electrolyte anion (tetraphenylborate, TPB[−]) into the aqueous phase. The interfacial adsorption of Cyt *c* from the aqueous bulk would facilitate the transfer of TPB[−] from the organic phase into the aqueous phase. The reverse current (sharp bell-shaped peaks) was explained in terms of desorption (or dissociation) of TPB[−] from Cyt *c* and the concomitant transfer back into the organic phase.⁷⁹ By analogy, in our studies, the voltammetric response could be due to the transfer of TPBCl[−] from the organic phase into the aqueous phase, facilitated by the dendrimer in the aqueous phase (forward peaks), and the back-transfer to the aqueous phase (sharp reverse peaks).

Figure 4 shows the electrochemical response of PAMAM-3.0 at pH 5.5 as a function of the concentration. The peak–peak

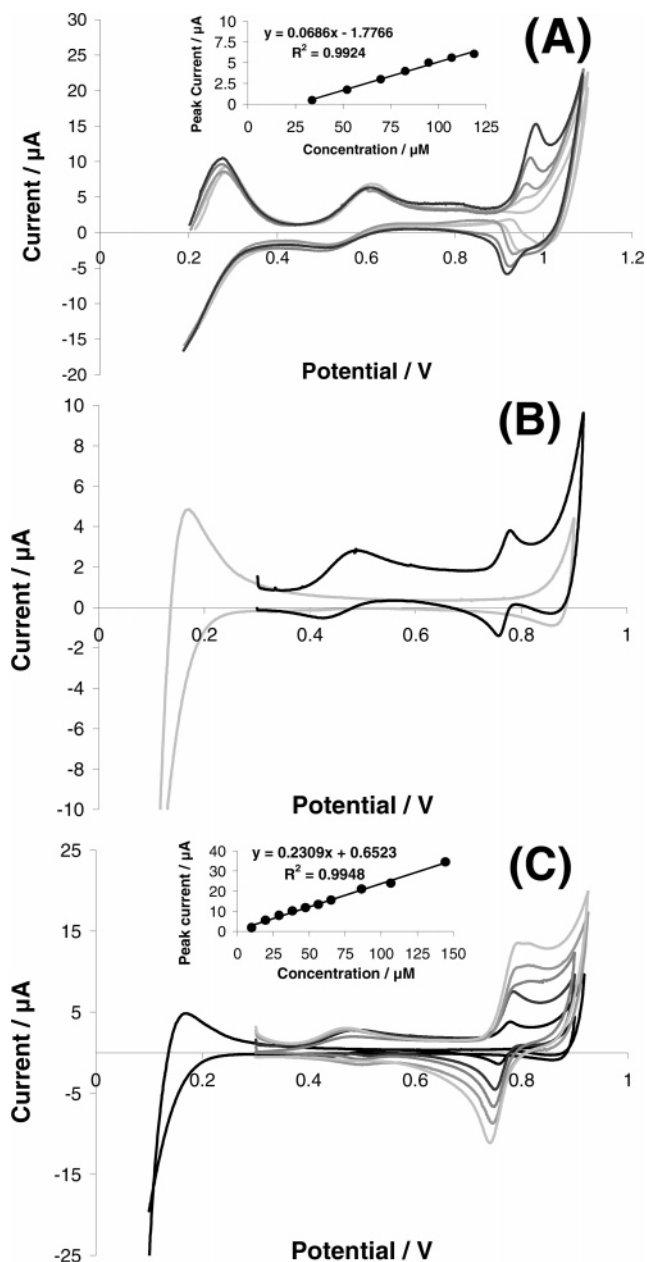


Figure 3. (A) Transfer of PAMAM-0 as a function of the concentration from 15 μM (light gray) to 107 μM (black): 15, 35, 52, 83, and 107 μM . The concentration of TEA⁺ in all experiments (except the blank) was 0.15 mM. (B) Electrochemistry of PAMAM-1.0: CV response of the blank (gray) and of 0.05 mM TEA⁺ and 10 μM PAMAM-1.0 (black). (C) CV response of the blank (black) and of 0.05 mM TEA⁺ and 10 (black), 20, 29, 38, and 47 (light gray) μM PAMAM-1.0. The scan rate in all experiments was 5 mV s^{-1} . The aqueous-phase electrolyte was 10 mM LiCl. The inset figures show the calibration curves.

separation at the lowest concentration studied is ca. 22 mV (at 3.5 μM), consistent with the transfer of a multiply charged species, as seen for PAMAM 1.0 and 2.0. Therefore, on the basis of the apparent reversible transfer of PAMAM, the charges of the transferring species were determined by the Randles–Sevcik equation (eq 1) using diffusion coefficients calculated using the Stokes–Einstein equation (eq 3) with dendrimer radii obtained from diffusion NMR measurements.⁷⁶ The net transferring charges are shown in Table 1.

It can be seen that there is quite a difference between the CV-calculated and the theoretical charges, suggesting that the apparent reversible transfer process is invalid. Therefore, the

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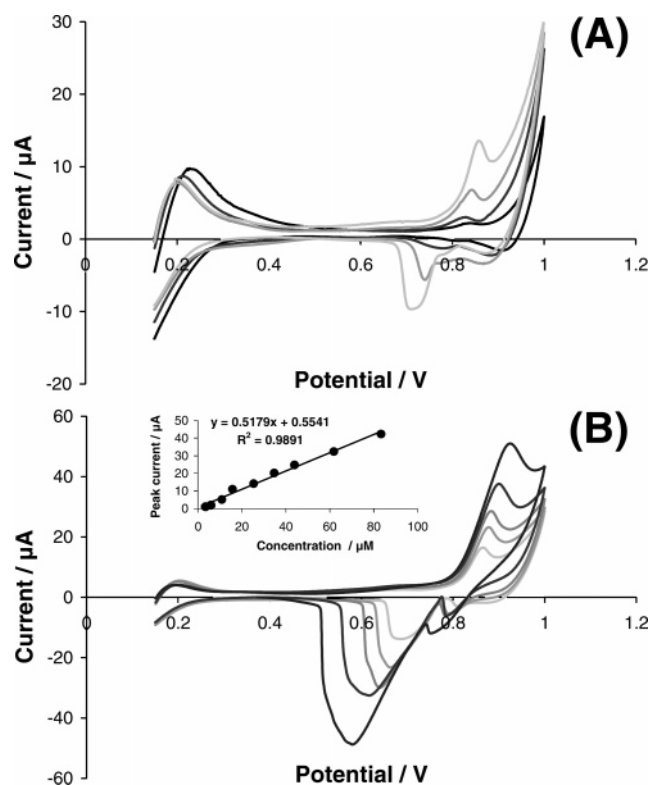


Figure 4. Electrochemistry of PAMAM-3.0 in 10 mM LiCl: (A) from black to light-gray cyclic voltammogram, 3.5, 6, 11, and 16 μM PAMAM-3.0; (B) from light-gray to black cyclic voltammogram, 25, 35, 44, 62, and 83 μM PAMAM-3.0. (scan rate 5 mV s^{-1}). The inset shows the calibration curve.

convolution technique was also applied to the PAMAM voltammetric data. Table 1 summarizes the values calculated from the convolution technique. The charge values obtained for the convolution analysis were closer to theory than those obtained from application of the Randles–Sevcik equation (CV), but there is still a discrepancy. The only exception seems to be PAMAM-0, where the CV and the convolution charge values are the same (Table 1). For reversible voltammetry, agreement between data from the Randles–Sevcik equation and the convolution technique is expected. It can therefore be concluded that the PAMAM dendrimers undergo an irreversible transfer at the ITIES. Lack of agreement between the convolution method charge and theory suggests that something other than simple ion transfer is taking place.

As found for the DAB-AM-*n* family, the calibration curves for the PAMAM dendrimers were linear over the following concentration ranges: 35–120 μM for PAMAM-0, 10–145 μM for PAMAM-1.0, 5–34 μM for PAMAM-2.0, and 3–83 μM for PAMAM-3.0. A general observation was that the organic phase became cloudy with increasing concentration of dendrimer in the aqueous phase, as was the case for DAB-AM-*n*.

Electrochemistry at pH 1.0. At pH 1.0 (0.1 M HCl aqueous phase), all the amino groups in the PAMAM dendrimers are protonated, which may increase the hydrophilicity of the dendrimers. In contrast with the DAB-AM-*n* family, all the PAMAM dendrimers showed an electrochemical response at this pH. The CV response of these dendrimers was quite different across the different generations. The lowest generation (PAMAM-0) produced a response similar to that shown for DAB-AM-*n* dendrimers using 0.1 M HCl as the aqueous phase. However, although the potential window limit overlaps greatly with the PAMAM-0 transfer peak, the dendrimer transfer peaks can be detected (Figure 5A). The CV response of PAMAM-1.0 was

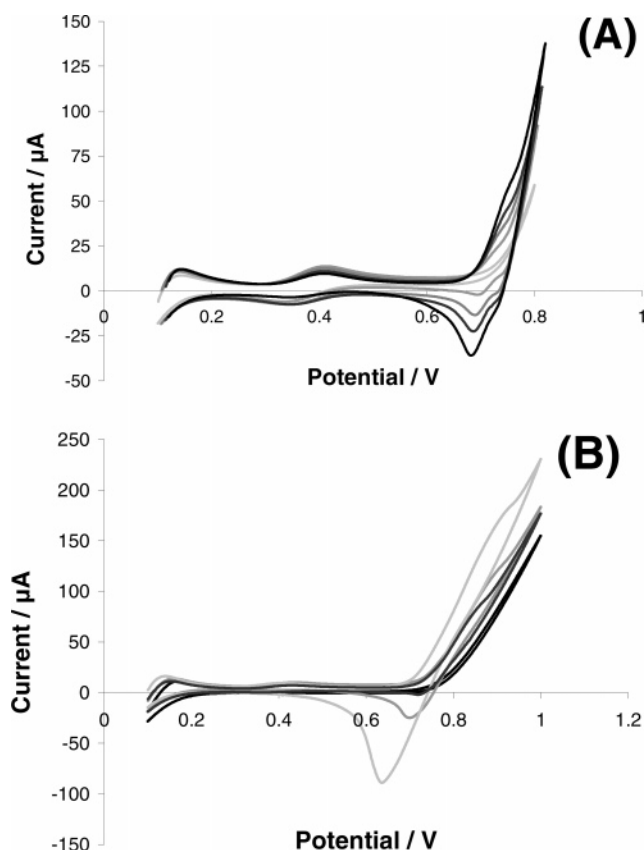


Figure 5. Electrochemistry of PAMAM dendrimers using a 0.1 M HCl aqueous phase: (A) from light gray to black, 0, 28, 96, 202, and 331 μM PAMAM-0 (the concentration of TEA^+ was ca. 0.3 mM); (B) 102 (black), 176 (gray), and 338 (light gray) μM PAMAM-1.0 (the concentration of TEA^+ was ca. 0.05 mM). The scan rate in all experiments was 5 mV s^{-1} .

quite similar to that of PAMAM-0 (Figure 5B). As in PAMAM-0, the forward peak overlapped with the positive limit of the potential window.

The electrochemistry of the higher generations of PAMAM, i.e., PAMAM-2.0 and PAMAM-3.0, was more complicated than that of the lower generations, as shown in Figure 6. The addition of increasing concentrations of dendrimer leads to an increased current in the forward sweep, although no peak was observed. In the reverse sweep, a peak was observed, due to a back-transfer process. The magnitude of this peak depended on the concentration of dendrimer present in the aqueous phase. The higher generations needed extension of the CV scan to higher potentials to generate a peak response on the forward half-cycle (Figure 6). This behavior appears to exclude organic-phase electrolyte transfer as a possible explanation of the observed voltammetry: the higher generations have greater charges and thus should in principle facilitate the transfer of more organic-phase electrolyte, not less (or none).

Discussion. These studies have shown that ionized dendrimers are electrochemically active at electrified liquid–liquid interfaces and that a new method for the redox-label-free electrochemical detection of low concentrations of dendrimers is available. The electrochemistry of the dendrimers varied with the pH and dendrimer family and generation. At pH 5.5, all DAB-AM-*n* and PAMAM dendrimers led to voltammetric ion transfer peaks. Table 2 summarizes the midpeak transfer potentials of all the dendrimers investigated, calculated after correction of the potentials of TEA^+ and the dendrimers versus the that of standard TEA^+ transfer in the water/1,2-dichloroethane system ($\Delta\phi^{\text{w}} = 0.044 \text{ V}$).⁷⁰

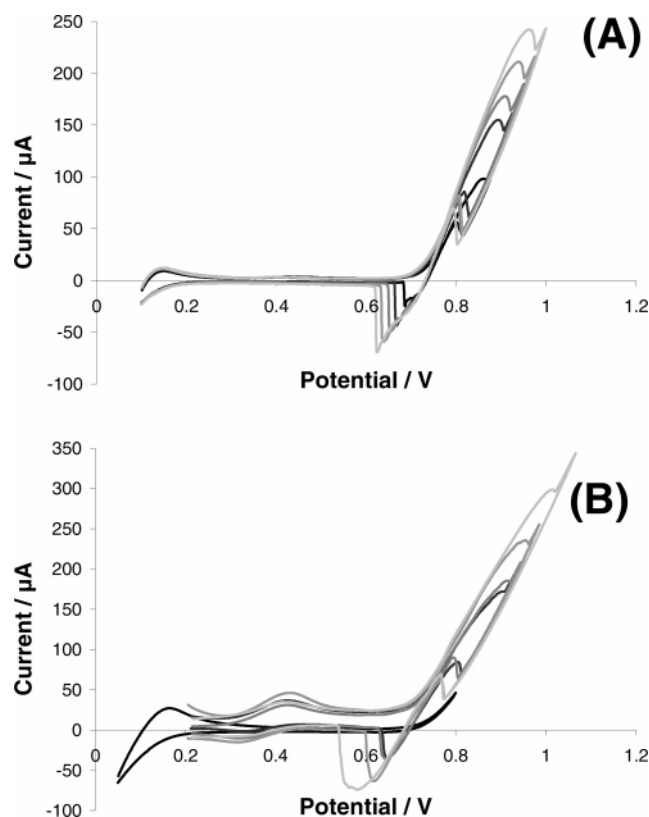


Figure 6. Electrochemistry of PAMAM dendrimers using a 0.1 M HCl aqueous phase: (A) from black to light gray, 34, 43, 52, 61, and 70 μM PAMAM-2.0 (the concentration of TEA^+ was ca. 0.05 mM); (B) CV response of the blank (black) and of PAMAM-3.0 from 9 μM (black) to 75 μM (light gray), 9, 11, 36, and 75 μM PAMAM-3.0 (the concentration of TEA^+ (except in the blank) was ca. 0.74 mM). The scan rate in all experiments was 5 mV s^{-1} .

The main points from the electrochemical analysis of dendrimers at the liquid–liquid interface are the following:

(1) For DAB-AM-*n* dendrimers, the peak–peak potential separations were greater than 59/ z_i mV, meaning that the Randles–Sevcik equation was not applicable for determination of the transferring species' charge. Therefore, the convolution technique was used. The charge values for these dendrimers obtained from the convolution technique were close to (but not identical to) the theoretical charge values.

(2) For PAMAM dendrimers, the CV experiments produced peak–peak potential separations of less than 59 mV, suggesting the transfer of multiply charged species and thus that application of the Randles–Sevcik equation was justified. However, it was found that charge values obtained from application of this equation and the convolution technique were significantly lower than the theoretical charge values.

(3) The voltammetric behaviors of both families are different, but some similarities exist: there is an increasing complexity of the voltammogram as the generation number increases, and the ability to detect low concentrations by a simple technique such as CV is possible. The degree of agreement between convolution-derived charge values and the theoretical ones was better for the DAB-AM-*n* family of dendrimers than for the PAMAM family; the transfer peaks for DAB-AM-*n* shifted to lower potentials with increasing concentration and with an increase in the dendrimer generation, but not those for PAMAM. PAMAM exhibited some electrochemistry at pH 1, but DAB-AM-*n* did not. These differences may be a reflection of the different physical structures of the two dendrimer families:

Table 2. Midpoint Transfer Potential ($\Delta_o^w\phi$)^a Calculated for All the DAB-AM-*n* and PAMAM Dendrimers^b

dendrimer	$\Delta_o^w\phi/\text{mV}$	dendrimer	$\Delta_o^w\phi/\text{mV}$
DAB-AM-4	467	PAMAM-0	439
DAB-AM-16	371	PAMAM-1.0	356
DAB-AM-32	347	PAMAM-2.0	343
DAB-AM-64	337	PAMAM-3	338

^a Midpoint transfer potential $\Delta_o^w\phi = (E_{p,f} - E_{p,r})/2$. ^b Data based on CV measurements, corrected for the formal TEA^+ transfer potential in the water/1,2-dichloroethane system ($\Delta_o^w\phi^\circ = 0.044 \text{ V}$).

Table 3. Lowest Concentrations Detected from CV Experiments Using 10 mM LiCl as the Aqueous Phase Electrolyte (pH 5.5)^a

dendrimer	lowest concn detected/ μM	sensitivity/ mA M^{-1}	dendrimer	lowest concn detected/ μM	sensitivity/ mA M^{-1}
DAB-AM-4	10	86	PAMAM-0	35	69
DAB-AM-16	2.5	791	PAMAM-1.0	10	231
DAB-AM-32	2.5	825	PAMAM-2.0	5	545
DAB-AM-64	2.5	828	PAMAM-3.0	3	518

^a Sensitivities (from CV calibration curves) included.

DAB-AM-*n* are more linear and open, and PAMAM are more globular.

(4) The transfer may be either that of the dendrimers themselves or dendrimer-facilitated organic-phase electrolyte anion transfer. If the former, then the transferring charge being lower than the theoretical charge is due to condensation of aqueous-phase electrolyte anion (Cl^-) around the dendrimer positive charges, so that the net charge transferred is less.

Table 1 summarizes the theoretical net charge of all these dendrimers at pH 5.5. A possible reason for the deviation of the convolution charge values from the theory may be the partial neutralization of their charge by condensation with aqueous-phase electrolyte anions, i.e., the presence of aqueous-phase electrolyte anions (Cl^-) condensing around the protonated amine moieties of the dendrimer molecule. Such electrolyte anions may transfer with the dendrimer, thus reducing the net charge (scenario 1). Another possible reason for the voltammetric transfer response might be the facilitated transfer of the organic-phase electrolyte anions (TPBCl^-) by the dendrimers present in the aqueous phase, in a way similar to that described by Osakai and co-workers⁷⁹ in the transfer of the organic-phase electrolyte anions facilitated by cytochrome *c* present in the aqueous phase (scenario 2). In the latter situation, the dendrimers would not transfer across the interface, but they would act as ionophores for TPBCl^- .

Of scenarios 1 and 2, both would show a dependence of the detection limit and calibration graph slope on the dendrimer size, as was observed (Table 3). However, by lowering the pH, an increase in the current and sensitivity (slope) should also be observed (a pH decrease would imply an increase of the dendrimer charge, facilitating the transfer of more TPBCl^- ions), which was not the case. Thus, our results exclude scenario 2 on the basis that higher generations of PAMAM (more highly charged) need higher applied potential differences to generate a transfer signal (at pH 1). Also, at pH 1, no transfer process for the DAB-AM-*n* dendrimers was observed, again inconsistent with organic-phase electrolyte anion transfer facilitated by the aqueous-phase dendrimer (highly charged at pH 1). Furthermore, with both families of generations, it was found that initiation of voltammetric experiments led to cloudiness at the interface which progressed gradually into the organic phase, signifying that some reactions

leading to insoluble products were occurring there and not in the aqueous phase. These observations led us to suggest that the mechanism of transfer is transfer of the dendrimer into the organic phase, with adsorption and interface destabilization occurring as the dendrimer generation increases. Furthermore, comparisons between the DAB-AM-*n* and the PAMAM families show that differences between the theoretical and the convolution charges are greater for the PAMAM family. This may be due to the different physical structures of the dendrimer families: the DAB-AM-*n* family is a more linear family of dendrimers, whereas the PAMAM dendrimers are more globular. Thus, the PAMAM family may be better able to trap electrolyte anions from the aqueous phase within their structures during transfer across the ITIES, leading to the disagreement between determined and theoretical charges.

We note some similarity to the study here of dendrimer transfer into a lipid bilayer,⁸⁰ whereby partial neutralization of the dendrimer charge makes it less polar and more susceptible to move into the organic phase.

Finally, Table 3 shows the lowest concentrations detected in these CV experiments using 10 mM LiCl as the aqueous-phase electrolyte (pH 5.5) and the analytical sensitivities attained for all these dendrimers. First of all, it can be seen that CV enables detection of relatively low concentrations of these multiply charged species quite easily, in contrast to the extreme difficulty encountered in achieving similar detection capability for a singly charged ion such as dopamine.⁸¹ The lowest concentrations

detectable decrease and the analytical sensitivities (slope of the calibration graph) increase with dendrimer size, implying that the detection of multiply charged species is a preferable application area for electrochemistry at the ITIES. Similar analytical trends were reported from Meyerhoff's potentiometric investigations.⁴⁵

In conclusion, it can be stated that dendrimers containing no redox-active functionality may be subjected to voltammetry at the liquid–liquid interface. This voltammetric behavior can be attributed to the presence of amine groups, which are protonated in aqueous solution at sufficient pH and enable the transfer of the dendrimer across the interface. The electrochemistry at the ITIES of these materials is based on their transfer from the aqueous phase into the organic phase, and the voltammetric behavior becomes increasingly complex with an increase in the generation number. The charge values determined for the transferring species depend on the dendrimer family, but indicate that some of the dendrimer charge is neutralized by electrolyte anions from the aqueous phase. The electrochemistry of these dendrimers at the ITIES can be used as model systems for ionized macromolecules; further examination of the mechanism of transfer is under way. They can be detected down to micromolar concentrations without difficulty, indicating the great analytical possibility of this approach. Further studies on the behavior of these dendrimers are under way, including assessment of charge-transfer kinetics at the ITIES.

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