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Calculation of one-electron reduction potentials for nitroheterocyclic hypoxia-selective agents

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Theoretical one-electron reduction potentials, E^1, have been determined for a set of eight nitroarene hypoxic cell radiosensitisers using a combination of classical statistical mechanics and quantum mechanical methods. Gas-phase electron affinities were calculated using *ab initio* Hartree–Fock calculations and relative hydration energies were computed using the free energy perturbation (FEP) method. The results were used to estimate the relative one-electron reduction potentials for these molecules in solution. In general, the computed results are in good agreement with experiment although further work is required to determine the limitations of the method. Nevertheless, the method shows sufficient promise to be of value in the rational design of improved oxidative agents for use as hypoxia-selective radiosensitisers and bioreductivity activated cytotoxins.

Accurate theoretical prediction of electrode potentials would be of great benefit in many areas of medicinal chemistry. In particular, the efficacy of many bioreductively activated chemotherapeutic agents can frequently be related to their redox behaviour. Radiotherapy is widely used to combat many cancers, although the effective local control of solid tumours is ultimately limited by the presence of a population of viable oxygen-deficient (hypoxic) cells which resists sterilisation by classical treatments.[1–3] Cells in hypoxic regions of solid tumours are often resistant towards the effects of ionising radiation owing to the lack of molecular oxygen which is required as an electron acceptor for the manifestation of damage to DNA. Fortunately, many electron-affinic nitroheterocycles function as mimics of oxygen in this process, and can therefore be effective radiosensitisers of hypoxic cells. Clinical strategies based on the combined use of hypoxia-selective agents (HSA) and radiotherapy (and/or chemotherapy) are currently being developed to exploit the ability of these compounds to enhance the sensitivity of the resistant hypoxic cell fraction selectively whilst having a minimal effect upon normal or oxygen-sufficient tissue.[2–5]

The efficiency of a compound in differentially sensitising oxygen-deficient or hypoxic cells towards the lethal effects of ionising radiation has been shown to be critically dependent upon its ability to behave as a one-electron oxidant.[2,4] The mechanism of hypoxic cell radiosensitisation involves one-electron transfer at a radiation-induced lesion, thereby effecting fixation of the chemical damage. A multiple linear regression analysis of 35 nitroaromatic and nitroheterocyclic compounds has predicted a semi-empirical structure–activity relationship[6] for radiosensitising potency *in vitro*:

$$-\log(C_{1.6}/\text{mol dm}^{-3}) = 6.54 + 8.21(E^1_7/\text{V}) \quad (1)$$

where $C_{1.6}$ is the concentration of added compound required to effect a fixed level (1.6-fold factor) of increased relative radiosensitivity, and E^1 is the one-electron reduction potential (normally determined at pH 7). Thus, a change of 100 mV in the reduction potential corresponds to a *ca.* 10-fold change in nett potency. The radiosensitising efficiency of an 'electron-affinic' compound towards hypoxic cells, both *in vitro* and *in vivo*, is hence largely predicted by the E^1 parameter.

The cytotoxic properties of many such agents have also been found to correlate with the one-electron reduction potential[7] such that the chronic aerobic cytotoxicity *in vitro* is given by:

$$-\log(C_{50\%}/\text{mol dm}^{-3}) = 6.59 + 8.40(E^1_7/\text{V}) \quad (2)$$

It is evident that increasing the hypoxic cell radiosensitising efficiency is associated with unwanted elevated cytotoxicity, suggesting that potential radiosensitisers should have an E^1 reduction potential in the -300 to -500 mV range.[1,2,6,7]

Misonidazole, a 1-substituted-2-nitroimidazole, was the first hypoxic cell radiosensitiser to be extensively investigated in the clinic. However, the overall results were disappointing owing to the inherent neurological toxicity of this heterocycle. Experimental studies in rodents have now established that the neurotoxic properties of misonidazole and other nitroimidazole sensitisers are directly related to the lipophilic properties of the compound.[8] This observation was exploited to develop agents with reduced toxicity by altering the nature of the substituent on the heterocycle ring. Etanidazole, a drug now in clinical trials,[9,10] was subsequently designed to be less neurotoxic by being highly polar and therefore less able to cross the blood-brain barrier. This demonstrates that judicious tailoring of the substituent on the electron-affinic nitroheteroarene ring should be able to reduce undesirable side-effects and thereby lead to the design of improved chemotherapeutic adjuncts for radiotherapy.

The correlation between the one-electron reduction potential and the radiosensitising efficiency of these heterocyclic compounds offers the possibility of using well established techniques in computational chemistry to design better radiosensitisers. Such derivatives may also have further application as hypoxia-mediated bioreductively activated cytotoxins.[1,2] Reynolds *et al.*[11–13] have calculated two-electron reduction potentials for a series of quinones (with an error of *ca.* ±25 mV) by combining gas-phase Hartree–Fock calculations with classical simulations in aqueous solution. In this paper we have applied a similar methodology and calculated the one-electron reduction potentials for a set of eight nitroheteroarenes. The present results generally agree well with experimental data (with a typical error of *ca.* ±45 mV). However, our investigations of different protocols for both the

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quantum and classical components of such calculations show that improving the accuracy of these calculations may not be a simple matter of using more computer time in increasingly more sophisticated quantum mechanical methods and/or longer classical simulations.

Theoretical methodology

Background

The most promising theoretical approaches for including the effects of solvation on chemical processes are based on quasi-quantum mechanical/classical mechanics (QM/CM) methods. The underlying assumption of such methods is that the nett change in energy for this process can be decomposed into two components, each of which can be calculated separately:

$$\Delta E = \Delta E_{QM} - \Delta E_{CM} \quad (3)$$

where ΔE is the total change in energy in the solution state, ΔE_{QM} is the calculated change in energy for the gas-phase species (determined by *ab initio* Hartree–Fock methods), and ΔE_{CM} is the change in solvation energy [determined by molecular mechanics (MM), molecular dynamics (MD) or Monte Carlo simulation procedures]. Such methods have already been used with considerable success to study chemical reactions in solution.[14–16] In the present study, we have adopted a similar procedure to that described by Reynolds *et al.*[11–13] to calculate the difference in one-electron reduction potential between two nitroheteroarenes, A and B.

The methodology adopted is as follows. The difference in reduction potential between two candidate molecules (A and B) can be derived using

$$A_{aq} + B_{aq}^{\bullet -} \leftrightarrow A_{aq}^{\bullet -} + B_{aq} : \Delta\Delta G = -nF(E_q^A - E_q^B) \quad (4)$$

where E_q^A and E_q^B are, respectively, the one-electron reduction potentials for the two ground state species. This process is determined from the following thermodynamic cycle:

$$\begin{array}{ccccc} A_g & + & B_g^{\bullet -} & \xrightarrow{\Delta\Delta G_g} & A_g^{\bullet -} & + & B_g \\ \downarrow \Delta_{hyd}G(A) & & \downarrow \Delta_{hyd}G(B^{\bullet -}) & & \downarrow \Delta_{hyd}G(A^{\bullet -}) & & \downarrow \Delta_{hyd}G(B) \\ A_{aq} & + & B_{aq}^{\bullet -} & \xrightarrow[\Delta\Delta G_{aq}]{} & A_{aq}^{\bullet -} & + & B_{aq} \end{array}$$

It follows from this cycle that the difference in electron affinities for A and B in aqueous solution is given by:

$$\Delta\Delta G_{aq} = \Delta\Delta G_g + [\Delta_{hyd}G(B) - \Delta_{hyd}G(A)] + [\Delta_{hyd}G(A^{\bullet -}) - \Delta_{hyd}G(B^{\bullet -})] \quad (5)$$

where $\Delta\Delta G_g$ is the difference in the gas-phase electron affinities for A and B, and can be determined from Hartree–Fock calculations. The $[\Delta_{hyd}G(B) - \Delta_{hyd}G(A)]$ term represents the relative free energy of hydration of A and B; similarly, $[\Delta_{hyd}G(A^{\bullet -}) - \Delta_{hyd}G(B^{\bullet -})]$ is the relative free energy of hydration for the corresponding radical anions.

The relative free energies of hydration may be estimated by performing classical simulations on each molecule in water and using the configurations generated to calculate the average energetic properties. Free energy perturbation (FEP) methods use statistical mechanical relationships[17,18] to calculate the free energy difference ($\Delta\Delta G_{XY}$) between two systems of molecules (X and Y) experiencing the same macroscopic constraints (*e.g.* temperature, volume and/or pressure):

$$\Delta G_{XY} = -RT \ln\langle\exp\{[U(r)_X - U(r)_Y]/RT\}\rangle X \quad (6)$$

where $[U(r)_X - U(r)_Y]$ is the potential-energy difference between the two systems with respect to a particular configuration r, and $\langle\ \rangle X$ indicates that the quantity in parentheses is an ensemble average over all configurations of system X.

It is usually impossible to generate all the configurations of a system and Boltzmann weight the probability of occurrence of each in order to find the exact ensemble average. Consequently, a Monte Carlo or MD simulation of the system is used to produce a (hopefully) representative sample of configurations which are used to estimate the true ensemble average.

If the low-energy equilibrium configurations of the two systems are substantially different, the average in eqn. (6) will converge slowly. In order to avoid this problem, one exploits the fact that free energy is a state function and constructs a series of (unphysical) systems intermediate between X and Y. By mutating system X into Y in a stepwise manner, the change in free energy can be computed at each step, and the difference in free energy between systems X and Y can be obtained by the summation of all steps. As we are interested in calculating the difference in free energy of hydration between two similar solutes, the intermediate states correspond to a stepwise mutation of solute A into B. The potential functions $U(r, \lambda)$, representing the interactions of the intermediate solutes are defined by introducing a variable λ, such that $U(r, \lambda) = U_A(r) + \lambda[U_B(r) - U_A(r)]$. Intermediate states are created by incrementing λ in steps of $\Delta\lambda$ from 0 to 1. Such linear transformation of the initial solute to the final solute, in conjunction with a small value for $\Delta\lambda$ (we have used $\Delta\lambda = 0.05$), has been found to produce estimates of the free energy difference for each individual step which generally converge quickly for mutations involving a single chemical group.

Theoretical calculations

Theoretical calculations were performed for the eight methyl-substituted nitroheteroarenes shown in Fig. 1. These compounds were chosen because they are structurally similar and exhibit a wide range in E^1 potential (Table 1), and are thus ideal for investigating the validity of theoretical methods. Using the methodology described we have estimated the change in free energy for the following half-cell reactions: **1** → **2**, **1** → **6**, **2** → **4**, **2** → **7**, **6** → **3**, **7** → **5** and **7** → **8**, where the

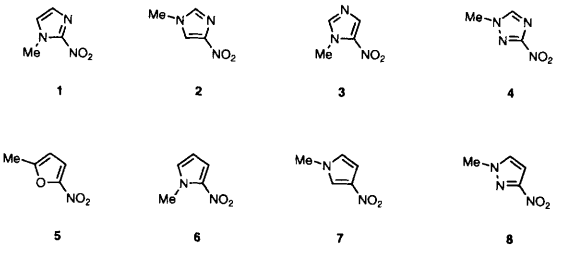

Fig. 1 The eight nitroheteroarenes examined in this redox study

Table 1 Experimental E^1 and ΔG° values for the simple methyl-substituted nitroheteroarenes

compound	$-E^1$/mV[a]	ΔG°_{expt}/kJ mol^{-1}
1	389[b,c,d]	37.53
2	527[d]	50.85
3	475[d]	45.83
4	325[e]	31.86
5	250–350[d,f,g] (330[h])	24.12–33.77 (31.55)
6	581[i]	56.06
7	657[i]	63.39
8	456[i]	44.00

[a] One-electron reduction potentials are referenced to the standard hydrogen electrode (SHE) at pH 7, with free energy change corresponding to $\Delta G^\circ_{expt} = -nFE^1$; estimated errors ±5–10 mV. [b] Ref. 38. [c] Ref. 39. [d] Ref. 40. [e] Ref. 41. [f] Ref. 42. [g] The redox potential is sensitive to the nature of the 2-alkyl furan ring substituent and is shown as a range from reported data. [h] Value for the 2-methoxymethyl analogue (from ref. 40). [i] Ref. 43.

reaction A → B corresponds to the $A^{\bullet -} + B \rightarrow A + B^{\bullet -}$ one-electron transfer process.

Free energy perturbation (FEP) calculations

Accurate estimates of hydration energies can only be obtained if the FEP simulations sample statistically representative regions of phase space. In particular, this can lead to difficulties if molecules A and B are too dissimilar; this problem can be partially alleviated by choosing pairs of molecules that are structurally closely related. Therefore, the series of half-cell reactions was chosen to minimise the structural change involved in each case. This, in principle, also allows one to calculate the relative electron affinity for any pair of molecules by summing the results for a series of mutations which converts one molecule to another. Inspection of Fig. 1 shows that the pairs of molecules involved in each reaction are generally isostructural. The one notable exception is the **1** → **2** reaction, which involves changing the position of the methyl group on the heterocyclic ring.

All simulations were run using the all-atom AMBER forcefield[19,20] and the AMBER suite of programs.[21] The FEP calculations for the neutral ground-state molecules were performed as follows. Each molecule was solvated in a rectangular box containing about 350 TIP3P water molecules.[22] Periodic boundary conditions and a non-bonded cut-off value of 1 nm were employed. The system was initially subjected to 4000 cycles of MM minimisation (using conjugate gradients), followed by 5 ps of MD equilibration at constant temperature (298 K) and volume. This was then followed by a further 5 ps of equilibration at constant temperature and pressure (298 K, 1 atm). In both equilibration phases, Berendsen's algorithm[23] was used to approximate the appropriate ensemble (NVT, NTP†). The SHAKE algorithm[24] was used to constrain bonds and allow an integration time step of 2 fs. FEP calculations were carried out over 21 windows, a number which we have previously found sufficient to avoid significant forward/backward hysteresis for FEP simulations on a variety of other systems.[25] Following the methodology employed by Reynolds *et al.*,[11,12] each window comprised 1 ps of equilibrium followed by 1 ps of data collection. The total time for the FEP simulations was 42 ps.

Recent studies have shown that these equilibration and sampling times are the minimum required to avoid errors due to correlation between successive windows.[26,27] However, there is no *a priori* reason to expect that the data collection time, in particular, is long enough to ensure convergence of the energetic averages. Consequently, longer FEP simulations (105 ps) were also run to assess the reliability of the shorter simulations. Again, 21 windows were used: 1.5 ps of equilibration followed by 3.5 ps of data collection. Differences between the results of these two sets of independent simulations arise from both statistical and systematic sources. These differences provide a good overall estimate of the error in the FEP results. In simulations where atoms are being created or destroyed, dummy atoms were used to maintain the topologies of A and B. The bond lengths involving dummy atoms were set to 0.02 nm; this choice is somewhat arbitrary, but previous reported simulations indicate that this value is suitable.[28]

A similar protocol was used for the radical anions. However, a larger simulation box containing about 630 water molecules, together with a longer non-bonded cut-off of 1.2 nm, was used in each case to allow for longer-range ordering of the solvent due to the negative charge on the solute. The results of other studies suggest that larger simulation boxes and longer cut-offs produce better results for the free energy differences between charged system.[29] It was necessary to use a shorter integration time step (1 fs) as the larger electrostatic forces present in the charged system caused the SHAKE algorithm to fail to converge when larger time steps were used. Atom-centred charges were calculated for both the ground states and the radical anions with a 6-31G* basis set using the method suggested by Singh and Kollman,[30] where a least-squares algorithm is used to produce a set of point charges which best reproduce the SCF electrostatic potential calculated at numerous points on the molecular surface. This approach has been found to produce fairly accurate values for the relative free energies of a large number of small molecules and ions.[31,32]

All 42 ps simulations were carried out on a Convex C2 computer (Birkbeck College), whereas the longer 105 ps simulations were performed using CRAY XMP and YMP supercomputers (ATLAS).

Hartree–Fock calculations

The FEP calculations yield only the intermolecular contribution to the difference in free energies between A and B. The intramolecular component has been calculated using *ab initio* Hartree–Fock calculations. All calculations were carried out using GAMESS-UK.[33] The geometries of the neutral (ground-state) molecules and the corresponding radical anions were optimised at the SCF level (RHF for the ground state and ROHF for the radical anions) using a split-valence 3-21G basis set.[34] These geometries were then used for more accurate calculations at the SCF and Møller–Plesset second-order perturbation theory[35] (MP2) levels using a 6-31G* basis set[36] (these calculations are here denoted 6-31G*//3-21G, corresponding to use of a 6-31G* basis set and the 3-21G optimised geometry). This approach has proved successful for the calculation of two-electron reduction potentials for a set of substituted quinones.[11–13] The difference in the calculated electron affinities (for A and B) yields only the change in enthalpy for the gas-phase reaction at 0 K. Therefore, the zero-point corrections (zero-point energy, entropy and thermal contributions) at 298 K were calculated by optimising the geometries of the neutral molecules and radical anions using the semi-empirical PM3 method[37] and performing frequency calculations on the optimised geometries. All *ab initio* calculations were performed on a CRAY XMP supercomputer (Rutherford Appleton Laboratory).

Results

Experimental one-electron reduction potentials (E^1 and ΔG°) for the eight compounds in Fig. 1, determined using pulse radiolysis-based equilibrium and/or kinetic methods,[38–43] are shown in Table 1. Reliable E^1 values have generally been reported only for compounds with higher alkyl substituents than a simple methyl group; thus, in general, we have assumed that replacement of the longer alkyl side-chain is relatively unimportant to the overall redox behaviour.[40] A wide range of E^1 data has been reported[39,40,42] for 5-nitro-2-furan derivatives (Table 1), but a reduction potential is available only for the strict 2-methoxymethyl analogue ($E^1_7 = -338$ mV [40]). Interpolation techniques based upon linear free-energy relationships have been used to derive E^1 values for candidate molecules[44] but could not be applied in the case of nitrofuran **5**, owing to the paucity of available data determined for structurally related furan analogues. Thus, we have assumed both mean (*i.e.* −300 mV) and estimated (−330 mV) values for the 2-methyl-5-nitrofuran, **5**, and these values are used for purposes of comparison.

The *ab initio* energies for the neutral ground-state molecules and their derived radical anion one-electron adducts (6-31G* SCF and MP2) are collected in Table 2, together with the

† NVT: constant number of particles, volume and temperature. NPT: constant number of particles, pressure and temperature.

Table 2 6-31G* SCF and MP2 energies[a] for the neutral molecules (RHF) and radical anions (UHF), and computed electron affinities (E_{ea})

	SCF energies/au[b]			MP2 energies/au[b]		
molecule	neutral molecule	radical anion	E_{ea} /kJ mol^{-1}	neutral molecule	radical anion	E_{ea} /kJ mol^{-1}
1	−467.296 86	−467.292 23	12.18	−468.648 85	−468.609 11	104.35
2	−467.307 32	−467.286 73	54.06	−468.652 13	−468.592 11	157.56
3	−467.304 03	−467.304 12	−0.25	−468.650 31	−468.615 26	92.01
4	−483.285 12	−483.272 13	34.10	−484.667 41	−484.614 34	139.33
5	−471.123 72	−471.120 23	9.25	−472.449 07	−472.399 60	129.87
6	−451.300 92	−451.292 73	21.51	−452.620 09	−452.573 26	122.93
7	−451.307 52	−451.285 34	58.24	−452.621 06	−452.558 36	164.60
8	−467.284 11	−467.267 48	43.64	−468.637 49	−468.576 35	160.54

[a] Only valence electrons were used in the MP2 calculations; the core electrons and the corresponding anti-bonding core orbitals were omitted. All geometries were optimised using the 3-21G basis set. [b] 1 au = 2625.50 kJ mol^{-1}.

calculated gas-phase electron affinities. The zero-point energies, thermal enthalpies and entropies are shown in Table 3. The computed FEP simulation results for the mutations: **1** → **2**, **1** → **6**, **2** → **4**, **2** → **7**, **6** → **3**, **7** → **5** and **7** → **8** are shown in Table 4. Theoretical electrode potentials have been calculated by combining the results in Tables 2–4. The zero-point corrections were calculated by summing the zero-point energy, thermal enthalpy and entropy ($-T\Delta S$ at $T = 298.15$ K)

Table 3 Calculated PM3 zero-point energies, thermal enthalpies (ΔH) and entropies (ΔS) for the neutral nitroarenes and their one-electron adducts (radical anions)

	ground state			radical anion		
molecule	zero-point energy /kJ mol^{-1}	ΔH /kJ mol^{-1}	ΔS /J mol^{-1} K^{-1}	zero-point energy /kJ mol^{-1}	ΔH /kJ mol^{-1}	ΔS /J mol^{-1} K^{-1}
1	261.83	24.21	396.98	254.19	24.07	378.94
2	260.76	24.23	390.43	251.54	25.13	384.51
3	262.03	23.30	371.27	253.29	23.98	373.74
4	228.62	23.91	388.12	222.06	24.23	388.92
5	265.18	23.80	383.88	261.09	24.21	380.52
6	295.32	23.53	372.23	285.91	24.14	374.32
7	293.98	24.32	388.36	289.30	24.38	375.53
8	261.62	24.23	397.91	254.48	24.09	379.65

Table 4 Calculated hydration enthalpies for 42 ps and 105 ps FEP simulations

	$\Delta_{hyd}H$ (42 ps)/kJ mol^{-1}		$\Delta_{hyd}H$ (105 ps)/kJ mol^{-1}	
mutation	neutral molecule	radical anion	neutral molecule	radical anion
1 → **2**	12.34 ± 0.73	38.12 ± 0.88	13.97 ± 0.26	38.28 ± 0.30
1 → **6**	−10.84 ± 0.16	−14.10 ± 0.01	−12.38 ± 0.18	−12.09 ± 0.14
2 → **4**	0.46 ± 0.59	4.23 ± 0.12	1.63 ± 0.08	4.39 ± 0.10
2 → **7**	−18.37 ± 0.46	−20.17 ± 0.33	−19.25 ± 0.15	−22.30 ± 0.20
6 → **3**	16.95 ± 0.45	11.21 ± 0.55	16.82 ± 0.20	10.33 ± 0.15
7 → **5**	−6.44 ± 0.12	−23.51 ± 0.46	−7.57 ± 0.01	−24.52 ± 0.28
7 → **8**	4.81 ± 0.36	9.96 ± 0.36	6.28 ± 0.26	10.17 ± 0.14

Table 5 Experimental and calculated relative one-electron reduction energies estimated from 6-31G*//3-21G SCF and MP2 gas-phase wavefunctions

		6-31G*//3-21G SCF energies /kJ mol^{-1}				6-31G*//3-21G MP2 energies /kJ mol^{-1}	
reaction	$\Delta\Delta G^{\circ}_{expt}$[a] /kJ mol^{-1}	$\Delta\Delta G^{\circ}_{calc}$[b]	*ab initio*	zero-point correction	hydration energy[b]	$\Delta\Delta G^{\circ}_{calc}$[b]	*ab initio*
1 → **2**	13.32	13.43 (11.97)	41.92	−4.14	−24.31 (−25.77)	24.77 (23.26)	53.26
1 → **6**	18.53	2.05 (5.61)	9.33	−7.03	−0.29 (3.26)	11.29 (14.85)	18.61
2 → **4**	−19.49	−22.64 (−23.60)	−19.96	0.08	−2.76 (−3.72)	−20.92 (−21.88)	−18.24
2 → **7**	12.54	13.01 (11.76)	4.18	5.77	3.05 (1.80)	12.89 (11.67)	10.17
6 → **3**	−10.23	−14.64 (−15.40)	−21.76	0.63	6.49 (5.73)	−23.81 (−24.56)	−30.92
7 → **5**	−34.44[c] (−31.55[d])	−33.93 (−33.81)	−48.99	−1.88	16.95 (17.07)	−19.66 (−19.54)	−34.73
7 → **8**	−19.39	−19.54 (−20.79)	−14.60	−1.05	−3.89 (−5.15)	−9.00 (−10.25)	−4.06

[a] Calculated from E^1 values, using $\Delta\Delta G^{\circ}_{expt} = -nF\Delta E^1$ where $n = 1$, with uncertainty values of ±2.41 kJ mol^{-1} for estimated ΔE^1 errors of ±25 mV (*cf.* ref. 40). [b] Computed energies refer to 105 ps simulations; values in parentheses refer to shorter 42 ps simulations. [c] Mean value from the ΔG°_{expt} range for **5** in Table 1. [d] Calculated using $E^1 = -330$ mV for compound **5**.

for each species. The difference in the electron affinity for molecules A and B (*i.e.* $\Delta\Delta H_g$) yields the enthalpic contribution at 0 K for the reaction

$$A_g + B_g^{\bullet -} \rightarrow A_g^{\bullet -} + B_g \quad (7)$$

The free energy for the gas-phase reaction ($\Delta\Delta G_g$) is obtained by including the zero-point corrections Thus, the theoretical electrode potential can be determined by adding the calculated hydration free energies ($\Delta\Delta G_{hyd}$, Table 4) for the reactions:

$$A_{aq} \rightarrow B_{aq}; \quad B_{aq}^{\bullet -} \rightarrow A_{aq}^{\bullet -} \quad (8)$$

to the computed difference in free energy for eqn. (7).

The theoretical electrode potentials are shown in Table 5, where the 6-31G*//3-21G SCF results have been used to calculate the enthalpic component of the gas-phase reaction [eqn. (7)]. Also included in Table 5 is a breakdown of the calculated electrode potentials into three separate components: $\Delta\Delta H_g$, $\Delta\Delta_{hyd}G$ and the differences in zero-point corrections. The 6-31G*//3-21G MP2 results were used to calculate the electrode potentials in terms of the $\Delta\Delta G^\circ$ free energy difference.

Discussion

Inspection of Table 5 shows that the $\Delta\Delta G^\circ_{calc}$ values determined from the gas-phase *ab initio* SCF calculations are generally in good agreement with experiment. Only one reaction (**1** → **6**) gave very poor results; the computed value of 2.05 kJ mol^{-1} (5.61 kJ mol^{-1} for the 42 ps simulation) compares very unfavourably with the experimental value (18.53 kJ mol^{-1}). In contrast, the predicted $\Delta\Delta G^\circ_{calc}$ values for the remaining half-cells agree reasonably well with experiment, with results from the 105 ps FEP simulations that are within ±5 kJ mol^{-1} of the experimental value. The results obtained with the shorter 42 ps FEP simulations are also surprisingly good, with the poorest correspondence obtained with the **6** → **3** half-cell.

Comparison of the calculated free energies of hydration (Table 4) indicates that the results from the 42 ps simulations compare very favourably to those from the longer 105 ps simulations, with the largest difference (2.13 kJ mol^{-1}) observed for the **2** → **7** mutation. This result is encouraging as it suggests that reliable free energies of hydration can be obtained with shorter FEP simulations.

In general, the contribution of hydration energy to the predicted electrode potential is relatively small. Indeed, the largest effect due to hydration is observed for the **1** → **2** reaction; this is not too surprising as it involves ring migration of the methyl substituent. Intuitively, the position of the alkyl group would be expected to have a marked influence on the structure of the primary solvation shell and thus to influence the free energy of hydration significantly. It is encouraging that the predicted result for the **1** → **2** half-cell is in good agreement with the available experimental data, since this emphasises the fact that the substituent position can influence the E^1 potential by (i) electronic interaction with the aromatic ring, and (ii) altering the structure of the primary solvation shell. Clearly, the topology of the solute has a marked influence on the solvation energy and ideally this should be included explicitly in any theoretical model used to calculate solvation energies.

In general, the effects of solvation are small when molecules A and B are isostructural (*e.g.* >CH is replaced by >NH or >N: *etc.*). One exception is the **7** → **5** reaction, where >CH is replaced by >O; presumably the difference in solvation energies can be attributed largely to the hydrophobic >CH group being replaced by the highly electronegative oxygen atom of the furan ring.

However, it should be stressed that these calculations are also likely to be sensitive to the protonation status of the radical-anion species, particularly as the pK_a values determined for certain one-electron reduced nitroarenes are reported to be in the range *ca.* 6–8.[45] Such behaviour has a marked influence upon the E^1 value determined at physiological pH.[40] Our present calculations have been restricted to the formal radical anions rather than the corresponding neutral protonated radicals. Uncertainties relating to both the pK_a values of the one-electron adducts and their likely structures in aqueous solution necessarily precluded a systematic study of possible protonation effects. Similarly, no attempt was made to examine the effects of co-solutes upon the redox behaviour, although the E^1 parameters are known to be sensitive to ionic strength and solvent.[40,45,46]

Electrode potentials calculated using MP2 electron affinities are collected in Table 5. The MP2 results are significantly worse when compared with experiment. In general, the MP2 results differ from the experimental electrode potentials by 8–12 kJ mol^{-1}. It is not clear why the MP2 results are so poor since calculations which include electron correlation are generally more reliable than SCF results. Inspection of Table 5 indicates that the largest contribution to the calculated electrode potential is invariably due to $\Delta\Delta H_g$. The good agreement between theory and experiment for the SCF results may possibly be due to a fortuitous cancellation of errors.

The poor value obtained using the SCF electron affinities for the **1** → **6** reaction may thus be the result of errors failing to cancel. More accurate calculations (*e.g.* MP3, MP4) using larger basis sets are required to identify the nature of the errors involved, but this was beyond the scope of the present study (and the available computational resources). Nevertheless, this result is a little disconcerting because the large error associated with the **1** → **6** reactions means that one cannot simply combine the results obtained for two reactions and expect to get an accurate result for another reaction. Thus, for example, combining the theoretical results obtained for the **1** → **6** and **6** → **3** reactions would produce an erroneous result for the **1** → **3** reaction.

In conclusion, our initial attempts at combining free energy perturbation (FEP) calculations with Hartree–Fock SCF theory in order to calculate one-electron reduction potentials in solution have been promising. Gas-phase calculations using a 6-31G* basis set in conjunction with FEP calculations using as little as 42 ps of simulation per pair of molecules generally predict E^1 values that are accurate to within ±45 mV (*i.e.* ±4.2 kJ mol^{-1}) of the reported potentials. Therefore, these results compare favourably to the two-electron reduction potentials calculated for a series of quinones,[11–13] where the predicted values were accurate to within ±25 mV (*i.e.* ±4.8 kJ mol^{-1} for an $n = 2$ process). However, the present results for the one-electron **1** → **6** half-cell indicate that the method is by no means wholly reliable and further work is required to identify the source(s) of the errors involved.

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References

1 T. C. Jenkins, in *Chemistry of Anti-Tumour Agents*, ed. D. E. V. Wilman, Blackie & Son, Glasgow, 1990, p. 342.
2 G. E. Adams, *Radiat. Res.*, 1992, **132**, 129.
3 S. Dische, in *Radiobiology in Radiotherapy*, ed. N. M. Bleehen, Springer-Verlag, London, 1989, ch. 15.
4 Collected papers, in *Radiation Sensitisers: Their Use in the Clinical Management of Cancer, Cancer Management*, ed. L. W. Brady, Masson, New York, 1980, vol. 5.
5 K. A. Kennedy, *Anti-Cancer Drug Des.*, 1987, **2**, 181.

6 G. E. Adams, E. D. Clarke, I. R. Flockhart, R. S. Jacobs, D. S. Sehmi, I. J. Stratford, P. Wardman and M. E. Watts, *Int. J. Radiat. Biol.*, 1979, **35**, 133.
7 G. E. Adams, E. D. Clarke, P. Gray, R. S. Jacobs, I. J. Stratford, P. Wardman and M. E. Watts, *Int. J. Radiat. Biol.*, 1979, **35**, 151.
8 T. H. Wasserman, T. L. Philips, G. van Raalte, R. Urtasun, J. Partington, D. Koziol, J. Schwade, D. Gangji and J. M. Strong, *Br. J. Radiol.*, 1980, **53**, 172.
9 C. N. Coleman, R. C. Urtasun, T. H. Wasserman, S. Hancock, J. W. Harris, J. Halsey and V. K. Hirst, *Int. J. Radiat. Oncol. Biol. Phys.*, 1984, **10**, 1749.
10 A. G. Beaman, W. Tautz and R. Duschinsky, *Antimicrob. Agents Chemoether.*, 1967, 5020.
11 C. A. Reynolds, P. M. King and W. G. Richards, *J. Chem. Soc., Chem. Commun.*, 1988, 1434.
12 C. A. Reynolds, P. M. King and W. G. Richards, *Nature (London)*, 1988, **334**, 80.
13 C. A. Reynolds, *J. Am. Chem. Soc.*, 1990, **112**, 7545.
14 J. Chandrasekhar, S. F. Smith and W. L. Jorgensen, *J. Am. Chem. Soc.*, 1985, **107**, 154.
15 S. J. Weiner, U. C. Singh and P. A. Kollman, *J. Am. Chem. Soc.*, 1985, **107**, 2219.
16 J. D. Madura and W. L. Jorgensen, *J. Am. Chem. Soc.*, 1986, **108**, 2517.
17 R. W. Zwanzig, *J. Chem. Phys.*, 1954, **22**, 1420.
18 D. L. Beveridge and F. M. Dicapua, *Annu. Rev. Biophys. Chem.*, 1989, **18**, 431–492.
19 S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta and P. J. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
20 S. J. Weiner, P. A. Kollman, D. T. Nguyen and D. A. Case, *J. Comput. Chem.*, 1986, **7**, 230.
21 AMBER (version 4). D. A. Pearlman, D. A. Case, J. C. Caldwell, G. L. Seibel, U. C. Singh, P. Weiner and P. A. Kollman, Department of Pharmaceutical Chemistry, University of California, San Francisco, USA, 1991.
22 W. L. Jorgensen, J. Chandrasekhar, J. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926.
23 H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola and J. R. Haak, *J. Chem. Phys.*, 1984, **81**, 3684.
24 J. P. Ryckaert, G. Cicotti and H. J. C. Berendsen, *J. Comput. Phys.*, 1977, **23**, 327.
25 M. A. Williams, Ph.D. Thesis, 1993, Birkbeck College, University of London.
26 D. A. Pearlman, *J. Comput. Chem.*, 1994, **15**, 105.
27 D. A. Pearlman and P. A. Kollman, *J. Chem. Phys.*, 1988, **91**, 7831.
28 D. A. Pearlman and P. A. Kollman, in *Computer Simulation of Biomolecular Systems*, ed. W. F. van Gunsteren and P. K. Weiner, ESCOM, Leiden, 1989, p. 101.
29 T. P. Straatsma and H. J. C. Berendsen, *J. Chem. Phys.*, 1988, **89**, 5876.
30 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129.
31 H. A. Carlson, T. B. Nguyen, M. Orozco and W. L. Jorgensen, *J. Comput. Chem.*, 1993, **14**, 240.
32 B. G. Rao and U. C. Singh, *J. Am. Chem. Soc.*, 1989, **111**, 3125.
33 GAMESS-UK: M. F. Guest, J. Kendrick, J. H. van Lenthe, K. Schoffel and P. Sherwood.
34 J. S. Binkley, J. A. Pople and W. J. Hehre, *J. Am. Chem. Soc.*, 1980, **102**, 939.
35 C. Møller and M. S. Plesset, *Phys. Rev.*, 1934, **46**, 618.
36 P. C. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213.
37 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209; 221.
38 G. E. Adams, I. R. Flockhart, C. E. Smithen, I. J. Stratford, P. Wardman and M. E. Watts, *Radiat. Res.*, 1976, **67**, 9.
39 P. Wardman and E. D. Clarke, *J. Chem. Soc., Faraday Trans. 1*, 1976, **72**, 1377.
40 P. Wardman, *J. Phys. Chem. Ref. Data*, 1989, **18**, 1637.
41 T. C. Jenkins, I. J. Stratford and M. A. Stephens, *Anti-Cancer Drug Des.*, 1989, **4**, 145.
42 M. A. Naylor, M. A. Stephens, S. Cole, M. D. Threadgill, I. J. Stratford, P. O'Neill, E. M. Fielden and G. E. Adams, *J. Med. Chem.*, 1990, **33**, 2508.
43 M. A. Naylor, M. A. Stephens, I. J. Stratford, A. Keohane, P. O'Neill, M. D. Threadgill, P. Webb, E. M. Fielden and G. E. Adams, *Anti-Cancer Drug Des.*, 1991, **6**, 151.
44 P. Wardman, in *Selective Activation of Drugs by Redox Processes*, ed. G. E. Adams, A. Breccia, E. M. Fielden and P. Wardman, Plenum Press, New York, 1990, p. 11.
45 P. Wardman, *Int. J. Radiat. Biol.*, 1975, **28**, 585.
46 P. Wardman, *Free Radical Res. Commun.*, 1990, **8**, 219.

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