Transport of Ions and Molecules in Biopolymeric Gels: Electroanalytical Studies

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Transport of ions and molecules was studied in hydrogels of uncharged and anionic biopolymers, agarose, and ι-carrageenan. Diffusion coefficients of neutral and ionic electroactive probes, the uncharged radical 2,2,6,6-tetramethyl-1-pipperidinyloxy, TEMPO, and Tl^+, were determined rapidly and precisely from steady-state, transport-limited currents at platinum and mercury microelectrodes. Transport properties of both probes were studied in gels as affected by temperature, ionic strength, and concentration of the biopolymer, and they were compared with those in solutions. The relation between macroscopic and local microscopic viscosity of biopolymeric gels was analyzed based on viscosity measurements and diffusion coefficient data.

Introduction

Transport of ions and molecules in polymeric gels is of interest for the description of many biological and synthetic systems, such as cellular membranes, biological matrixes, ion-exchangers, new materials for power sources, and gel-based sensors. The widely accepted topological definition of a gel is a three-dimensional network constituted of basic elements connected in some way and swollen by a solvent. As a rule, only systems wherein the solvent is the major component are considered to be gels. A gel possesses the unique property of incorporating and retaining a proportion of liquid molecules outweighing by far the proportion of the basic, added component. In some cases, a gel can contain up to 99% solvent. This is a rather unusual way in which large amounts of liquid can be maintained "solid", and therefore, gels possess many advantages characteristic of both the liquid and solid states of matter. The list of substances that can form gel networks includes biopolymers, synthetic polymers, and many inorganic compounds. In this work, we focus on gel systems based on biopolymeric networks. Synthesis, physical properties, and applications of polymeric gels have been reviewed by several authors,[1−5] and the potential usefulness of polymer gels for drug delivery systems,[6,7] selective sorbents,[8,9] and such electrochemical devices as batteries and sensors[10−16] is well recognized and seems to be very promising, thus justifying more detailed studies of their properties.

An important group of biological polymers forming gels are polysaccharides; those chosen for these studies are shown in Figure 1. Agarose is an uncharged polysaccharide, while ι-car, ι-carrageenan, is a charged sulfated polysaccharide with ideally two charges per repeating (disaccharide) unit.[17,18] They both are used as stabilizers, binders, and gelling agents in the food and cosmetic industry because of their thickening and gel-forming properties, and agarose is used widely in separation techniques. There are few reports on the transport of ions and molecules in biopolymeric gels. The reported values of the diffusion coefficients of small molecules and ions in these materials differ significantly from the identity in solutions and gels,[19,20] to differences up to 50%.[21]

Diffusion of simple ions and molecules in polymeric solutions has been studied using radioactive tracer techniques[22] and NMR spectroscopy.[23−26] Recently steady-state voltammetry at microelectrodes has been successfully employed for diffusion measurements in polyelectrolyte solutions[27−34] and colloidal systems.[35,36]

In our studies, the diffusion coefficient of electroactive probes, D, is determined from the diffusion-limited steady-state current, i_d, at disk microelectrodes[37]

$$i_d = 4nFCDr \tag{1}$$

where C is the concentration of electroactive ion, r is the radius of the microelectrode, n is the number of electrons transferred, and F is the Faraday constant. Electrodes of small size (diameter in the range of several micrometers) yield steady-state voltammograms on time scales of seconds and make measurements possible in resistive media, such as solutions with low or no supporting electrolyte. In addition, the steady-state current at microelectrodes is directly proportional to the flux of reactant. Thus, the voltammetric signal is very sensitive to changes in the value of the diffusion coefficient. This method has advantages of simplicity, low cost, and very high throughput in comparison with NMR spectroscopy or use of a radioactive tracer.

We report here on the transport of the uncharged electroactive radical 2,2,6,6-tetramethyl-1-pipperidinyloxy, TEMPO, and the monovalent cation, Tl^+, in solutions and gels of the uncharged and anionic biopolymers, agarose and ι-car, respectively. The aim of this work is to develop precise methodology for the determination of diffusion coefficients of electroactive probes in biopolymeric gels, to determine diffusion coefficients of neutral and ionic probes in these biopolymeric gels, as affected by temperature, ionic strength, and biopolymer concentration, and to analyze the influence of macroscopic viscosity of biogels on transport properties of molecules and ions in these systems.

Experimental Section

Chemicals and Solutions. Agarose was purchased from Fluka (gelling temperature 29−33 °C, % sulfate < 0.1%) and

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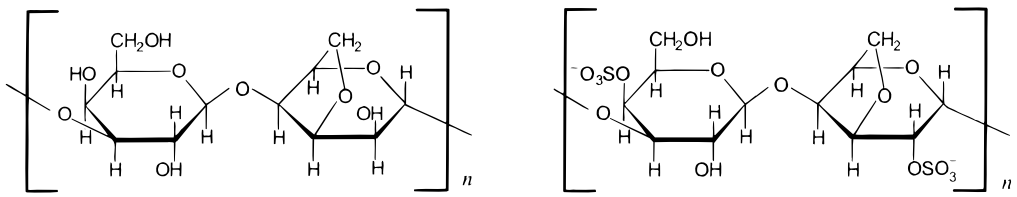

Figure 1. Repeating monomer units of agarose and ι-carrageenan, ι-car.

was used as received. ι-carrageenan, ι-car, sodium salt was purchased from Fluka. The samples of ι-car were dialyzed (Spectra/Por 3, MWCO 3500) against ultrapure water (Milli-Q, Millipore Corporation) for 5 days at 5 °C. The water was changed approximately 5 times per day. Final solutions were passed through 5.0 μm filters (Whatman) and lypholized under vacuum at the temperature of liquid nitrogen. Purified compounds were stored at 5 °C to inhibit bacterial growth. All other reagents were of reagent grade purity and were used as received. Ultrapure water (MilliQ, Millipore) was used in all rinses and preparations of solutions.

Gel Preparation. Agarose was dissolved in deionized water at 50 °C; then, the solution was cooled to 35 °C and the appropriate amount of TEMPO or $TlNO_3$ was added. Also, if required, an appropriate amount of $NaNO_3$ was added at 35 °C. Agarose solutions were gelled at 5 °C for 2 h. ι-Carrageenan was dissolved in water at 25 °C, and the appropriate amount of TEMPO or $TlNO_3$ was added. The gelation process of ι-car was initiated by addition of an appropriate amount of $LiClO_4$. The minimum concentration of $LiClO_4$ for which gelation was observed was 0.02 M.

Voltammetry. Electrochemical measurements were carried out with a three-electrode system in a jacketed glass cell enclosed in an aluminum Faraday cage. The temperature of solutions was controlled using a refrigerated circulator (Isotemp model 1016P, Fisher). Staircase voltammetry was applied with a model 283 potentiostat (EG&G PARC) controlled via a PC computer. Staircase voltammetry parameters were as follows: step height (ΔE) 5 mV, frequency (f) 1 Hz. Under these conditions, the limiting current for thallium cation reduction at disk electrodes of 15 μm radius does not exceed the steady-state value by more than 3%.[38]

The working microelectrodes were 5 μm radius platinum disk (Project Ltd., Warsaw, Poland) and mercury film (silver-amalgam-based) disk microelectrodes of 15 μm radius. The method of the preparation of those electrodes has been described in detail.[27,28,39] Optical inspection of the state of the electrode surface was accomplished with an inverted microscope for reflected light (Nikon model Epiphot-200).

A platinum quasireference electrode or a saturated calomel electrode, SCE, was used as a reference electrode. The stability of the Pt quasireference electrodes in aqueous solutions for the potential range of Tl^+ reduction has been described previously.[28] The counter electrode was a platinum wire. All solutions were deoxygenated before voltammetric scans and blanketed with temperature-controlled water-saturated argon.

Viscosity. Viscosity measurements were performed using Ostwald viscometers placed in a temperature controlled jacketed glass cell. Viscometers were calibrated using deionized water at 1, 25, and 40 °C.[40]

Results and Discussion

Agarose Gels. Figure 2 presents steady-state voltammograms for the oxidation of TEMPO at a platinum disk microelectrode

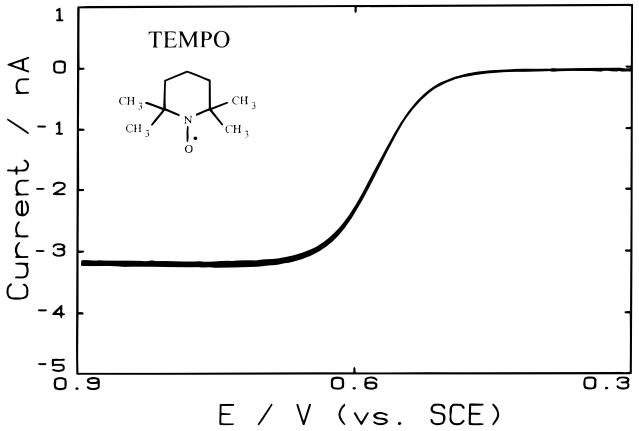

Figure 2. Steady-state voltammograms (11 curves) of oxidation of 3 mM TEMPO free radical in 1% agarose gel without supporting electrolyte: Pt disk microelectrode, $r = 5$ μm, 25 °C.

in 1% agarose gel with 0.1 $NaNO_3$. As one can see, steady-state waves of TEMPO oxidation are very well defined. The reproducibility of limiting currents is excellent, with relative standard deviation, rsd, of 0.6% calculated for 15 voltammograms. Steady-state voltammograms of TEMPO oxidation look almost identical in 1% agarose gel without electrolyte, in equivalent solution of $NaNO_3$ without agarose, and in pure water without agarose. However, the rsd was higher in solutions without agarose, 1.5% and 2.0% in 0.1 $NaNO_3$ solution and water, respectively. This difference in rsd values illustrates how the solidlike structure of agarose gel protects the system against any distortions by convection. As expected for uncharged TEMPO, migrational contribution to limiting currents was not observed in gels or solutions without supporting electrolyte. Diffusion coefficient values for TEMPO at 25 °C, calculated from steady-state currents according to eq 1, are 6.2×10^{-6}, 6.1×10^{-6}, 6.3×10^{-6}, and 6.2×10^{-6} cm^2/s for agarose gel without electrolyte, agarose gel with 0.1 M $NaNO_3$, water, and 0.1 M $NaNO_3$ solution, respectively. Although the macroscopically observed viscosity of the agarose gel is several orders of magnitude larger than that of the solution without agarose, the values of the diffusion coefficient of TEMPO are the same within 1.6%, i.e., within experimental error.

We also studied the dependence of the diffusion coefficient of TEMPO on the concentration of agarose in the gel. Results for five concentrations of agarose gels are presented in Table 1, and they are compared with data reported for metal ions in agarose gels. As one can see, for low concentrations of agarose in the gel, not higher than 1.5%, the diffusion coefficient of TEMPO remains constant within 7%. It decreases for higher concentrations of agarose. The comparison of the ratio of the diffusion coefficient in the gel to that in the solution, $D_{gel}/D_{solution}$, for TEMPO with data reported for Pb(II) and Cd(II)[21] shows that in 1.5% agarose gel our results are significantly higher than those for Pb(II) and Cd(II); however, our results for 3% agarose gel agree very well with those reported for metal

TABLE 1: Comparison of Diffusion Coefficient of TEMPO for Various Concentrations of Agarose in the Gel[a]

concentration of agarose (%)	D_{gel} (TEMPO) cm^2/s	$D_{gel}/D_{solution}$[b] this work	$D_{gel}/D_{solution}$ (from ref 21)
0.5	6.1×10^{-6}	0.97	
1.0	6.2×10^{-6}	0.98	
1.5	5.8×10^{-6}	0.92	0.56 (Pb(II)), 0.50 (Cd(II))
2.0	3.9×10^{-6}	0.62	
3.0	2.6×10^{-6}	0.41	0.36 (Pb(II)), 0.39 (Cd(II))

[a] 3 mM TEMPO, agarose gel without electrolyte, Pt disk, $r = 5$ μm, 25 °C. [b] $D_{solution} = 6.3 \times 10^{-6}$ cm^2/s, TEMPO in water.

TABLE 2: Dependence of Limiting Currents for Oxidation of TEMPO in 1% Agarose Gel on Radius of Pt Disk Microelectrodes[a]

r (μm)	i_d (nA)[b] voltammetry	i (nA)[c] chronoamperometry	i_d/r (nA/μm)	D_{TEMPO} (cm^2/s)[d]
5.5	7.1	6.9	1.29	6.1×10^{-6}
10	14.0	13.7	1.40	6.6×10^{-6}
26	34.6	34.9	1.33	6.3×10^{-6}

[a] 5.5 mM TEMPO, 1% agarose gel with 0.05 M $LiClO_4$, 25 °C. [b] Plateau current, staircase voltammetry, $\Delta E = 2$ mV, $f = 2$ Hz. [c] Measured after 100 s at +0.85 V. [d] Calculated from eq 1 from voltammetric current.

ions. This dependence of the diffusion coefficient of TEMPO on the concentration of agarose in the gel illustrates the influence of the structure of the polymeric network on the diffusion of species dissolved in the solvent. For low concentrations of macromolecules and consequently large distances between polymeric segments in the gel network, diffusion of small probes is influenced only by the composition of the solution immobilized in that network. For higher concentrations of macromolecules, the diffusion process becomes sensitive to the network structure. Transport properties of large probes (e.g., macromolecular probes), however, might depend on the gel structure even for very low concentrations of polymeric gels, and studies of this are now underway in our laboratory.

To make sure that the voltammetric experiment with microelectrodes is not restricted only to a very small volume of a free solution and that diffusivity of electroactive probes is measured in the bulk of the gel, we performed steady-state voltammetric and chronoamperometric experiments for TEMPO in 1% agarose gel using three different sizes of microelectrodes, 5.5, 10, and 26 μm in radius. Table 2 shows limiting currents obtained by both techniques. As one can see, limiting steady-state currents measured at the plateau of the voltammetric waves do not differ from those obtained after 100 s at +0.85 V (potential on the plateau of TEMPO oxidation wave) by more than 4%. The ratio of the steady-state current to the radius of the disk, i_d/r, is constant (±5%) for all sizes of the microelectrodes used. This indicates that the height of the oxidation wave of TEMPO in 1% agarose gel is diffusion-controlled under these experimental conditions. The estimated thickness of a diffusion layer, δ, for a disk microelectrode under steady-state conditions is $(r(\pi/4))$.[37] Therefore, δ can be estimated as 4.3, 7.8, and 20.4 μm for the radius of the disk of 5.5, 10, and 26 μm, respectively. Distances reported (from cryo electron microscopy measurements) between polymeric threads (approximate diameter of 10 nm) in 1% agarose gels are in the range of 0.05−0.3 μm.[4] Comparison of the thickness of the diffusion layer with distances between polymeric segments in the gel shows that the microelectrode voltammetric measurement should be representative of the bulk of the gel.

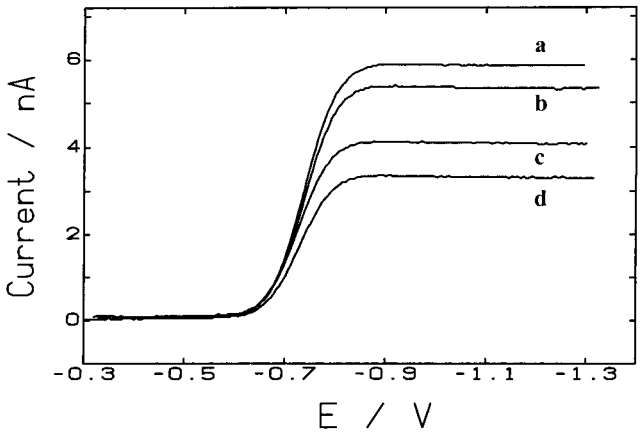

Figure 3. Steady-state voltammograms of reduction of 0.3 mM Tl^+ in 1% agarose gel (a) without added supporting electrolyte and with (b) 1.5×10^{-5}, (c) 1.5×10^{-4}, and (d) 0.03 M $LiClO_4$: Hg film disk electrode, $r = 15$ μm, 25 °C.

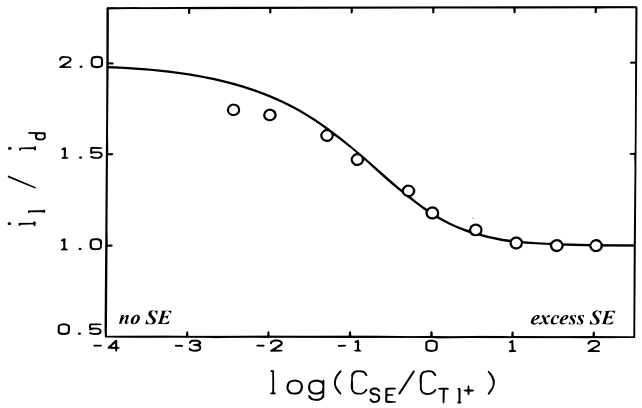

Figure 4. Dependence of normalized limiting currents (wave height in the presence of excess supporting electrolyte = 1) on concentration of supporting electrolyte (SE), $LiClO_4$, for 0.3 mM Tl^+ reduction in 1% agarose gel. (—) theoretical plot for +1−1 substrate in univalent electrolyte, eq 2.[43]

Steady-state voltammograms for the reduction of thallium ion at a mercury film disk microelectrode in 1% agarose gel with various concentrations of $LiClO_4$ are presented in Figure 3. Steady-state plateaus of these voltammograms are very well defined and reproducible, with rsd values of 1.4% and 1.7% calculated for 0.3 mM Tl^+ from five voltammograms for agarose gel with 0.05 M $LiClO_4$ and gel without electrolyte, respectively. The shape of voltammograms is identical in solutions without agarose, with rsd values calculated from five voltammograms of 1.8% and 2.2% for 0.05 M $LiClO_4$ and water without electrolyte, respectively. The diffusion coefficient of thallium cation in the gel with 0.05 M $LiClO_4$ was determined, using eq 1, as 1.85×10^{-5} cm^2/s at 25 °C. The value of 1.90×10^{-5} cm^2/s was obtained from a solution of 0.05 M $LiClO_4$ without agarose. Those two values agree well with previously reported values of 2.0×10^{-5} cm^2/s for solution at infinite dilution at 25 °C[41] and 1.97×10^{-5} cm^2/s in 0.04 M $LiClO_4$ solution at 25 °C.[42]

In the 1% agarose gel without supporting electrolyte, steady-state currents of Tl^+ reduction were significantly higher than those in the gel with excess $LiClO_4$. This increase of current is due to the migrational component to the total flux of Tl^+ in the absence of supporting electrolyte. Figure 4 presents the dependence of the limiting current of Tl^+ reduction in the agarose gel on the concentration of supporting electrolyte, $LiClO_4$. This plot shows the ratio of the limiting current to diffusional current,

i_l/i_d, as a function of the ratio of the concentration of supporting electrolyte to the concentration of Tl^+, $\log(C_{SE}/C_{Tl^+})$. Experimental data are compared with the theoretical prediction for the steady-state current for the one-electron reduction of a +1 charged cation in solution of univalent supporting electrolyte:[43]

$$i_l/i_d = 2 + 2C_{SE}/C_{Tl^+} - 2[C_{SE}/C_{Tl^+}(1 + C_{SE}/C_{Tl^+})]^{1/2} \quad (2)$$

As one can see from Figure 4, the experimental data are very close to those predicted by theory. Transport behavior of Tl^+ in agarose gel with low concentrations of supporting electrolyte is the same as it is in aqueous solution without polymer.[42] Note, that the experimental point for the lowest value of $\log(C_{SE}/C_{Tl^+})$ is obtained in the gel without added supporting electrolyte and the value of C_{SE} for this point is the estimated concentration of monovalent cations in deionized water (approximately 1×10^{-6} M[28]). There are some differences between experimental data and theoretical predictions for the gel with very low concentrations of electrolyte, $\log(C_{SE}/C_{Tl^+}) < -2$. The reason for this could be the background level of ions present in agarose gel. Agarose is an uncharged polymer; however, small numbers of ionic groups (mainly sulfate groups) can be expected in the sample. For the agarose used in our experiments, the percentage of sulfate groups is reported as lower than 0.1%. This number would result in the presence of an approximately 0.005 mM concentration of counterions (probably Na^+ or K^+) from the dissociation of polymer sulfate groups, and these cations would act as additional supporting electrolyte. The concentration of adventitious monovalent cations in the agarose gel determined from the experimental data for Tl^+ (i_l value without added electrolyte) is 0.0045 mM. It is within 10% of the percentage of sulfate groups reported by the supplier. This methodology, therefore, could be useful for the estimation of ionic impurities in uncharged polymeric solutions and gels.

***ι*-Carrageenan Gels.** Steady-state voltammograms of TEMPO oxidation at Pt microelectrodes in the 0.5% *ι*-car gel are very well defined and reproducible, with rsd values of 0.9% calculated from nine voltammograms in 0.2 M $LiClO_4$. The shape of voltammograms and the half-wave potential are identical to those in agarose gel; see Figure 2. Steady-state currents increase with increasing TEMPO concentration, and the dependence of the limiting current on the concentration of TEMPO can be described by the linear equation i (nA) = 3.36C (mM) − 0.04. The diffusion coefficient of TEMPO calculated from the slope of this concentration calibration curve is 5.8×10^{-6} cm^2/s, and it is very close to that in 1% agarose gel. It is also very close to that of 5.9×10^{-6} cm^2/s, determined in 0.2 M $LiClO_4$ solution without *ι*-car.

Reduction of thallium ion at a mercury film disk microelectrode in 0.5% *ι*-car gel with 1 M $LiClO_4$ results in very well-defined steady-state voltammograms, with shapes identical to those from the agarose gel; see Figure 3. The reproducibility of limiting currents is very good, with rsd values not higher than 1.9%, calculated for 0.3 mM Tl^+ from five voltammograms. We investigated the dependence of steady-state currents of Tl^+ reduction in 0.5% *ι*-car on temperature. The limiting current increases with increasing temperature, and since the concentration of Tl^+ and the size of the microelectrode are constant, this illustrates changes in the diffusion coefficient of Tl^+; see eq 1. Figure 5 compares diffusion coefficients for Tl^+ in 0.5% *ι*-car gel with 1 M $LiClO_4$ with those in 1 M $LiClO_4$ solution without *ι*-car. There are no significant differences between the diffusion coefficient values from the gel and from solution without *ι*-car. There are several parameters that influence changes of the diffusion coefficient as a result of temperature change. First, the diffusion coefficient changes with temperature as described by the Stokes−Einstein equation:

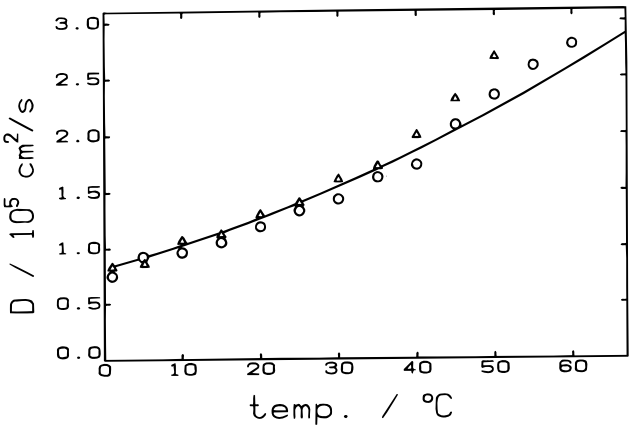

Figure 5. Dependence of the diffusion coefficient of Tl^+ on temperature: (○) 0.3 mM Tl^+ in 0.5% *ι*-car gel with 1 M $LiClO_4$; (△) 0.3 mM Tl^+ in 1 M $LiClO_4$ solution; (—) calculated from eq 3 and the experimental diffusion coefficient at 25 °C for Tl^+ in 1 M $LiClO_4$ solution.

$$D = kT/6\pi r_0\eta \quad (3)$$

where k is the Boltzmann constant, T is the absolute temperature, r_0 the radius of the diffusing species, and η is the viscosity. Second, the viscosity of the solution changes with temperature and influences the value of the diffusion coefficient as predicted by eq 3. Third, the diffusion coefficient may change as a consequence of the change in the structure of the *ι*-car gel network. It should be pointed out here that *ι*-car gel undergoes a thermoreversible sol−gel transition in the temperature range from 45−55 °C.[17,18] This transition temperature depends on the concentration of polymer and on the ionic strength of the system.

We have calculated changes in the diffusion coefficient of an ideal species based on eq 3, as a ratio of temperature, T_{exp}, and viscosity of 1 M $LiClO_4$ solution at this temperature, η_{exp} (at T_{exp}). We then plot the normalized diffusion coefficient of Tl^+, D_{Tl^+} (at 25 °C) × [T_{exp}/η_{exp} (at T_{exp})] as a function of temperature (solid line in Figure 5). Note, that the only viscosity values used for this normalization are those of the solution with $LiClO_4$, not those of the gel. Both experimental sets of diffusion coefficients values (from the gel and the solution) follow well the prediction of eq 3. What one also can see in Figure 5 is that the melting process of the 0.5% *ι*-car gel does not influence the diffusion coefficient of the thallium cation.

We studied the dependence of the Tl^+ diffusion coefficient in *ι*-car gel on the concentration of supporting electrolyte, $LiClO_4$. The dependence of the diffusion coefficient of Tl^+ in 0.5% *ι*-car gel and in equivalent aqueous solution without polymer is presented in Figure 6. There is no difference between diffusion coefficients in the gel and in the solution for concentrations of $LiClO_4$ higher than 0.2 M. Below this concentration, the diffusion coefficient of the Tl^+ probe is significantly lower in *ι*-car gel than in the solution without polymer. There are two effects that could influence Tl^+ transport in this system with a low concentration of supporting electrolyte. First, in the absence of supporting electrolyte or with very low supporting electrolyte concentration, the limiting current of Tl^+ reduction could be controlled by both diffusional and migrational transport, as described by eq 2. Note, however, that migrational effects would increase the total current. However, the concentration of Tl^+ is very low, 0.0003 M, and therefore, purely

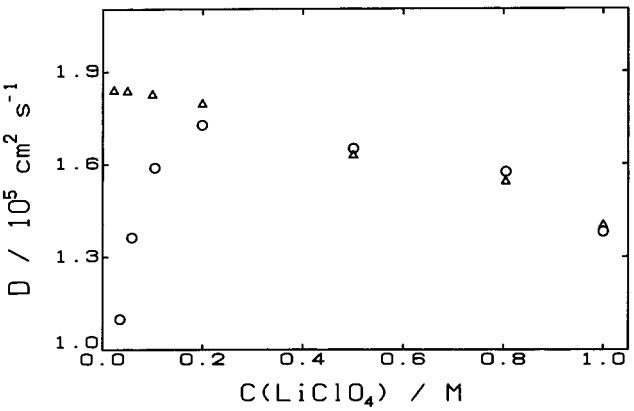

Figure 6. Dependence of the diffusion coefficient of 0.3 mM Tl^+ on $LiClO_4$ concentration in (○) 0.5% ι-car gel and (△) solution without ι-car both at 25 °C.

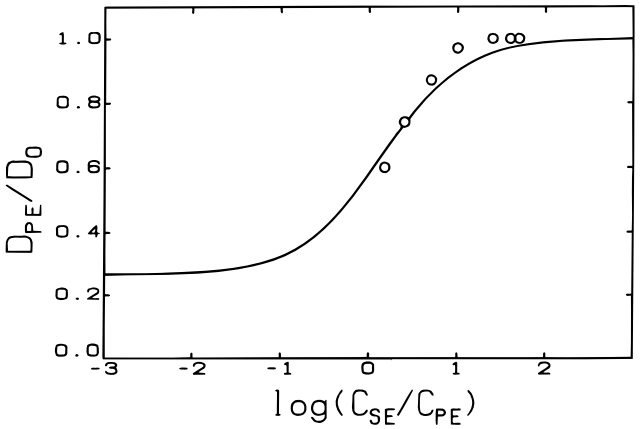

Figure 7. Dependence of the normalized diffusion coefficient of Tl^+ probe (○) in 0.5% ι-car gel, D_{PE}/D_0, on the concentration of electrolyte, $LiClO_4$, $\log(C_{SE}/C_{PE})$. (—) calculated according to the Manning's theory, with $b = 0.22$ nm.

diffusional currents are expected even for as low a concentration of supporting electrolyte as 0.03 M.[43] Since the lowest concentration of $LiClO_4$ used in our experiments was 0.03 M, we can assume that reduction currents of Tl^+ are controlled only by diffusional transport.

The second effect that can be expected in polyionic systems of very low ionic strength is an attractive electrostatic interaction between polyion and counterion (or probe ion of the opposite charge). This effect is know as "counterion condensation" or "counterion binding". For polyionic solutions, electrostatic interactions between polyions and counterions depend on the charge density of the polyion and the charge of counterion.[44,45] Two major theories, a linear charge model proposed by Manning[46−50] and a cylindrical cell model based on the Poisson−Boltzmann equation,[51,52] predict the changes of transport properties of counterions as affected by the charge density of the polyion and the ionic strength of solution. It has been shown that Manning's theory describes these changes very well, even for such complex systems as biological polyelectrolytes, including ι-car.[34] It has also been shown that probe ions in very low concentration can be used to study electrostatic interactions in polyionic solutions.[29,30] Unfortunately, there is no simple theoretical model that could be applied to describe electrostatic interactions in three-dimensional networks of polyions, such as ι-car gel.

We have compared our experimental results from ι-car gel with the theoretical predictions of Manning's theory for linear polyelectrolytes. According to Manning's line charge model,[46−50] the self-diffusion coefficient of the counterion depends on the dimensionless charge density, λ, given by

$$\lambda = e_0^2/4\pi\epsilon^\circ\epsilon bkT = \lambda_B/b \qquad (4)$$

where e_0 is the elementary charge, ϵ° is the permittivity of vacuum, ϵ is the dielectric constant of the solvent, b is spacing between charges of the polyion, k is the Boltzmann constant, T is the absolute temperature, and λ_B is the Bjerrum length, that is, the spacing between two singly charged ions for which the electrostatic energy of interaction is equal to kT.

Manning's theory treats polyions as infinitely long line charges. Counterions are assumed to condense onto the polyion chain as required to avoid exceeding the critical charge density, λ_c (for monovalent counterions $\lambda_c = 1$).[46−50] These condensed counterions are inside the hydration layer; therefore, their mobility is that of the polyion, usually negligible in comparison with the mobility of free counterions. Uncondensed counterions and coions are subject to Debye−Hückel interactions with the polyions. The diffusion coefficient ratio for monovalent counterions is[47]

$$D_{PE}/D_0 = [1 - (1/3)A(1; \lambda^{-1}\ 1/X)] \qquad \text{for } \lambda > 1 \quad (5)$$

where D_{PE} and D_0 are diffusion coefficients of the monovalent counterion in solutions with and without polyelectrolyte, respectively, X is the ratio of the concentration of added monovalent salt to the equivalent concentration of polyelectrolyte (i.e., the molar concentration of polyion multiplied by the charge per polyion), $X = C_{SE}/C_{PE}$, and $A(\lambda; 1/X)$ is given by

$$A(\lambda; 1/X) = \sum_{\substack{m_1=-\infty \\ (m_1,m_2)\neq(0,0)}}^{\infty} \sum_{m_2=-\infty}^{\infty} [\pi\lambda^{-1}(m_1^2 + m_2^2) + 1 + 2X]^{-2} \qquad (6)$$

where m_1 and m_2 are integers.

In salt-free solution, for $X \to 0$,

$$D_{PE}^0/D_0^0 = 0.867\lambda^{-1} \qquad \text{for } \lambda > 1 \qquad (7)$$

The mechanism of gelation of ι-car has been proposed as coil → helix → double helix → condensation to gel network.[17,18,53,54] Therefore, in calculations based on Manning's theory, we take into account the double helix form of ι-car, the form that condenses to a three-dimensional network, with $b = 0.22$ nm.[55−58] At 25 °C and with the dielectric constant, ϵ, of 78.3, $\lambda_B = 0.716$ nm and the charge density of the double helix form of ι-car, calculated according to eq 4, is 3.25. For a salt-free solution under the same conditions, the ratio D_{PE}^0/D_0^0 (eq 7) for the double helix form of ι-car is 0.267. Figure 7 presents comparison of our experimental results for the transport of Tl^+ probe ions in 0.5% ι-car gel as a function of the concentration of $LiClO_4$ and the theoretical dependence of D_{PE}/D_0 values as predicted by Manning's theory for linear polyelectrolytes. As one can see, our experimental results from ι-car gels are very close to those predicted by Manning's theory for linear polyelectrolytes. This is unexpected since the electrostatic characteristic of three-dimensional polyionic gel should be different than that of a linear ionic polymer. However, since a physical model for the gel should differ from that for the linear polyelectrolyte, the only conclusion that can be made here is that the effect of electrostatic interactions on the transport

TABLE 3: Comparison of the Diffusion Coefficient of Tl^+ and Macroscopic Viscosity of 0.5% ι-car Gel with 1 M $LiClO_4$ and 1 M $LiClO_4$ Solution without Polymer at 1 and 25 °C

	solution (1 M $LiClO_4$)		gel (0.5% ι-car, 1 M $LiClO_4$)		
temp, °C	viscosity, cp	$D(Tl^+)$, cm^2 s^{-1}; expt	viscosity, cp	$D(Tl^+)$, cm^2 s^{-1}; expt	$D(Tl^+)$, cm^2 s^{-1}; calcd[d]
1	1.73	8.29×10^{-6}	4372	7.48×10^{-6}	3.34×10^{-9}
25	1.02	1.40×10^{-5}	1704	1.38×10^{-5}	7.45×10^{-9}

[d] Calculated based on macroscopic viscosity of the gel from the Stokes−Einstein equation, eq 3.

properties of counterions in ι-car gel is very similar to that predicted for the double helix linear form of that polyion.

Macroscopic versus Local Viscosity of Gels. The macroscopic viscosity of biopolymeric gels is several orders of magnitude higher than that of aqueous solutions without polymer. Table 3 presents viscosity values for 0.5% ι-car gel with 1 M $LiClO_4$ at two temperatures, 1 and 25 °C. It also shows the viscosity of 1 M $LiClO_4$ solution without polymer. As one can see, the macroscopic viscosity of ι-car gels at 1 °C is over 2500 times higher than that of a solution without polymer. For gels with higher concentrations of ι-car or for agarose gels, this difference is even larger. On the basis of the Stokes−Einstein equation and the macroscopic viscosity of 0.5% ι-car gel, the calculated diffusion coefficient of Tl^+ should be of the order of 3×10^{-9} and 7×10^{-9} cm^2/s at 1 and 25 °C, respectively. However, the experimental diffusion coefficients are much larger, and there are no differences in diffusion coefficients of Tl^+ or TEMPO between the gel and the solution without polymer. In other words, the local, microscopic viscosity of the gel that influences transport of small ions and molecules is that of the solution (or solvent) immobilized in the gel network. On the basis of the viscosity of 1 M $LiClO_4$ solution and the experimental diffusion coefficient for Tl^+ in the ι-car gel at 25 °C (see Table 3), we have estimated the value of the ionic radius, r_0, of Tl^+ in 0.5% ι-car gel with 1 M $LiClO_4$ from the Stokes−Einstein equation (eq 3). The calculated value is 1.55 Å, and it is within 4% of that reported for Tl^+ in aqueous solutions, 1.49 Å.[59]

Summary

We have shown that steady-state voltammetry with microelectrodes can be used effectively in transport studies in polymeric gels. This technique requires only simple and inexpensive equipment and can be quickly completed. The measurement of steady-state transport-limited currents is robust; it permits studies under very wide ranges of ionic strengths and temperatures, and it allows accurate and precise determination of the diffusion coefficient, as described by eq 1.

We have determined the diffusion coefficient of the uncharged probe TEMPO in two different gels, agarose and ι-car. We have shown that, for low concentrations of the polymer (approximately 1%), the three-dimensional structure of the polymeric network does not influence the transport properties of this probe and does not change or modify electrooxidation at the platinum microelectrodes. For higher concentrations of the biopolymer and consequently shorter distances between polymeric segments in the gel, the diffusion coefficient of TEMPO decreased. We have also shown that the macroscopic viscosity of agarose and ι-car gels does not influence diffusion of small molecules and ions and that, for low concentrations of the polymer, the diffusion coefficients of those species depend on the composition of the solution immobilized in the gel network, or in other words on local microscopic viscosity of the system.

For the charged electroactive probe, Tl^+, we have shown that in the neutral gel, agarose, thallium reduction is identical to that in aqueous solutions, with the same effects of migration in systems of low ionic strength. We have also indicated that this voltammetric method could be used for the estimation of low concentrations of ions in uncharged gels. In the polyionic gel, ι-car, we have shown that electrostatic interactions between the polyanion and thallium cation significantly change the transport properties of Tl^+. Since there is a dearth of appropriate models, we have not compared our experimental results with theories for three-dimensional polyionic systems. However, we have found that the change of the diffusion coefficient of the Tl^+ probe in the ι-car gel is similar to that predicted by Manning's theory for a monovalent counterion in solutions of the double helix form of ι-car, with an assumption of the linearity of the polyion.

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