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Monoprotonated Sapphyrin–Pertechnetate Anion Interactions in Aqueous Media

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The addition of aqueous pH 7 solutions of 7.2×10^{-3} M pertechnetate to dilute aqueous 2.5% MeOH solutions containing a water-solubilized sapphyrin, 3,12,13,22-tetraethyl-8,17-bis[bis(hydroxyethyl)-amino)carbonylethyl]-2,7,18,23-tetramethylsapphyrin (1), gives rise to spectroscopic changes in the UV–Vis spectrum of 1 that are consistent with anion-binding and sapphyrin deaggregation. The spectroscopic changes induced by pertechnetate were found to differ dramatically from those induced by the addition of either pure water or dilute nitric acid; however, they were found to parallel those seen when sodium phosphate was added to solutions of 1 under analogous experimental conditions. Fits of the spectroscopic titration data to a 1:1 binding profile revealed that the effective *K* describing the interaction of pertechnetate anion with 1 was ca. $3900 \pm 300\ M^{-1}$; this value compares to the effective *K* of $23000 \pm 3000\ M^{-1}$ that describes the corresponding interaction of sodium phosphate with 1.

Keywords: Pertechnetate anion; Sapphyrin; Phosphate anion; Anion binding; Aqueous media

INTRODUCTION

The use of radioactive materials as fuel for the production of power for either general use or in satellite and space exploration applications is beleaguered with radioactive waste management risks [1]. We recently demonstrated that expanded porphyrins may be used to bind actinides and are currently working to exploit these systems in the control of potential waste risk issues [2–5]. Separately, we have discovered that sapphyrins (e.g. **1**–**3**) and other expanded porphyrins can act as anion receptors in their protonated forms [6–9]. Such findings have led us to consider that oligopyrrolic species could be used to recognize and remove radioactive technetium, in the form of pertechnetate anion, $^{99}TcO_4^-$, from radioactive waste streams.

While technetium, in the form ^{99m}Tc, is commonly used as an industrial tracer and/or in human diagnostics, ^{99m}Tc from these applications represents only a secondary source of radioactive pertechnetate waste [10]. The most significant source of technetium by far is from the nuclear fuel cycle. In fact, approximately 6% of the total fission product yield is ^{99}Tc, which adheres to the surfaces of the reactor and must be removed with nitric acid washes [11,12]. The ^{99}Tc, largely in the form of the pertechnetate anion (TcO_4^-), ends up in the final "raffinate" waste stream during the separations processes used to limit the quantities of high level waste that are produced [11–13].

Pertechnetate anion is the predominant form of technetium not only in nuclear waste streams, but also in other oxic and aqueous environments. It is highly soluble in water and mobile in the environment, and as is the case with I^-, this monovalent anion is not retained by cation exchange [14]. Not surprisingly, geological barriers are ineffective in impeding its mobility as diffusion is relatively unhindered via coordination to minerals [11,14,15].

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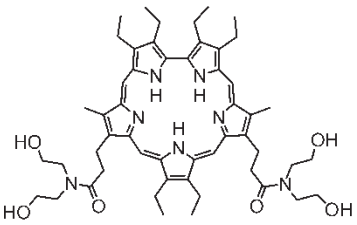
This raises concerns that TcO_4^--derived radioactivity may be introduced into groundwater and that technetium could enter into the food chain via, for example, sea-dwelling plants [14,16,17]. Compounding the issue is the fact that the half-life of technetium in its ground ^{99}Tc state is 213000 years. As a consequence, pertechnetate is grouped with high-level waste species [13].

Incorporated into anion-exchange resins and anion-specific extraction agents or immobilized on solid supports, appropriately designed pertechnetate receptors might allow reprocessing-derived waste streams to be produced that are free of pertechnetate. Unfortunately, this goal is far from being realized. This is perhaps not surprising given the fact that, in contrast to the more established field of cation coordination, the area of anion recognition is still relatively new. The effects of size, charge, and anion shape on selectivity and binding strength have yet to be defined in either general terms or for various individual anions (e.g. TcO_4^-) [18,19].

To date, quaternary ammonium cations have been used extensively for pertechnetate extraction, although these species are limited in that they must be used in very acidic pH regions so that the polyaza hosts remain protonated [19]. In addition, while some of these systems have been shown to be selective for iodide based on size exclusion, they have not been demonstrated to be selective for pertechnetate in particular over other anions such as nitrate or sulfate, which are present at much higher concentrations in raffinate waste streams [12,19,20]. To develop systems with improved selectivity, Farrell *et al.* prepared a series of amino-azacryptands, as well as several of their open chain analogs, and used them to effect the extraction of pertechnetate and perrhenate from neutral pH buffered solutions [21]. Other macrocycles have also been used as extractants for pertechnetate. In their work, Beer *et al.* demonstrated a tripodal tris(benzo-15-crown-5) ligand with a tren cap and 3 benzo-15-crown-5 coordinating units capable of extracting pertechentate from aqueous solutions into chloroform based on a cooperative cation–anion binding system using sodium [22]. Atwood and co-workers used another promising approach towards pertechnetate receptor generation. These researchers synthesized several π-metallated cyclotrivertrylenes and found that one of their compounds, **4**, did indeed act as a receptor for perrhenate and pertechnetate anions [23,24]. In spite of this favorable result, it is not clear whether these systems are selective for pertechnetate over other anions that would be present in the raffinate waste stream or whether they would prove viable in applications. Nevertheless, this work is significant in demonstrating how, in principle, selective pertechnetate anion binding can be achieved via the use of appropriately designed anion receptors [23,24].

Toward the same end, resin-based systems are also being developed with an eye toward increasing the ease of pertechnetate removal. One such system is Reillex™-HPQ (cf. general structure, **5**). This resin has been studied extensively with simulated, as well as actual, high level wastes by Schroeder and his co-workers at Los Alamos National Laboratory [20]. Another resin system based on a ligand, given the appellation SuperLig 639, is currently being studied for its ability to remove ^{99}Tc from highly alkaline wastes by Hassan and co-workers at the Savannah River Technology Center. This resin, whose structure is proprietary, was demonstrated to be highly selective for pertechnetate even in the presence of large quantities of nitrate, sulfate, and phosphate. It was also found to be capable of removing more than 80% of the pertechnetate anion from radioactive waste solutions, a capacity level that makes it potentially useful in actual waste treatment scenarios [25].



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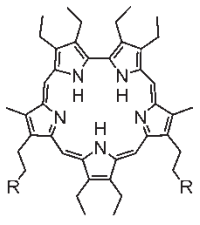
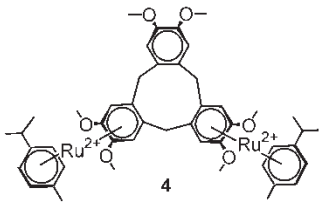


2 R = H
3 R = CH_2OH



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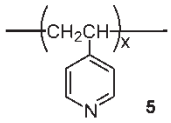

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Another series of receptors that might be useful in the area of pertechnetate recognition and removal are the expanded porphyrins. Expanded porphyrins are porphyrin analogs containing larger central cores, a greater number of π-electrons in their conjugated peripheries, or more than four pyrrole, or pyrrole-like heterocyclic subunits. Among the interesting attributes of some expanded porphyrins is their ability to bind anions. While we were the first to describe such a phenomenon, specifically the binding of fluoride anion by diprotonated sapphyrin [26,27], others have also begun to study the properties of these kinds of systems. For instance, Tabata *et al.* recently described the use of protonated sapphyrins as analytical reagents for the fluorimetric determination of fluoride anion in aqueous solutions

[28,29]. This latter work, in combination with the recent reports from Pillai *et al.*, wherein ^{99}Tc complexes of pyrrole- and thiophene-based systems were described [30], and our own findings that sapphyrin binds phosphate-type anions in highly polar media [7,31,32], led us to consider that sapphyrin might function as a pertechnetate anion receptor. Here, we report the results of first studies designed to explore this possibility.

Sapphyrin (e.g. **1**–**3**) is the most venerable of all known expanded porphyrins. First reported by R. B. Woodward in 1966, it was obtained as a by-product during early efforts directed toward the total synthesis of vitamin B_{12} [33]. Characterized by a conjugated 22 π-electron periphery and spectroscopic properties that are characteristic of heteroannulene-type aromaticity, it also contains a central core that is ca. 35% larger than that present in porphyrin. Given the presence of this larger core and its five potentially donating nitrogen atoms, it was originally expected that sapphyrins would display a rich metallation chemistry. To date, this promise remains largely unrealized [34,35]. On the other hand, early studies from our laboratory, carried out in conjunction with the Ibers group, revealed that the diprotonated form of sapphyrin **2** formed a 1:1 in-plane complex with fluoride anion in the solid state and acted as a highly effective receptor for this species in both dichloromethane and methanol. Subsequent studies revealed that the mono- and diprotonated forms of this and other sapphyrins also bound chloride and phosphate-type anions (e.g. phosphonates, mononucleotides) in the solid state and in organic solution, with the latter being bound particularly well [27,36,37]. Although not as high as for fluoride anion, the high affinities displayed towards phosphate-type anions [6,38] were rationalized in part as being due to an ability to form complexes wherein the phosphate oxyanion is bound via multiple hydrogen bonds, including five in a "helicopter-like" fashion among other motifs (cf. Fig. 1) [32]. Their ability to bind phosphate allowed water-soluble sapphyrins, such as **1**, to be used for both the recognition [39–41] and light-based cleavage of DNA [42–44]. This, in turn, spawned efforts to synthesize and study water-soluble versions of sapphyrin, both as possible phosphate anion sensors [31,45,46] and as potential phosphate and chloride anion carriers [47,48]. At neutral pH, sapphyrin exists in its monoprotonated form ($pK_{a1} = 4.8$, $pK_{a2} = 8.8$ for sapphyrin **1**) [6].

Studies of the solution phase behavior of water-soluble sapphyrins led to the identification of three limiting forms, specifically an extended aggregate (characterized by a dominant, Soret-like absorption feature at ca. 410 nm), a dimer (characterized by a λ_{max} at ca. 420 nm), and a monomer (characterized by a λ_{max} at ca. 450 nm) [6,9]. For most of the sapphyrin species modified for increased water solubility, including the prototypical system **1** that is the focus of the present study, it is the extended aggregate that dominates at neutral pH. This is true even at relatively low concentrations (i.e. $[\mathbf{1}] \approx 10^{-5}$ M). On the other hand, the addition of organic co-solvents (e.g. methanol) or surfactants (e.g. SDS: dodecyl sulfate, sodium salt), as well as simple protonation, serves to shift the solution phase equilibrium towards the dimeric and monomeric forms. It was also found that deaggregation could be effected by the addition of appropriate anions, a phenomenon that has been extensively studied in the case of phosphate-type anions [7,9,31].

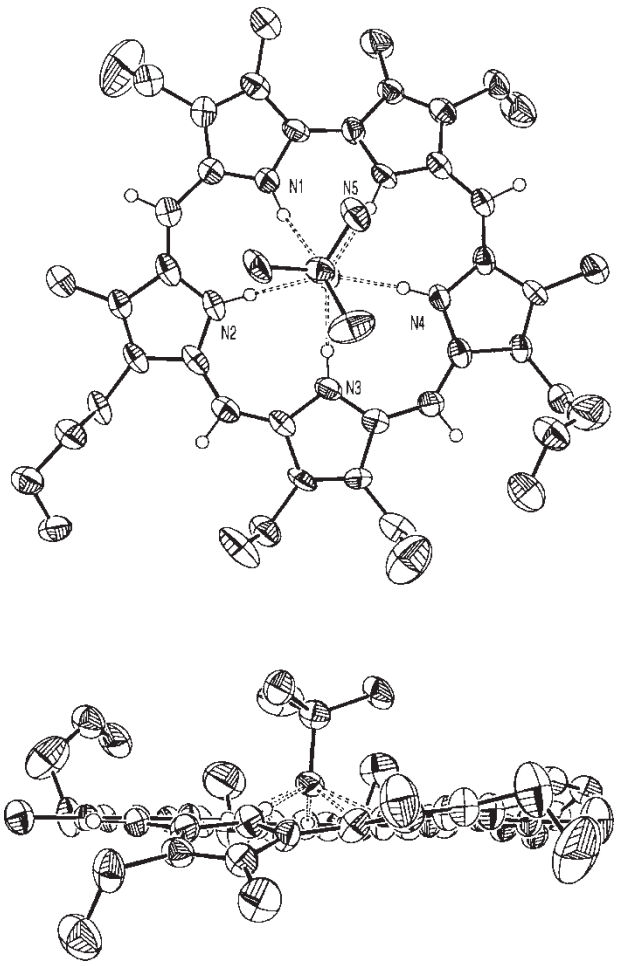

FIGURE 1 X-ray structure of the phosphate anion complex stabilized in the solid state by the diprotonated form of sapphyrin **3**. This figure was produced using coordinates downloaded from the Cambridge Crystallographic Data Centre and corresponds to a structure first reported in Ref. [6].

In early work, it was noted that the addition of phosphate and phosphonate anions to buffered, pH 6.1, aqueous solutions of the water-soluble sapphyrin **1**, gave rise to changes in the visible spectrum that were best interpreted in terms of a coupled anion-induced deaggregation and concurrent dimer formation. The effective equilibrium constant for this process, representing the build-up

of the latter anion-bound species, was found to be on the order of 100–350 M^{-1} [6]. More recently, it was found that the addition of sodium phosphate at very high phosphate-to-sapphyrin ratios, solutions of **3** buffered to pH 7.0 (PIPES: 1,4-piperazinebis-(ethanesulfonic) acid, sodium salt; buffer; 25 mM) and containing 150 mM of NaCl led to an enhancement of the fluorescence spectral features characteristic of the highly emissive monomeric sapphyrin forms. Effective equilibrium constants for this process, which represent the "tail end" of the overall anion-induced aggregate → monomer deaggregation conversion process, were found to be on the order of 5–20 M^{-1} [9].

While not the focus of the latter study, it was noted that the addition of phosphate at low phosphate-to-sapphyrin ratios gave rise to changes in the visible spectrum that were in accord with the binding of anions to the aggregated form without the concomitant build-up of dimeric species. (Although, it is considered likely that a certain degree of deaggregation was being induced.) Thus, at least three limiting parts to what is an obviously complex equilibrium process can be identified in the case of phosphate, namely binding to the initial aggregated form, anion-induced formation of a dimer, and, at very high anion-to-phosphate ratios, the generation of one or more anion-bound monomeric forms. In the context of the proposed pertechnetate binding, it was the first of the events, presumably characterized by the highest effective equilibrium constants, that was considered most interesting. Thus, the scientific questions we sought to address were whether sapphyrin **1** would bind pertechnetate at low-to-moderate pertechnetate ratios and, if so, how the effective equilibrium constant for the presumed binding and deaggregation process might compare quantitatively with that produced by phosphate anion under identical experimental conditions.

RESULTS AND DISCUSSION

The fact that sapphyrin **1** was found to bind phosphate-type anions in aqueous media, both at pH 6.1 and at pH 7.0 in the presence of excess sodium chloride, led to the consideration that it might also serve as a receptor for pertechnetate, another tetrahedral anion. To the extent this proved true, it was thought that spectroscopic methods could again be used to follow the associated binding and deaggregation process and allow for the calculation of an effective equilibrium constant.

Unlike phosphate, pertechnetate is radioactive and available in only a limited number of chemical forms. Needless to say, this fact serves to limit the nature and number of the experiments that could be used to probe the sapphyrin–pertechnetate anion interactions. In particular, it proved necessary to limit the size and quantity of the samples for this study. Thus, the effects of pH, if any, on the observed spectral changes were monitored using pH paper and by means of appropriate control experiments. Although the stock pertechnetate solutions used in the present study were diluted to the point where the pH was 7 and no change in pH was seen over the course of the titrations, concerns remained that possible acid-induced changes in the spectral features of sapphyrin could serve to mask any associated with the putative pertechnetate anion binding and deaggregation process. Unfortunately, the use of buffers to control the effects of pH during the "titrations" designed to probe the effects of pertechnetate binding to sapphyrin proved impractical; several commonly used buffers were found either to bind to the pertechnetate anion or to the monoprotonated sapphyrin core. Thus, control experiments, involving dilutions of stock sapphyrin solutions with pure water and, separately, additions of nitric acid, were carried out. In both cases, the spectral changes seen were unlike those seen upon addition of pertechnetate anion (*vide infra*).

Of the various water-soluble sapphyrins prepared to date, the tetrahydroxy diamide **1** is perhaps the most extensively investigated. Thus, it was chosen for use in the present study. As with other sapphyrins, this particular system exists in its monoprotonated form at pH 7.

Because no fluorimeter was available in the radiological laboratories where the experiments with pertechnetate were carried out, the solution phase interactions between pertechnetate and sapphyrin **1** were followed by UV–Vis absorption spectroscopy. This technique, which monitors the first part of the overall anion-induced deaggregation process, was expected to determine the effective equilibrium constants to be considerably larger than those recorded using fluorescence-based methods. On the other hand, since the interactions at low-to-moderate pertechnetate-to-sapphyrin ratios are likely to be dominated more by binding than deaggregation, they were the ones that were considered the most interesting in terms of determining whether sapphyrin **1** might have a role to play as a potential pertechnetate anion receptor.

The previous studies of phosphate binding to **1** at pH 7 focused on exploring equilibrium events occurring at high anion-to-sapphyrin ratios (as detailed above). In these, PIPES buffer was used to ensure that neutrality was maintained [7,9]. In the case of the corresponding pertechnetate studies, buffers could not be used (*vide supra*). Further, 2.5% methanol (v/v) was added to the aqueous sapphyrin solutions to ensure solubility

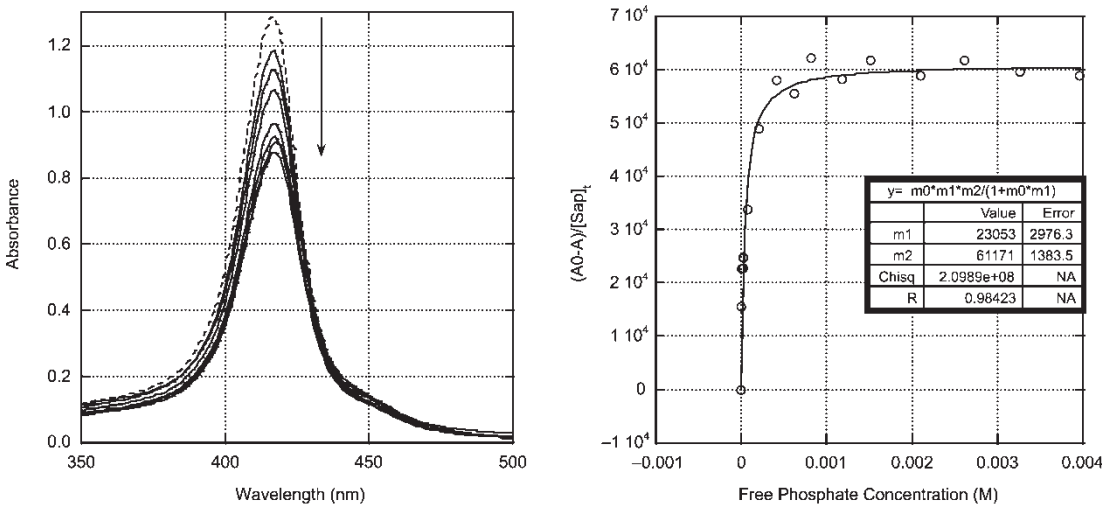

FIGURE 2 A: absorbance spectra of tetrahydroxy sapphyrin (**1**) (6.6 μM) recorded in the presence of 0–4 mM phosphate anion (P_i) in 2.5% methanol–water (pH 7.0). No external buffers were present in these solutions. The sapphyrin concentration was held constant throughout the series by using a concentrated aqueous sodium phosphate stock solution containing 6.6 μM sapphyrin. B: experimental data points and computer generated fit to a standard 1:1 binding profile.

was retained throughout the course of the titrations.[†] Thus, for comparison, titrations of sapphyrin **1** with phosphate were performed under conditions analogous to those used in the case of pertechnetate.

These control titrations were performed by adding aliquots of 9.8 mM aqueous sodium phosphate solution (pH 7; mixture of mono- and dibasic forms) containing 6.6 μM sapphyrin (to maintain a constant sapphyrin concentration throughout the titration) to aqueous 2.5% methanol pH 7 solutions of sapphyrin **1** (6.6 μM). As shown in Fig. 2, such additions, corresponding to titrations of sapphyrin **1** with $0 \rightarrow 4$ mM phosphate, gave rise to a sharp, monotonic reduction in the intensity of the Soret band ascribed to the aggregate form of **1**. These spectral changes were found to fit well to a 1:1 binding isotherm (ΔA vs. $[P_i]$, where ΔA corresponds to the change in absorbance and $[P_i]$ the free, or unbound, phosphate concentration). Standard curve fitting procedures then gave rise to an effective K of $23000 \pm 3000\,M^{-1}$. This K value, coupled with the dilute sapphyrin solutions employed, meant that the $[P_i]$ term in the binding expression could initially be approximated by the total phosphate concentration, $[P_i]_{tot}$. The K value obtained using this approximation ($20000\,M^{-1}$) was then used to estimate a set of corrected $[P_i]$ values. Using these latter values, a new K was calculated. This process was repeated until convergence was reached.

In contrast to what was observed at pH 6.1, but consistent with the previous qualitative observations made at pH 7.0 in the presence of PIPES buffer and excess NaCl, essentially no build-up in spectral features ascribable to the formation of dimeric (or monomeric) forms of the sapphyrin was observed over the full course of the above titrations. This lack of appreciable conversion to easily identified deaggregated forms of sapphyrin, leads us to assign the effective equilibrium constant, K, to a process that involves primarily *binding* to the aggregated form of sapphyrin, just as we assigned the earlier fluorescence-based changes observed at high phosphate-to-sapphyrin ratios to processes that involve primarily final *deaggregation* of various less-well-associated sapphyrin species [9]. To the extent such an assignment is correct, the calculated K value, although formally reflecting a process involving both binding and deaggregation, can be used to obtain a semi-quantitative indication of how well phosphate binds to sapphyrin at low phosphate-to-sapphyrin ratios, thereby providing a numeric calibration point against which the putative interactions with pertechnetate may be judged.

Prior to commencing studies with pertechnetate, it was considered appropriate to test also for possible pH effects. While phosphate at pH 7 acts as its own

[†]Failure to add small quantities of methanol led to the observation of a sapphyrin-green precipitate, after the course of the titration. Seeking to better characterize this precipitate, a solution of **1** containing a ca. 8-fold excess of pertechnetate anion was allowed to stand overnight. Centrifugation of the resulting precipitate, followed by gamma counting using a scintillation counter, revealed that approximately 60% of the pertechnetate anion was contained in the precipitate while the remaining 40% was in the supernatant. Such observations are consistent with the formation of a sapphyrin–pertechnetate complex, but also reflect in whole or in part the entrapment of pertechnetate within the precipitate.

buffer (approximately a 3 to 2 ratio of mono- and dibasic forms), this is not so in the case of monoanionic pertechnetate. Further, the absorption spectra of water-soluble sapphyrins are known to be a sensitive function of pH, with an increase in the proton concentration leading to production of the deaggregated (i.e. dimeric and monomeric) forms. Thus, dilute, aqueous solutions of sapphyrin **3** (as well as its precursors **1** and **2**) made up as above at an initial pH of 7 in the presence of 2.5% methanol, albeit in the absence of a buffer, were "titrated" with dilute solutions of aqueous nitric acid. In all cases, the spectroscopic changes observed were very different from those seen upon the addition of phosphate or pertechnate (*vide infra*), obviating concerns that adventitious protonation effects were masking those associated with anion binding. Specifically, little decrease in the absorption feature at ca. 410 nm ascribed to the aggregate was seen; instead, essentially complete conversion to the dimer was seen even at the lowest nitric acid concentrations (ca. 1×10^{-6} M), followed by a subsequent decrease in the absorption band characteristic of this latter species, as illustrated in Fig. 3 for the specific case of sapphyrin **3**. Qualitatively similar results obtained with other strong acids support the assertion this is an acidity effect rather than an anion effect (data not shown).

On the basis of this information, studies of pertechnetate binding to sapphyrin **1** were performed. Towards this end, a stock solution of sapphyrin **1** was prepared in 2.5% aqueous methanol, at pH 7, to give an absorptivity of close to 1 at the Soret-like maximum in a 1 cm cuvet. (The actual concentration of the stock sapphyrin solution was 4.64×10^{-5}; for further details, see the "Experimental Section".) This solution was then titrated with pH 7 aqueous 7.2×10^{-3} M solutions of sodium pertechnetate with the changes in the UV–Vis spectral features being monitored. Unfortunately, the realities of working with the radioactive pertechnetate solutions precluded the addition of sapphyrin to the TcO_4^- stock solutions (as was done in the case of the phosphate control experiments; *vide supra*). Thus, the concentration of sapphyrin decreased over the course of the titrations, as noted below.

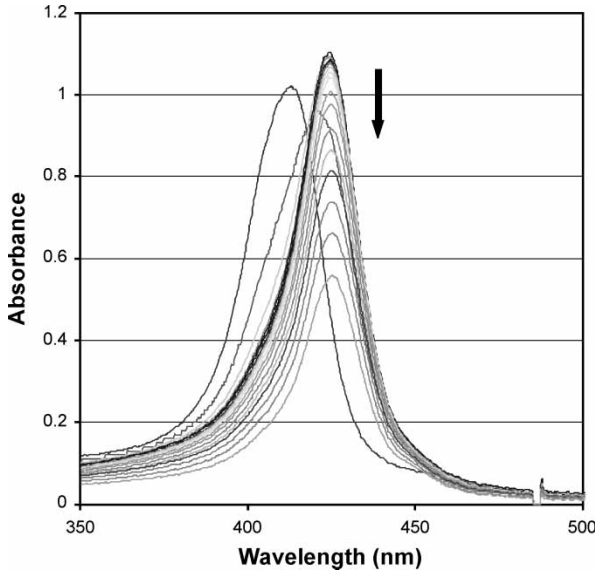

FIGURE 3 Absorbance spectra of tetrahydroxy sapphyrin (**1**) (4.64×10^{-5} M) recorded in the presence of increasing concentration of nitric acid (0–0.5 mM, HNO_3) in 2.5% methanol–water. No external buffers were present in these solutions. The initial shift from ca. 410 to 425 nm at a concentration of 1×10^{-6} M HNO_3 is followed by continued decrease in the absorbance with increasing concentration of acid.

Figure 4 shows the changes in the UV–Vis spectrum of sapphyrin **1** (4.64×10^{-5} M in pH 7 H_2O containing 2.5% v/v MeOH) when titrated with increasing quantities of a 7.2 mM stock solution of pertechnetate anion (pH 7 as judged by pH paper; counter cation mostly sodium but not fully defined; cf. "Experimental Section"). As can be seen by inspection of this figure, the intensity of the Soret-like absorption feature at ca. 410 nm decreased in intensity in direct analogy to what was observed in the case of phosphate. During the course of this titration, the volume of the sapphyrin solution, initially 2.0 mL, increased to 3.5 mL. This change in volume served to reduce the effective sapphyrin concentration (sum total of all species present in solution) and, possibly, induce a level of dilution-induced deaggregation that could serve to mask the effects of those engendered by the addition of pertechnetate.

Because of this concern, a control "titration", involving dilution with millipure, pH 7 water, was performed. This demonstrated that there was a significant change in optical features of a sapphyrin **1** solution (aqueous; pH 7; 2.5% MeOH v/v) as it was diluted from 2 to 3 mL; however, as indicated in Fig. 5, the change in absorbance at 413 nm is only appreciable at higher sapphyrin concentrations. Indeed, once sapphyrin concentrations of ca. 4.5×10^{-5} M are reached, a break in the curve is observed and almost pure Beer's Law-like behavior ensues (i.e. as the sapphyrin solution is subject to further dilution). This "phase transition" is in some ways analogous to a critical micelle concentration and could reflect the presence and disappearance of liposome-like higher order aggregated species. The crucial points are two-fold. First, that adding water produces an effect that is far less significant than that of adding pertechnetate anion and, second, that over the bulk of the concentration regime where the addition of pertechnetate anion induces large spectral changes, adding water gives rise to effects that are no different from simple, linear Beer's Law-type dilution.

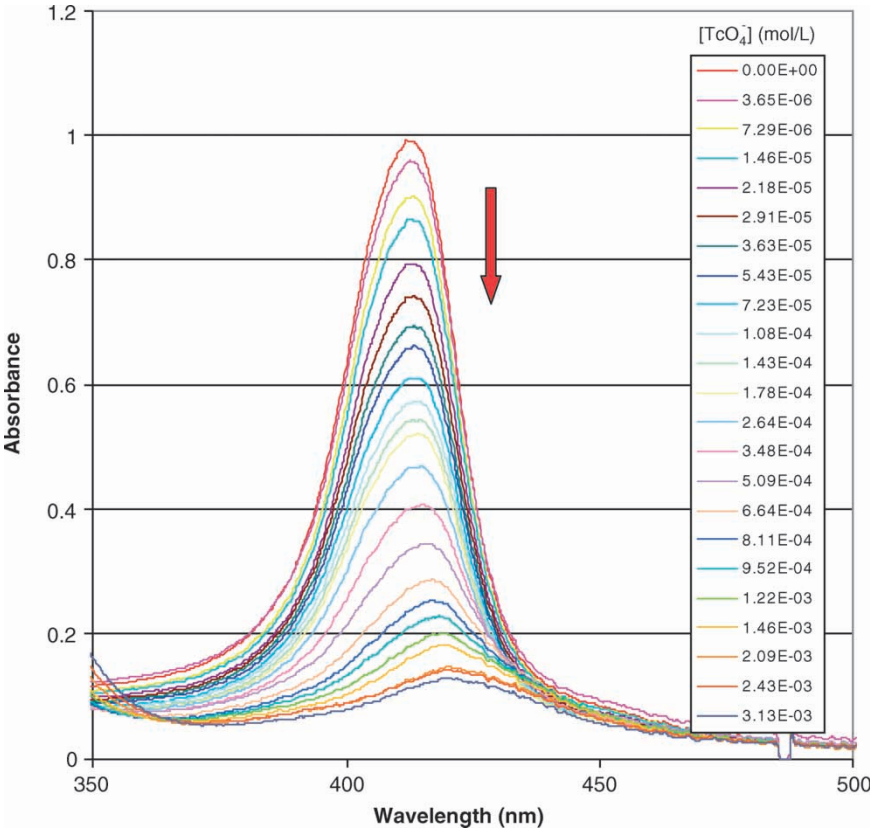

FIGURE 4 Spectral changes observed when an aqueous unbuffered solution (pH 7) of sapphyrin **1** (4.65×10^{-5} M) containing 2.5% methanol is titrated with a 7.2×10^{-3} M TcO_4^- solution.

The above considerations have an important consequence, namely that by choosing absorbance vs. pertechnetate concentration data points corresponding to sapphyrin concentrations at or below ca. 4.5×10^{-5} M and applying an appropriate correction for the observed Beer's Law-like behavior caused by dilution, it is possible to fit the pertechnetate titration data to 1:1 binding profiles. Towards this end, the linear region of the absorbance vs. sapphyrin concentration curve obtained upon dilution with pure water was least-squares fit to a line of the form $y = 22700[\text{sap}] - 0.254$, and used to provide a corrected A_o (at 413 nm) for each of the absorption points, A (again at 413 nm), corresponding to each given sapphyrin and pertechnetate concentration ([sap], [Tc]). The difference between these corrected A_o values and the experimental absorbance values was then divided by the total sapphyrin concentration ([sap]) at each point to correct for dilution effects. In accord with Eq. (4.5) of Connors (modified by dividing both sides by [sap]), which corresponds to an idealized 1:1 binding profile [49], a plot of the resulting values vs. [Tc] was then constructed. A computer fit of the resulting experimental points yielded an effective K value of $3900 \pm 300\ \text{M}^{-1}$, as shown in Fig. 6. This does not represent a true equilibrium constant, per se, but rather a preliminary attempt to quantify the binding/deaggregation phenomenon.

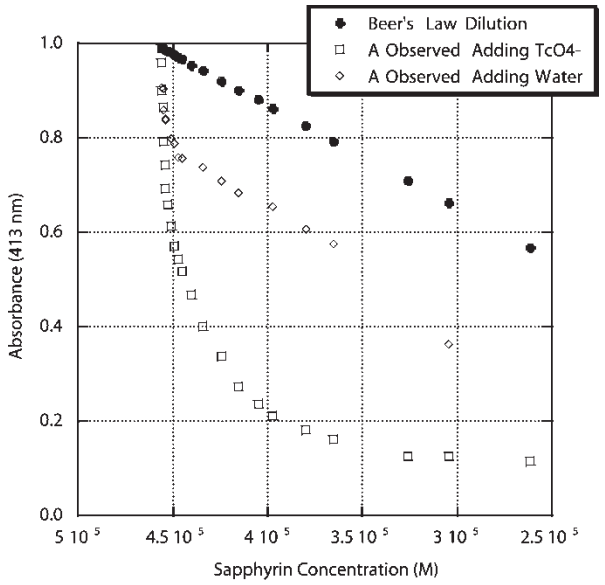

FIGURE 5 Absorbance at 413 nm as a function of concentration of sapphyrin **1** for a simple Beer's law dilution, for a 4.6 μM solution of sapphyrin **1** titrated with TcO_4^-, and for a 4.6 μM solution of sapphyrin **1** diluted with pure water.

Although this value is considerably smaller than that seen in the case of phosphate, this finding is not

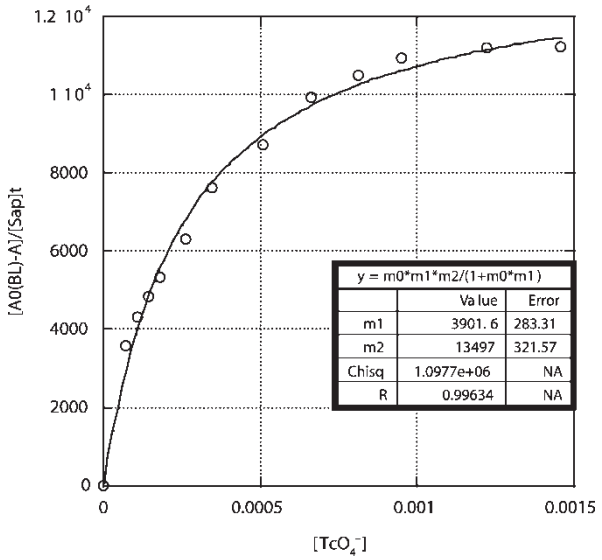

FIGURE 6 Plot of $(A_o - A)/[Sap]_t$ vs. $[TcO_4^-]$ for the titration of sapphyrin **1** with pertechnetate anion, including the computer-generated curve fit used to estimate the equilibrium constant *K*.

surprising in light of the greater net charge density present on phosphate and the higher basicity of the corresponding oxyanion. Nonetheless, the present semi-quantitative analysis underscores the fact that sapphyrins act as effective receptors for pertechnetate anion in neutral aqueous media.

CONCLUSIONS

In the developing field of anion-recognition chemistry, there are still a great number of fundamental challenges, including in particular the design of ligands that are selective for tetrahedral anions such as pertechnetate, that are environmentally relevant. Here, key questions are what should be the basis for such the design, as well as what kinds of donor systems provide for the best level of binding and selectivity for a given level of charge. In this report, evidence has been put forth that supports the contention that water-solubilized monoprotonated sapphyrins (e.g. **1**), although highly aggregated at neutral pH in aqueous media, are capable of interacting strongly with the pertechnetate anion, displaying effective *K* values for the combined binding and deaggregation process that are of the order of $4000\,M^{-1}$ in the case of the prototypical tetrahydroxy sapphyrin **1**. The fact that pertechnetate is bound well by sapphyrin is not surprising since, like phosphate, it is a tetrahedral anion. Likewise, that pertechnetate is less well bound is also not surprising, given that the net charge density on the pertechnetate oxyanion is less than that present in the case of phosphate. In any event, the strong nature of the interaction between aggregated water-soluble sapphyrins and the pertechnetate anion argues in favor of the sapphyrins finding potential application in the areas of pertechnetate recognition, sensing, and remediation. Studies designed to explore the merits of this possibility are currently in progress.

EXPERIMENTAL

The water-soluble sapphyrin, 3,12,13,22-tetraethyl-8,17-bis[bis(hydroxyethyl)amino)carbonylethyl]-2,7,18,23-tetramethylsapphyrin (**1**), used in this study was prepared and purified in the Sessler labs at the University of Texas at Austin using published procedures [50–52]. Methanol and all other organic solvents were used as received from Aldrich. Water was purified via a Beckman Millipure Ion Exchange system, verified to be pH neutral with a pH meter, and used immediately. The 0.072 M pertechnetate anion solution was provided by Oak Ridge National Laboratory, and the counter ion was mostly sodium. There was no complete assay of this substrate, which may also contain nitrate. UV–Vis characterization of radioactive samples took place at the laboratories at Los Alamos National Laboratory. When required, standard radiochemical procedures were used and operations were performed inside HEPA-filtered fume hoods designed for containment of radioactive materials. UV–Vis characterization of the sapphyrins with pertechnetate was done using a Cary 500 UV–Vis spectrometer with a remote sample holder to prevent contamination of the source and instrument and to contain the cells within the hood. Care was taken to exclude air and CO_2 whenever possible.

UV–Vis Characterization of Sapphyrin–Pertechnetate Anion Recognition Study

Stock solutions of the pertechnetate substrate suitable for use in the titrations with sapphyrin were prepared using a stock solution obtained from Hanford. Specifically, a 1 mL aliquot of this 0.072 M pertechnetate solution was diluted to 10 mL with deionized water yielding a stock 7.2×10^{-3} M TcO_4^- solution, which was shown to be neutral by pH indicating paper.

Stock solutions of the sapphyrin were prepared by dissolving and diluting **1** (16.0 mg) to 10 mL in a 10 mL volumetric flask with MeOH. This solution was then diluted further by adding 39 parts millipure H_2O to one part of the original solution. This gave a 4.64×10^{-5} M solution that is 2.5% in methanol, which was prepared fresh prior to each experiment and kept sealed when not in use.

The general procedure for the pertechnetate binding studies involved making sequential additions of titrant using an autopipette to a 2 mL aliquot of the stock solution in the spectrometric cell. A pipette was used to agitate the solutions after each subsequent addition prior to collecting the UV–Vis spectral data. The data was then collated and combined to demonstrate changes in the spectra based on changes in the concentration of the pertechnetate titrant. The effects of dilution were accounted for by mathematical manipulation of the data.

Calculations of Effective Equilibrium Constants, *K*

Effective equilibrium constants were calculated using Eq. (4.5) of Connors [49] with the exception that the term $[sap]_t$, referring to the total sapphyrin concentration, was moved from the right (x) to left (y) side of the equation by dividing both sides by this term. The resulting equation, of the form, $y = m0{\cdot}m1{\cdot}m2/(1 + m0{\cdot}m1)$, was computer fit using Kaleidegraph version 3.5.2, yielding the equilibrium constant, K, as $m1$. This equation requires $[anion]_f$, the concentration of the free anion, as the input parameter. Under the conditions of the present experiments, this term may be estimated as the total anion concentration, $[anion]_t$, when the K value is less than ca. $5000\,M^{-1}$. For large K values, the free anion concentration was estimated using an interative procedure wherein the K value was first calculated using the assumption that $[anion]_f = [anion]_t$. The resulting K was then used to calculate the $[anion]_f$ using the equilibrium expression and the mass balance equation, assuming a 1:1 equilibrium. The $[anion]_f$ value obtained in this way was then used to redetermine the K value, which, in turn, was used to re-estimate the free anion concentration. This procedure was repeated iteratively until convergence was observed. In the case of the pertechnetate anion binding studies, variations in the sapphyrin concentration and the effects of water-induced deaggregation were accounted for using the procedure described in the text proper.

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