Uranium(VI) Solubility and Speciation in Simulated Elemental Human Biological Fluids

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The complete understanding of the human body response to uranium contamination exposure is vital to the development of exposure analysis and subsequent treatments for overexposure. Thermodynamic modeling has traditionally been used to study environmental metal contaminant migration (especially uranium and other radionuclides), allowing examination of chemical processes difficult to study experimentally. However, such techniques are rarely used in the study of metal toxicology. Chemical thermodynamics has a unique and valuable role in developing models to explain metal metabolism and toxicology. Previous computational models of beryllium in simulated biological fluids have been shown to be useful in predicting metal behavior in the human body. However, previous studies utilizing chemical thermodynamics in understanding uranium chemistry in body fluids are limited. Here, a chemical thermodynamic speciation code has been used to model and understand the chemistry of uranium in simulated human biological fluids such as intracellular, interstitial, and plasma fluids, saliva, sweat, urine, bile, gastric juice, pancreatic fluid, and a number of airway surface fluids from patients with acute lung conditions. The results show predicted uranium solubility, and speciation varies markedly between each biological fluid due to differences in fluid composition, ionic strength, and pH. The formation of uranium hydroxide, phosphate (sodium/potassium autunite), and calcium uranate was observed in most of the fluids. The results of this work, supported by experimental validation, can aid in understanding the metabolism and toxic effects of uranium with potential applications to biological monitoring as well as chelation treatment of uranium body burden.

Introduction

Uranium has been widely used in applications beyond the nuclear industry. For example, the manufacture of Fiestaware tableware, popular until the 1960s, included uranium oxides resulting in a decorative orange color. The high density of depleted uranium (DU, 19.0 g/cm^3, almost twice that of lead at 11.3 g/cm^3) is exploited in balancing cargo weights in transport aircraft and ships, radiation shielding in shipping containers for radiopharmaceuticals, gyroscopes of inertial guidance systems, and in armor-piercing ammunition and armor plating. The human body naturally contains approximately 90 μg of uranium (*1*), and overexposure to uranium results in both chemical and radiological toxicity.

The radiological component of uranium toxicity is largely dependent on the isotopic proportions of the exposure. Higher proportions of enriched uranium result in radiological toxicity. Most uranium isotopes decay by emission of α-particles that have a large amount of energy to dissipate upon interaction with nearby biological materials. Therefore, uranium radiological toxicity is caused by internal exposure through inhalation, ingestion, or subcutaneous penetration. Uranium is known to be a bone seeker and readily replaces calcium in the bone structure, which can elevate long-term internal radiological exposure, slow the dissolution/exchange with interstitial fluid, and create the potential for bone cancer (*2*).

The chemical toxicity of overexposure is dictated by the chemical nature of the uranium exposure, which in turn affects uranium absorption, distribution, deposition, and excretion. For example, Scott (*3*) has described UF_6, UO_3, uranium sulfates, and carbonates as highly transportable from the lungs, whereas UO_2, U_3O_8, uranium carbides, and hydrides are only slightly transportable. Accidental exposures to soluble uranium are reported in the literature (*4*), of which many cases involved the inhalation of gaseous UF_6 leaking from uranium enrichment systems. Chemical toxicity primarily affects damage to the proximal tubule of the kidney, resulting in nephritis and proteinuria (*5*). The mechanism of nephrotoxicity may involve binding of uranium to the brush border membrane in the distal portion of the proximal tubules (*5*). The degree of renal damage is dependent upon the level of intake, and if overexposure is limited, kidney recovery generally can take several weeks provided there is no subsequent reexposure. High levels of exposure cause high urinary uranium levels, the greatest renal damage (as evidenced by albuminuria, red cells and casts in the urinary sediment, and elevated blood urea nitrogens),

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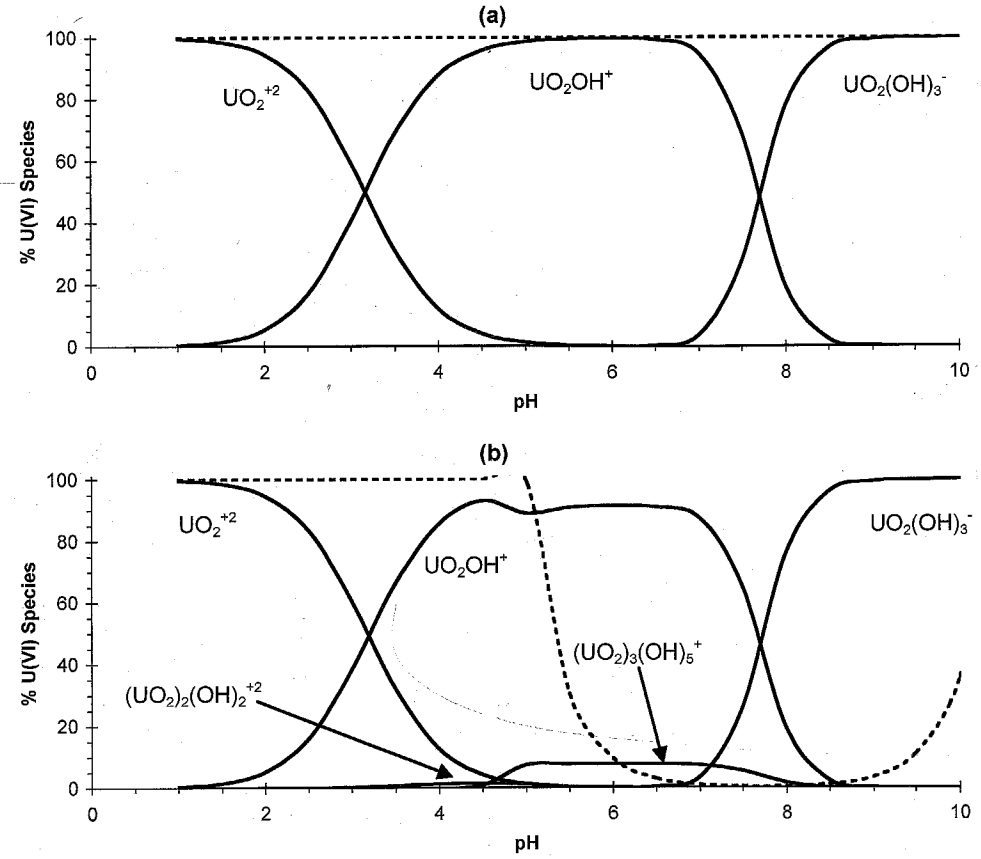

Figure 1. Uranium speciation and solubility with varying pH in the U(VI)−H_2O system at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

and death from renal failure (*2*). Research experience with chronic low-level exposure has been less conclusive. While earlier researchers (*3*) reported virtually no nephrotoxicity with chronic low-level exposures, a later study of uranium mill workers suggests that long-term low exposures can result in B_2 microglobinuria and amino aciduria (*6*), which is consistent with low grade renal toxicity involving the proximal tubule. However, there is a paucity of research on long-term health effects of low-level chronic exposure.

Recently, the use of DU ammunition and possible links between the exposure of military personnel to DU fine particles and Gulf War Syndrome (*2*, *7*−*9*) has generated much interest in the medical and environmental fate of DU. What is clear is that personnel exposed to DU shrapnel do exhibit elevated urinary uranium concentrations (*10*, *11*). However, there is much debate over the association of DU exposure and Gulf War Syndrome, especially when multiple potential environmental exposures (chemical/biological agents and vaccines) are also taken into account (*12*, *13*). Bleise (*14*) reported that with the exception of the crews of military vehicles having been hit by DU penetrators, no body burdens above the range of values for natural uranium have been found and that no observable health effects are expected.

Hexavalent uranyl compounds are generally slightly more soluble than the tetravalent uranium analogues, and the aqueous uranyl complexes are thermodynamically more stable in aerobic environments. The uranyl ion is also amphoteric; that is, it reacts with both acids and bases to form a mixture of positively and negatively charged species. The current treatment for uranium over exposure is outlined by the Radiation Emergency Assistance Center/Training Site provided by Oak Ridge National Laboratory and suggests that patients be given an infusion of 250 mL of a 1.4% sodium bicarbonate solution (*15*, *16*), increasing the pH of urine and consequently enhancing uranium excretion. At neutral and higher pH, exceptionally stable uranyl carbonate complexes are formed, $UO_2(CO_3)_2^{-2}$ and $UO_2(CO_3)_3^{-4}$. In 1949, it seemed somewhat doubtful that a more effective uranium poisoning treatment than bicarbonate would be found (*17*). In 2000, Stradling (*18*) reported that since bicarbonate administration is not effective for uranium dust and aerosol exposure, development of chelators remains an outstanding problem. Bicarbonate treatment is by no means specific for uranium, and several researchers have examined the role of chelation therapy as a treatment for reducing uranium body burden (*19*−*22*). New generations of chelators can be designed to be both effective and selective in removing toxic metals from the body while leaving other essential metals untouched (*23*, *24*). Over the past decade, considerable progress has been made in evaluating new treatment regimes and chelators for some actinides, but research to find new methods of uranium decorporation should be expedited through collaborations between clinicians and researchers (*25*). To develop an effective uranium chelator, it is also crucial to first understand the chemistry and distribution of uranium in body fluids. It is important to understand both the chemical environments that uranium will encounter during and after exposure and the chemical effects that might alter the stability and efficiency of uranium chelation and excretion. An overview of the contribution of chemical speciation to internal dosimetry is given by Paquet (*26*), who concludes that

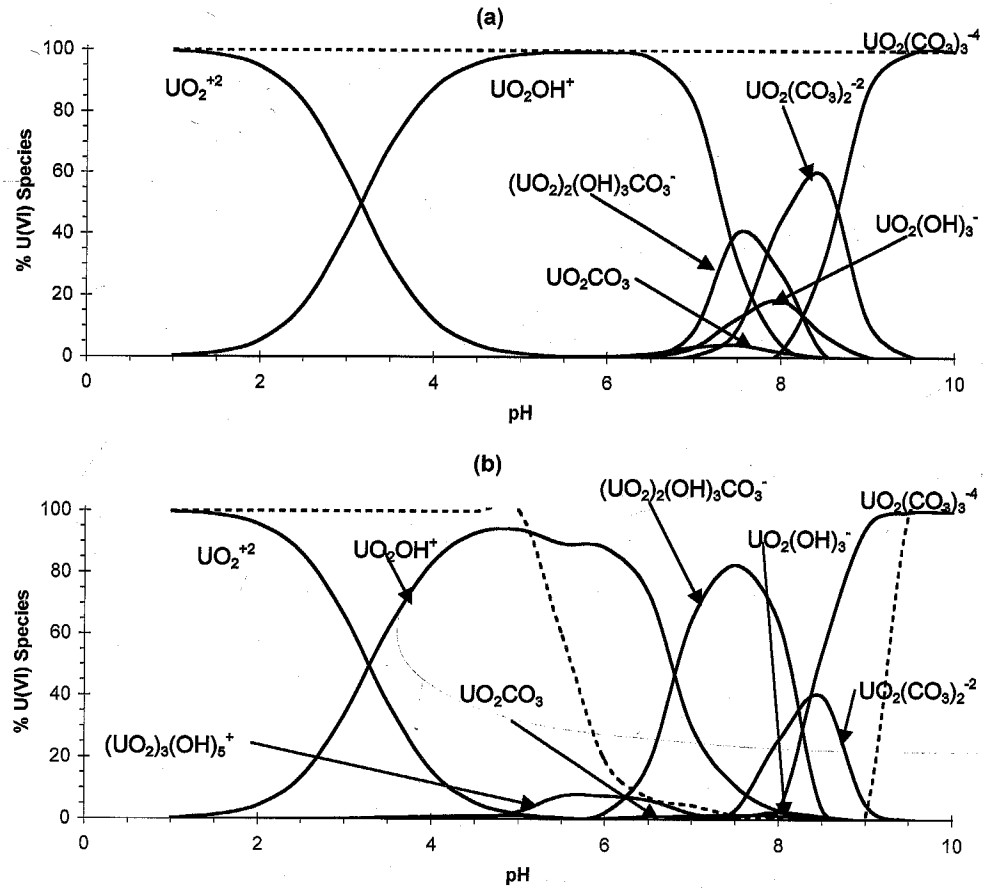

Figure 2. Uranium speciation and solubility with varying pH in the U(VI)−H_2O−CO_3 system at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

computer speciation modeling can be used to provide a first estimate of species distribution at equilibrium and that metal speciation with regard to human internal dosimetry will remain a challenge for the coming decades. Furthermore, previous models of uranium speciation in fluids using chemical thermodynamics (*27*, *28*) fall short of a complete assessment of uranium chemical interactions using thorough elemental fluid compositions, considering only a portion of the potential elemental interactions. Comprehensive chemical thermodynamic models have been used to study the environmental behavior of uranium(VI) with regard to nuclear waste disposal and serve to provide a better understanding of uranium chemistry (*29*, *30*).

This paper describes the speciation of uranium(VI) in a number of simulated elemental human body fluids by using chemical thermodynamics speciation and solubility modeling using methods previously described to study beryllium speciation in body fluids (*31*). The work is of importance to the understanding of uranium metabolism and toxicology. Modeling can provide an important insight into uranium chemistry in biological fluids and consequently aid in the development of chelation techniques or other methods to treat overexposures. Furthermore, if results can be applied to predicted urinary excretion of uranium, they may have application to biological monitoring for uranium exposure.

Materials and Methods

The elemental composition of human body fluids (intracellular fluid, interstitial fluid, plasma water, saliva, sweat, urine, bile, gastric juice, and pancreatic fluid) used in this study has been previously described (*31*). Interstitial fluid and airway surface fluid (ASF) concentrations are in the millimolar range and not nanomolar as stated. For ASF, only the cases were investigated as follows: (a) normal ASF, (b) ASF collected after treatment with an anticholinergic therapy, (c) ASF collected after treatment with benzodiazepine, and (d) ASF while in a hypersecretory state.

Model input files were generated using the PRODEFA2 code (*32*) for each of the body fluids. The thermodynamic data in the THERMO.DBS database (*32*) that accompanies the MINTEQA2v4 model were compared for compatibility and updated with critically reviewed chemical thermodynamics data from the NIST database (*33*, *34*) and Nuclear Energy Agency (NEA) thermodynamic uranium data set (*35*). Two concentrations of uranium were used to simulate the low uranium particle solubility in the biological environment (1 μM, 238 μg/L) and acute poisoning (1 mM, 238 mg/L), and the reaction temperature was set to 37 °C. For comparison, the estimated typical uranium lung burden in a Gulf War DU-positive veteran is 340 μg (*36*), while an occupationally exposed lung deposition is 3.1 mg (*37*). In cases where fluids are normally in contact with air, the system was equilibrated with atmospheric CO_2 gas (0.03%) to maintain carbonate concentrations especially in the cases where carbon was not detailed in the elemental composition. For a simulated fluid model, any element with a concentration less than 5% of the total lower uranium concentration was removed from the input data set. Once each model was programmed with element concentrations and pH, the MINTEQA2 code was run and the uranium speciation data were transferred to Microsoft Excel for data analysis.

The effect of pH on the uranium speciation was investigated for each fluid, and speciation was studied more rigorously at the actual biological pH of each fluid. For comparison, speciation models of uranium in water (CO_2 free) and air/water systems were also performed.

Table 1. Summary of Fluid pH, Modeled Uranium Speciation, Solubility, and Precipitation

biological fluid	pH	U(VI) speciation in dilute solutions		U(VI) speciation in concentrated solutions		U(VI) solubility	solubility limiting phase
intracellular	6	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	100%	12.8 mM	$K_2(UO_2)_2(PO_4)_2{\cdot}6H_2O$
	7.4	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	100%	4.9 mM	$K_2(UO_2)_2(PO_4)_2{\cdot}6H_2O$
interstitial	7.4	$UO_2(CO_3)_3^{-4}$	92%	$UO_2(CO_3)_3^{-4}$	92%	0.93 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
		$UO_2(CO_3)_2^{-2}$	8%	$UO_2(CO_3)_2^{-2}$	8%		
plasma	7.4	$UO_2(CO_3)_3^{-4}$	91%	$UO_2(CO_3)_3^{-4}$	91%	0.91 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
		$UO_2(CO_3)_2^{-2}$	8%	$UO_2(CO_3)_2^{-2}$	8%		
saliva	6	$UO_2(OH)_2aq$	78%	$(UO_2)_3(OH)_5^{+}$	58%	41.8 μM	$UO_2(OH)_2$
		UO_2OH^{+}	11%	$UO_2(OH)_2aq$	24%		
		UO_2CO_3aq	4%	$(UO_2)_4(OH)_7^{+}$	5%		
				$(UO_2)_2(OH_3)CO_3^{-}$	5%		
				UO_2CO_3aq	3%		
	7	$UO_2(OH)_2aq$	81%	$UO_2(OH)_2aq$	73%	0.44 μM	$CaUO_4$
		$(UO_2)_2(OH)_3CO_3^{-}$	7%	$(UO_2)_2(OH)_3CO_3^{-}$	16%		
		$UO_2(OH)_3^{-}$	5%	$UO_2(OH)_3^{-}$	4%		
		UO_2CO_3aq	4%	UO_2CO_3aq	4%		
sweat	4.2	UO_2^{+2}	86%	UO_2^{+2}	41%	4.9 mM	$UO_2(OH)_2$
		UO_2OH^{+}	11%	$(UO_2)_2(OH)_2^{+2}$	23%		
				$(UO_2)_3(OH)_5^{+}$	21%		
				$(UO_2)_2(OH)_3^{+}$	9%		
				UO_2OH^{+}	4%		
	7.5	$UO_2(OH)_2aq$	58%	$(UO_2)_2(OH)_3CO_3^{-}$	82%	80 μM	$CaUO_4/UO_2(OH)_2$
		$(UO_2)_2(OH)_3CO_3^{-}$	22%	$UO_2(OH)_2aq$	12%		
		$UO_2(OH)_3^{-}$ (9%)	9%				
		$UO_2(CO_3)_2^{-2}$	7%				
pancreatic	8	$UO_2(CO_3)_2^{-2}$	41%	$(UO_2)_2(OH)_3CO_3^{-}$	78%	0.35 mM	$UO_2(OH)_2$
		$UO_2(CO_3)_3^{-4}$	33%	$UO_2(CO_3)_2^{-2}$	9%		
		$UO_2(OH)_2aq$	12%	$UO_2(CO_3)_3^{-4}$	8%		
		$UO_2(OH)_3^{-}$	8%	$UO_2(OH)_2aq$	3%		
		$(UO_2)_2(OH)_3CO_3^{-}$	4%				
	8.3	$UO_2(CO_3)_3^{-4}$	63%	$UO_2(CO_3)_3^{-4}$	52%	0.74 mM	$UO_2(OH)_2$
		$UO_2(CO_3)_2^{-2}$	23%	$(UO_2)_2(OH)_3CO_3^{-}$	36%		
		$UO_2(OH)_2aq$	5%	$UO_2(CO_3)_2^{-2}$	9%		
		$UO_2(OH)_3^{-}$	4%				
bile	7.8	$UO_2(HPO_4)_2^{-2}$	34%	$(UO_2)_2(OH)_3CO_3^{-}$	84%	0.44 μM	$CaUO_4$
		$UO_2(CO_3)_2^{-2}$	29%	$UO_2(OH)_2$	7%		
		$UO_2(OH)_2aq$	22%	$UO_2(CO_3)_2^{-2}$	4%		
		$UO_2(OH)_3^{-}$	10%				
		$UO_2(CO_3)_3^{-4}$	3%				
gastric juice	1	UO_2^{+2}	88%	UO_2^{+2}	88%	1.3 M	$(UO_2)_3(PO_4)_2$
		$UO_2H_2PO_4^{+}$	6%	$UO_2H_2PO_4^{+}$	6%		
		UO_2Cl^{+}	5%	UO_2Cl^{+}	5%		
	3.5	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	94%	0.44 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
				UO_2^{+2}	3%		
				UO_2F^{-}	2%		
urine	4.2	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	100%	8.4 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
	8	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	100%	2.0 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
ASF	7	$UO_2(HPO_4)_2^{-2}$	100%	$UO_2(HPO_4)_2^{-2}$	100%	3.0–9.1 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$
SLF	7.4	$UO_2(CO_3)_3^{-4}$	92%	$UO_2(CO_3)_3^{-4}$	91%	2.3 mM	$Na_2(UO_2)_2(PO_4)_2{\cdot}8H_2O$ /
		$UO_2(CO_3)_2^{-2}$	7%	$UO_2(CO_3)_2^{-2}$	8%		$CaUO_4/UO_2(OH)_2$

Results and Discussion

The results obtained by chemical thermodynamic modeling allow examination of the aqueous chemistry of uranium with regard to the elemental constituents of the biological fluids. The results show how the soluble uranium is chemically distributed after reacting with other constituents of the elemental biological fluids. For reference, the speciation of low and high uranium in CO_2-free water at 37 °C is shown in Figure 1. At lower concentrations of uranium, hydrolysis starts at approximately pH 3 and is dominated by the formation of monomeric hydroxides such as UO_2OH^{+}, aqueous $UO_2(OH)_2$, and $UO_2(OH)_3^{-}$. At a higher uranium concentration, dimers [$(UO_2)_2(OH)_2^{+2}$], trimers [$(UO_2)_3(OH)_5^{+}$], and even tetramers [$(UO_2)_4(OH)_7^{+}$] are formed in addition to the monomeric hydroxides. However, the speciation diagrams of the dilute and concentrated uranium systems at biological pH are similar and mainly consist of UO_2OH^{+}. In the presence of CO_2, uranium carbonate complexes begin to form at pH 7 (Figure 2) and compete with hydrolysis to form the dominant species. Of note is the presence of a mixed hydroxycarbonate species, $(UO_2)_2(OH)_3CO_3^{-}$, which formed at pH 7–8. The chemistry of uranium in both H_2O and HCO_3 systems is rather well-defined, especially in environmental chemistry, and is summarized by several researchers (*38–43*). The thermodynamic data chosen for the models included in this work are critically reviewed (*35*), and models are therefore consistent with the NEA consensus of uranium chemistry in aqueous (and bicarbonate) systems.

The uranium speciation, solubility, and precipitation in both dilute and concentrated solutions are shown in Table 1 for the important pH ranges for each biological fluid. In cases where speciation varies within the pH range, a speciation diagram is presented.

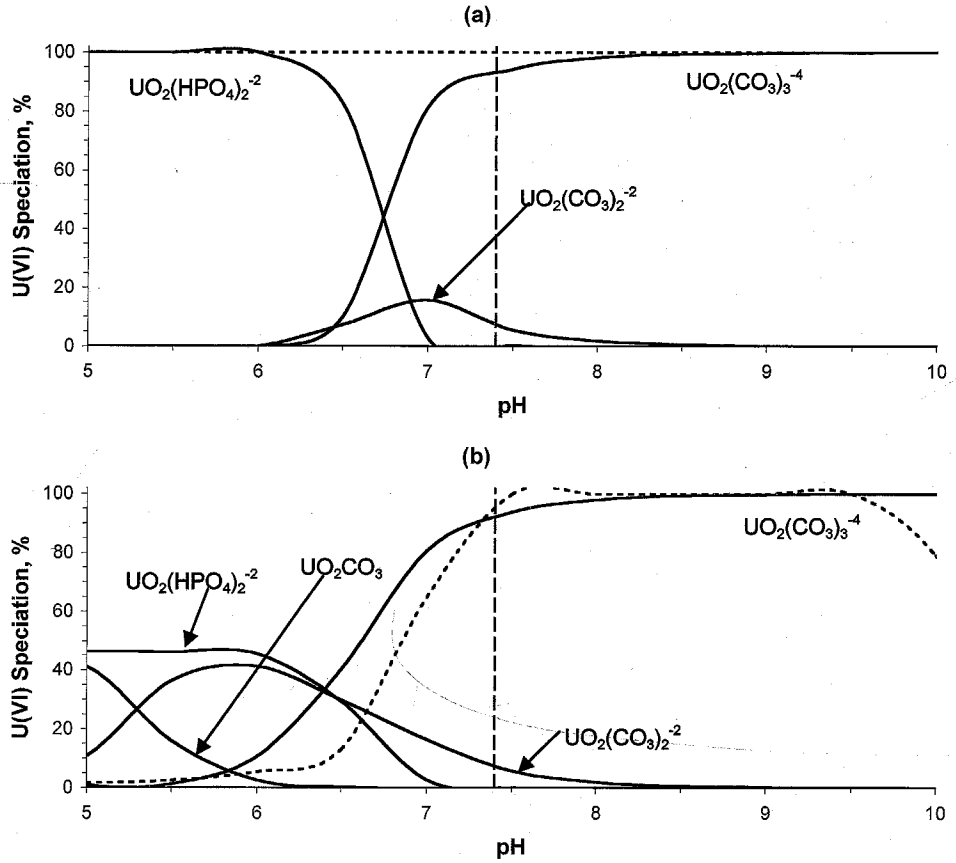

Figure 3. Uranium speciation and solubility with varying pH in simulated interstitial and plasma fluids at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

Intracellular and Extracellular Fluids. In simulated intracellular fluid, the major uranium species is $UO_2(HPO_4)_2^{-2}$ at both high and low uranium concentrations (Table 1). This is a consequence of the high concentration of phosphate ions in solution and the high affinity between uranyl and phosphate ions. Phosphate complexation also limits the formation of dimeric and trimeric uranium hydroxide species. $K_2(UO_2)_2(PO_4)_2 \cdot 6H_2O$ (potassium-autunite) is predicted to form and therefore limits the uranium solubility. There are several reports of uranium phosphate needlelike precipitates within the cell (*44*, *45*). Specifically, intracellular phosphate was subsequently bound to uranium as a precipitate within the cytoplasmic compartment (*45*). Even lower pH ranges may be encountered (perhaps as low as pH 4) in the phagocytic lysosomal environments of macrophages within intracellular fluid. After phagocytosis of particulate matter, the macrophages are involved in the retention of these particles in the alveolar compartment (*46*). However, the phosphate complex remains the major species even at a lower pH until pH 1, where the uncomplexed uranyl ion begins to dominate. At much higher pH values, carbonate complexation of uranium begins to dominate the speciation.

Carbonate species dominate much of the uranium chemistry in simulated interstitial fluid (Table 1 and Figure 3). The phosphate concentration in interstitial fluid is lower than in that of intracellular fluid, and so, phosphate complexation is not sufficient to compete with the high carbonate affinity for uranyl ions at this neutral pH. The solubility of uranium(VI) in interstitial fluid is limited by the formation of $Na_2(UO_2)_2(PO_4)_2 \cdot 8H_2O$ (sodium-autunite). A similar case is true for uranyl ions in simulated plasma fluid (Table 1 and Figure 3). Rothstein (*47*) reported a 40-fold increase in uranium solubility in plasma as compared to water, caused by the formation of a soluble carbonate complex.

Uranium in plasma may enter the glomerulus and pass into the peritubular capillaries or the tubular lumen where it is subsequently filtered into the urine or bound by the brush border membranes of epithelial cells (*5*). Work by Chevari (*48*) has shown that in blood, uranium is bound as 50% carbonate and citrate complexes, 30% protein complexes (transferrin and albumin), and 20% erythrocyte complexes. Scott (*3*) found that about 60% of uranium(VI) is carried as a soluble anionic complex such as bicarbonate; the remainder is bound to plasma protein (*3*) such as transferrin (*5*). However, as carbonate complexes are rapidly removed in the glomerulus, the transferrin complex dissociates and becomes less important in uranium speciation with respect to carbonate (*5*). Furthermore, brush border membranes contain anionic sites that attract cationic uranyl speices (*5*, *49*–*51*), which may in turn compete with calcium ions (*52*). Thus, there is equilibrium between uranium deposition on the cell surface and subsequent removal to lumen and urine (*5*).

Our results suggest that the concentration of uranium in elemental cellular fluids would be higher inside the cell than outside the cell. The results are consistent with those noted by Mirto (*45*) in that uranium enters the cell as a carbonate species and is then precipitated inside the cell as a phosphate mineral. The difference between the speciation of these three cellular fluids is largely due to the difference in phosphate and carbonate concentrations. At pH values less than the reference pH 7.4, phosphate

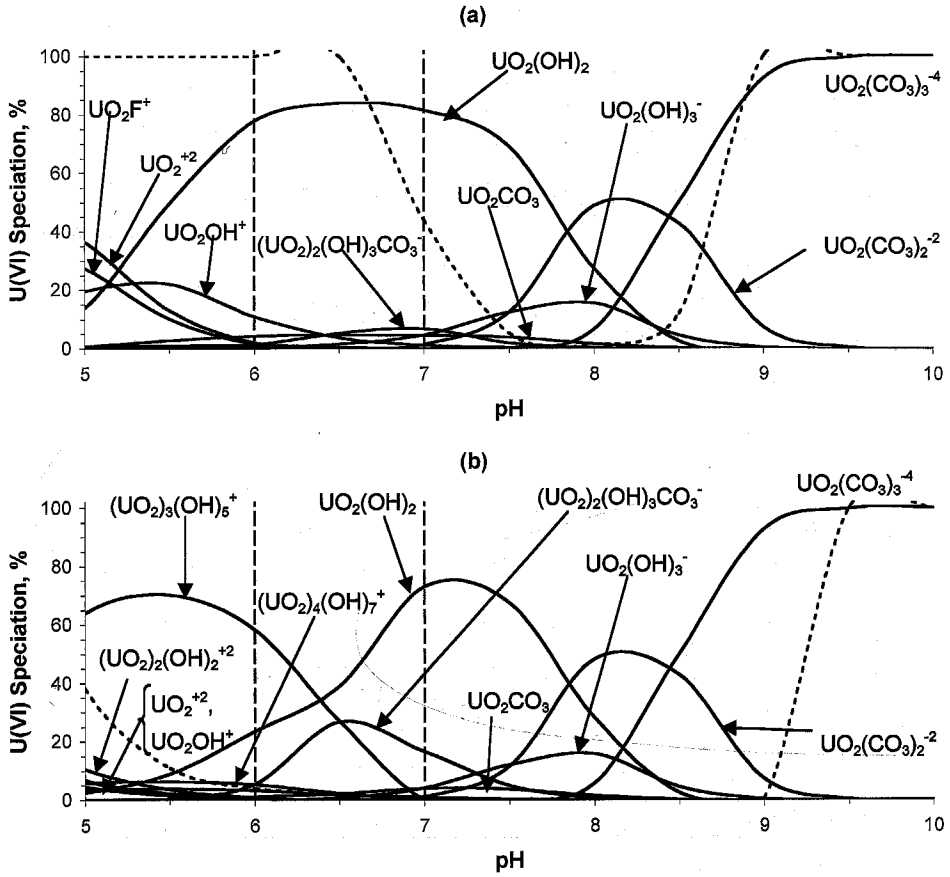

Figure 4. Uranium speciation and solubility with varying pH in simulated saliva at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

complexation of uranium is clearly more important in both extracellular fluids. The results also have implications on the uranium solubility and speciation involved in kidney toxicity. The uranium CL_{50} (concentration leading to 50% cell death) of renal LLC-PK_1 cells is 0.85–0.90 mM (*45*).

Saliva. The modeled speciation of uranium in simulated saliva is shown graphically in Figure 4. As can be seen in Table 1, the solubility of uranium(VI) at each pH boundary is limited by the formation of uranyl hydroxide [$UO_2(OH)_2$], which forms at pH 5–6.5, generating a uranium solubility of 41.8 μM at pH 6.0. Above pH 6.5, $CaUO_4$ (calcium uranate) begins to form. Within the reference pH limits for saliva, the mixed hydroxycarbonate species becomes more important with increasing pH until a maximum of 27% at pH 6.5 where it begins to decrease with increasing amounts of aqueous $UO_2(OH)_2$. By contrast, recent studies (*26*, *53*) found that uranyl speciation in saliva was dominated by a mixture of phosphate and carbonate species at pH 7.5.

In a study of uranium distribution in skeletal tissues, Hamilton (*54*) found uranium concentrations to be 7 ng/g for unashed teeth. Additionally, porcelain, prosthetic teeth historically contained uranium compounds to imitate the natural color, tone, and fluorescent properties of human teeth. However, such prosthetic use is no longer approved in dental clinics. The average uranium concentrations in several Japanese denture brands are 3.6, 9.4, and 18 ppm and 82 ppm in a U.S. brand (*55*). Furthermore, O'Riordan (*56*) found a mean uranium dental composition as high as 410 ppm with a maximum uranium concentration of 1000 ppm. Clearly, the effect of saliva on the chemistry of uranium in teeth and dentures is relevant to this field.

Sweat. In simulated sweat, uranium at dilute concentrations (Table 1 and Figure 5) is present in solution as a mixture of uranyl, hydroxide, and carbonate ions. Clearly, with such wide reference pH limits (4.2–7.5), the speciation and solubility of uranium(VI) change greatly depending upon the exact pH. At the lower pH, uranium speciation is dominated by hydroxide and uncomplexed uranyl ions, while at high pH, uranium speciation is dominated by carbonate complexation. The solubility of uranium in simulated sweat is limited by the formation of solid $UO_2(OH)_2$ at lower pH values, and $CaUO_4$ and $UO_2(OH)_2$ precipitate at neutral to basic pH values.

There is evidence that uranium compounds can permeate through the skin and that surface area, contact time, concentration, and chemical composition can greatly increase the absorption (*57*, *58*). In a study of uranium worker exposure during the 1950s, it was found that the mean incidence of hand contamination of production and maintenance workers was 4.9 and 7.7%, respectively, with a reported upper incidence value of 12.2% (*59*). Our model is relevant to industrial uranium workers and also military handlers of DU weapons and highlights the likely uranium chemical species responsible for skin permeation.

Pancreatic Fluid and Bile. The speciation of uranium(VI) in simulated pancreatic fluid (Table 1 and Figure 6) is a good example of how the speciation of uranium can change dramatically with very small changes in pH. Within 0.3 pH units, the speciation varies between

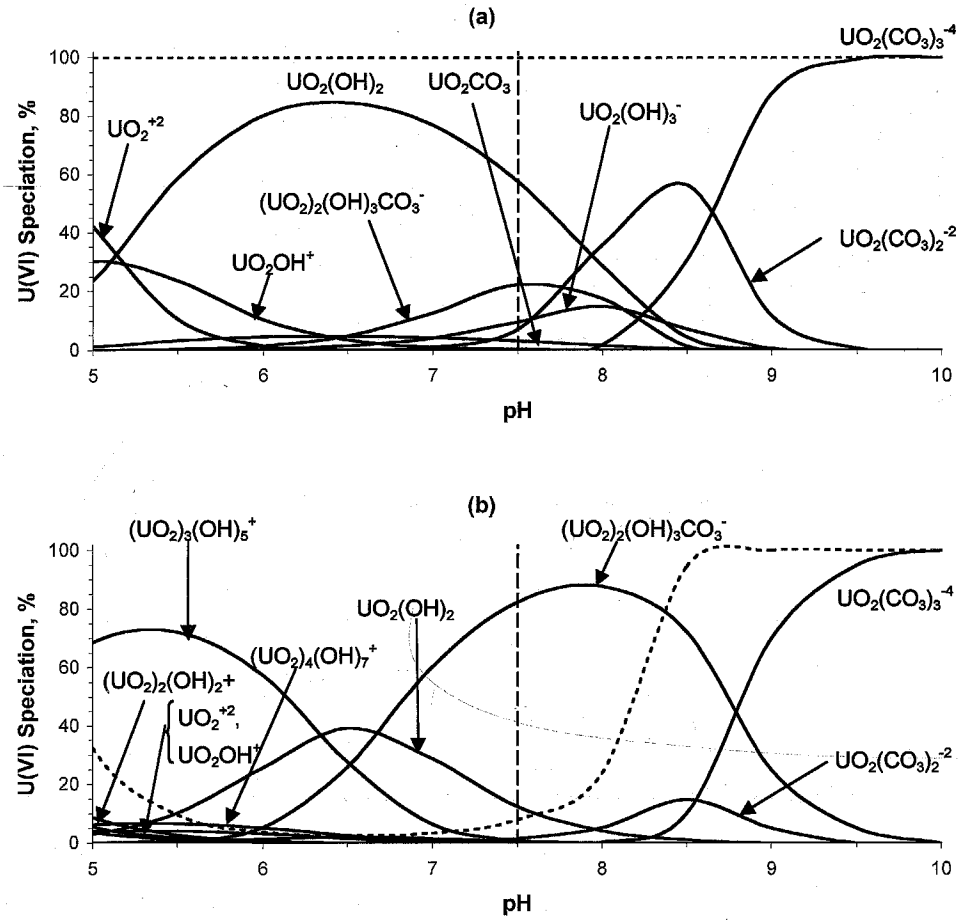

Figure 5. Uranium speciation and solubility with varying pH in simulated sweat at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

hydroxide and carbonate complexes. Similarly, the solubility of solid $UO_2(OH)_2$ differs by a factor of 2. The speciation of uranium in simulated bile is shown in Figure 7 and summarized in Table 1. Dominating species include phosphate, carbonate, and hydroxide complexes, and solubility is limited by the formation of a $CaUO_4$ precipitate. Predictions also show that in simulated elemental bile, sodium-autunite precipitates from pH 4.5 to pH 8.5, $UO_2(OH)_2$ precipitates from pH 5 to pH 8.5, and $CaUO_4$ precipitates from pH 7.0 to pH 9.5.

Although other biological components of bile (e.g., steroid acids and bilirubin) are not considered in this elemental model (to the most part) due to lack of thermodynamic data, their presence in bile may well impact the speciation and solubility of uranium. More research is needed to understand the chemistry of uranium in the presence of bile, steroid acids, and bilirubin. Both pancreatic fluid and bile are not considered key components in uranium toxicity.

Gastric Juice. The phosphate complexation of uranium is also important in the speciation of uranium in simulated gastric juice (Table 1 and Figure 8). Uranium(VI) is predicted to be mostly present as the uncomplexed uranyl ion, but some phosphate complexation does occur even at this low pH. The solubility of uranium(VI) in simulated gastric juice is limited by the formation of a solid $(UO_2)_3(PO_4)_2$ salt between pH 1 and pH 3 and by sodium-autunite between pH 3 and pH 9.

The absorption of uranium in the gastrointestinal (GI) tract has been the study of much investigation and has been summarized by several researchers (*60*−*62*). Their conclusions are that uranium absorption is small, typically between 0.02 and 6% (*61*, *62*), with a reasonable central estimate of 1−2% (*60*, *62*). The variation in absorption factors depends on the parameters of each study (human vs animal, chemical form, exposure route, age, diet, etc.). Furthermore, Leggett (*62*) summarizes the results of others and concludes that absorption generally decreases with the solubility of the uranium compound, such that soluble ionic uranium salts such as uranyl nitrate, uranyl fluoride, and ammonium diuranate are more absorbed than insoluble compounds such as uranium(IV) chloride, oxides, and fluoride. We believe that in addition to the solubility of these compounds, GI absorption is dependent on the pH and chemical composition of gastric juice and the formation (and thus solubility) of uranyl phosphate salt. Absorption of aqueous uranium species into the GI wall is modeled here in the form of uranyl phosphate and uncomplexed uranyl ions.

Urine. Model speciation of uranium in simulated urine shows uranium bound completely as $UO_2(HPO_4)_2^{-2}$ throughout the pH range (Table 1). As with intracellular fluid models, solubility is largely due to the high phosphate concentration with respect to uranium and the high affinity of uranyl-phosphate ions. The uranium(VI) solubility limiting phase in simulated urine is predicted to be sodium-autunite. At higher pH values (above pH 9), the phosphate complex begins to diminish while the tricarbonate species begins to dominate the uranium speciation.

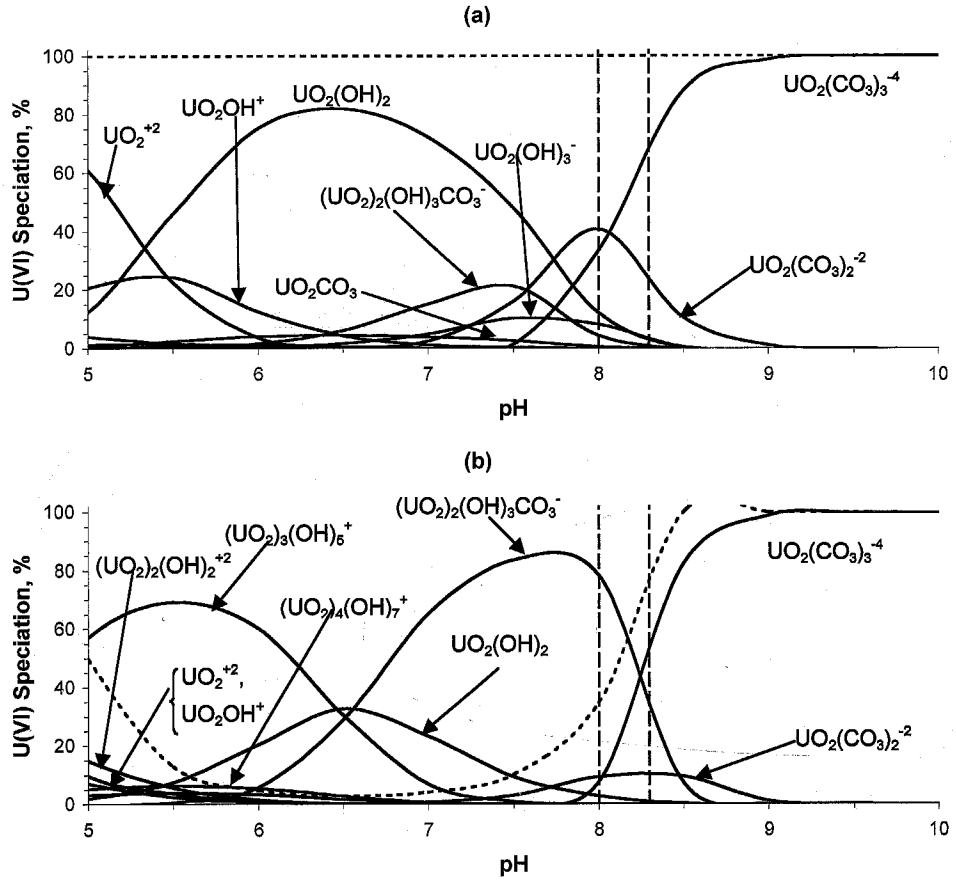

Figure 6. Uranium speciation and solubility with varying pH in simulated pancreatic fluid at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

The pH and carbonate concentration greatly affect the transfer of uranium from the plasma to the urine (*5*, *17*, *63*). Recall that in plasma, carbonate complexation does not dominate uranium speciation until pH 6.5. The effects of pH are 3-fold, namely, the amphoteric nature of the uranyl ion (its ability to form anionic hydroxide species), the increased carbonate concentration with increasing pH, and the affinity for uranyl-carbonate affinity in more alkaline conditions. Hence, when carbonate concentrations are high or the pH is above 6.5, uranium absorption is low in the kidney tubules resulting in high urinary excretion (*5*, *17*, *63*).

Examples of uranium concentration in urine are reported in several units, typically μg/L urine, μg/g creatinine, and μg/day. Excretion data are converted using 1.7 g creatinine/day and 1.4 L urine/day (*64*). A selection of urinary uranium concentrations reported in the literature (*65*–*69*) is shown in Table 2 for a variety of exposures. Results such as these can be applied as an indirect measure of worker exposure controls. Urine bioassays can also be used to measure excretion during medical follow-up of an accidental overexposure. Thus, chemical thermodynamics modeling provides potentially useful knowledge in the development of metal bioassay techniques in human samples as a measurement of human exposure (*70*). For example, the hand exposure previously mentioned in our study of the effect of sweat on uranium chemistry resulted in urinary uranium concentrations of 34.3 and 32.6 μg/L for production and maintenance workers, respectively. Urinary uranium concentrations as high as 3.5 mg/L have been observed (*71*) for workers exposed to highly soluble uranium compounds. As with GI absorption, uranium concentrations in urine vary depend on the exposure type, age, and diet (*62*).

Lung/ASF. Chemical thermodynamic modeling of ASF showed somewhat similar results for all of the cases of interest. In each case, the dominating species was found to be $UO_2(HPO_4)_2^{-2}$ and the solubility was above the initial uranium model input concentrations (Table 1). However, as with previous examples, we were able to predict uranium(VI) solubilities in ASF by adding further uranium to the model calculations. The results predict that the solubility of uranium(VI) at pH 7.0 in ASF is greatest in hypersecretory ASF (9.1 mM), followed by postanticholinergic ASF (6.1 mM), normal ASF (6.0 mM), and postbezodiazepine ASF (3.0 mM). This difference is due to the availability of phosphate in each ASF and the subsequent precipitation of sodium-autunite. At pH values higher than 9.0, uranium speciation becomes increasingly dependent on carbonate complexation. Additional ASF models were available (such as sustained irritation ASF, acute airway infection ASF, cystic fibrosis ASF, and severe asthmatic ASF) but did not contain phosphate composition data and so are not reported here for reasons of consistency. Cooke (*72*) found that UO_3 solubility in simulated lung fluid (SLF) (*73*) was approximately 1.0 mM at 37 °C. Recent work has also shown that rat alveolar exposure to uranium induces TNF_α (cytokine) secretion and MAPK (protein kinase) activation, potentially leading to chronic inflammatory lung disorders (*74*). The LC_{50} for rat alveolar macrophages is 0.5 mM (*75*).

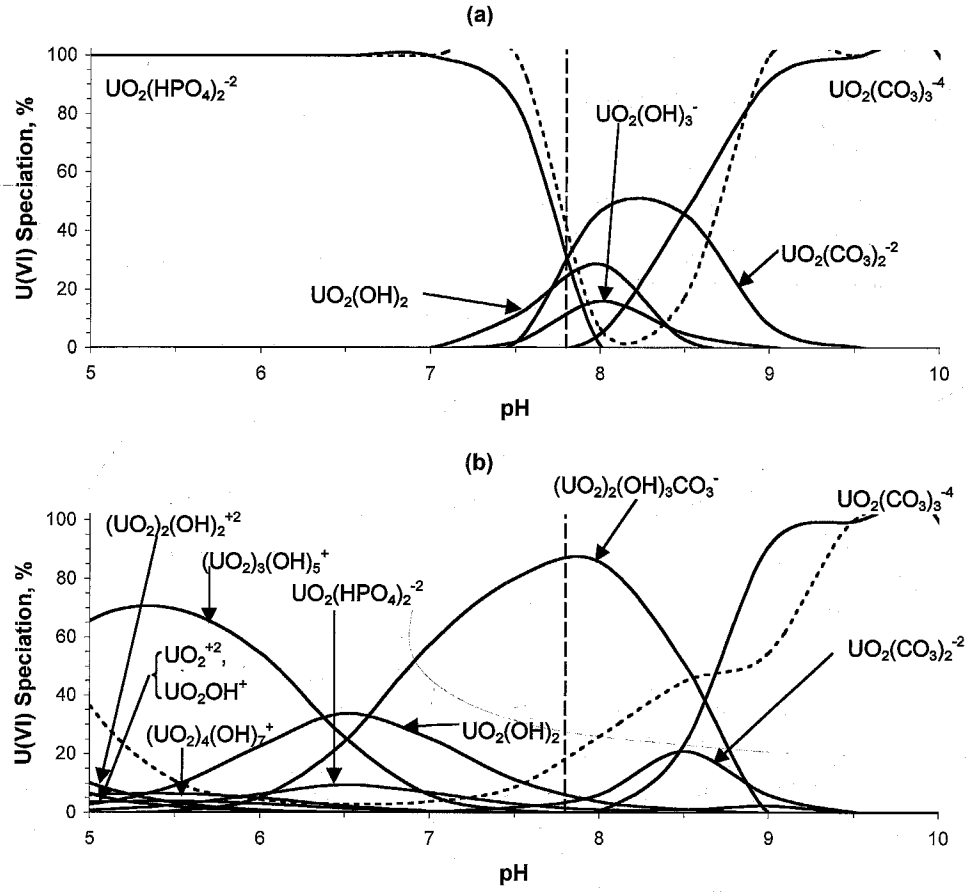

Figure 7. Uranium speciation and solubility with varying pH in simulated bile at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

A further alternative to ASF as a lung fluid model is that of SLF (*76*–*78*). Here, the composition of the fluid is similar to that of interstitial fluid but includes citrate and acetate compounds to simulate protein components in the fluid. Uranium is known to form complexes with both citrate and acetate, and these effects can be observed in Figure 9. However, at the reference pH of SLF (pH 7.4), citrate and acetate are not at a significant concentration to be able to compete with carbonate complexation (Table 1). The results are therefore similar to those observed in extracellular fluid (interstitial and plasma) models. However, the solubility of uranium(VI) in SLF is 2.3 mM and is somewhat higher than that of interstitial fluid (0.93 mM). At lower pH regions, the uranium speciation can be explained in terms of a mixture of phosphate, carbonate, and even citrate complexes. Recall that Scott (*3*) and Leggett (*5*) suggested that in the case of uranium(VI), about 60% is carried as a soluble bicarbonate complex with the remainder bound to plasma protein. Our results show that in order to recreate 40% protein binding, citrate concentrations need to be higher than those reported by Moss if an elemental simulated fluid is to be used. Indeed, Moss (*76*) stated that under the conditions studied, protein did not seem to be a significant sink for uranium. Alternatively, it may be that citrate is not a good analogue for protein binding of uranium. Kalkwarf (*78*) found that using citrate and acetate as previously described (*76*) failed to affect the dissolution rate of a hexavalent uranium ionic compound (ammonium diuranate) in SLF. Our conclusion is therefore that citrate and acetate are not reasonable analogues to use for uranium protein interactions in thermodynamic modeling and that more research and discussion are needed to determine acceptable analogues.

Model Assumptions and Limitations. The modeling presented in this work requires several assumptions and is subject to the following limitations: (i) A thermodynamic equilibrium state is assumed. This assumption is valid because reaction kinetics of ion interactions in homogeneous fluids are almost instantaneous and based on the probability of ions existing in the same space at the same time. Additionally, kinetics depends on the rate of electron transfer to form a chemical bond (electrostatic or covalent) followed by the subsequent structural rearrangement of atoms in space to yield the most stable structure. However, much larger kinetic effects are seen in heterogeneous fluids, especially in the reaction between solids and liquids. Depending on the mineral, the chemical, and the physical environment, such reactions can occur over time periods ranging from seconds to thousands of years. Reaction kinetics is not considered in this model.

(ii) Typical elemental biological fluids are assumed. The variation in human fluid composition and its effect on in vivo uranium chemistry is acknowledged. For example, decreasing the partial pressure of CO_2 in extracellular fluid models would lead to a decrease in uranium carbonate species and a decrease in sodium autunite solubility. Age, prolonged illness, prescribed medications, environment, diet, and lifestyle all influence fluid composition in the human populations. While some biological fluids are subject to rapid changes that would affect uranium chemistry in vivo, homeostasis in both fluids and cells minimizes such changes.

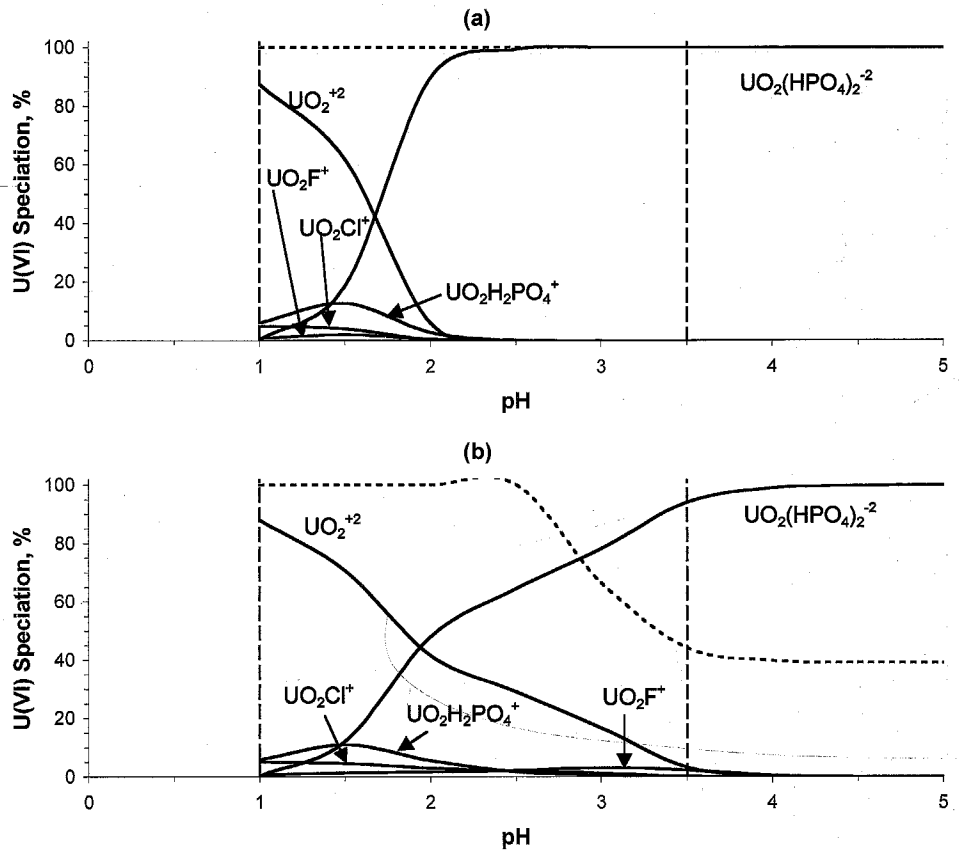

Figure 8. Uranium speciation and solubility with varying pH in simulated gastric juice at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

Table 2. Examples of Urinary Uranium Concentrations Relating to a Variety of Exposures

exposure type	urinary range (μg/L)	urinary median (μg/L)	ref
drinking water	1–5650	0.078	*65*
occupationally exposed group (and control group)	0.056–5.31/ (0.003–0.049)	0.240 (0.009)	*66*
chronic inhalation exposure	9–57	21	*67*
occupationally exposure, lung	5.5–26.0		*38*
uranium miners, Egypt	8.4–29.2		*68*
Gulf War DU exposure	0.001–0.525	0.012	*10*
Gulf War DU exposure	0.001–3.515	0.012	*69*

(iii) Elemental fluid compositions are assumed. The modeling reported in this article is limited to the elemental (simple ion) speciation and solubility. No thermodynamic assessment of uranium binding to proteins or intracellular constituents is made (with the exception of citrate and acetate as potential analogues). Transferrin is known to bind to actinides (*26*, *48*, *79*), and a conditional formation constant ($\log K_f = 16$) has been proposed (*27*) as a first estimation for a $(UO_2)_2Tf$ species. While the authors acknowledge the research by Scapolan and others and the possible presence of uranyl-transferrin in some biological fluids, the complex was not included in the model for the following reasons: (i) the charge on the uranyl-transferrin species was not determined; therefore, its use would be incongruous with chemical thermodynamic modeling; (ii) the complex has not yet been critically reviewed by sources used in this model (*33*–*35*); and (iii) transferrin forms a stronger complex with iron [$\log K_f = 21$ at pH 7.4 (*80*)] and in certain chemical environments would therefore bind preferentially with iron rather than uranium. In a biological system, there is typically more available transferrin as compared to iron, thus leaving an excess of available binding sites for potential binding with uranium. Furthermore, the upper and lower limits of the pH range of the biological fluids studied here are in some cases much different to the normal biological pH of 7.4. The pK_a of transferrin differs depending upon the nature of the binding site. The apparent pK_a of transferrin is approximately 7.4, but iron binding occurs at different sites on the transferrin molecule at pH 6.0 and pH 8.5 (*81*). We believe that at lower pH, the affinity of transferrin for both iron and uranium would be less because of increased protonation of the functional groups on the transferrin protein molecule. At higher pH, we believe that the hydroxide and carbonate complexation of uranium will predominate over transferrin complexation.

Thus, the models presented in this work are the best possible given the lack of complete thermodynamic data on protein interaction. We strongly recommend the further characterization of thermodynamic data for uranium–protein interactions and their inclusion in future uranium/biological fluid thermodynamic models.

Conclusions

Uranium speciation and solubility in simulated biological fluids are dependent on the pH, uranium concentration, fluid composition, and ionic strength of each system. In addition, uranium solubility is several times higher inside a cell than outside a cell, depending upon the phosphate concentration and pH of intracellular fluid. The solubility of uranium is controlled by potassium-autunite in intracellular fluid and sodium-autunite in extracellular fluid. Carbonate, hydroxide, and phosphate

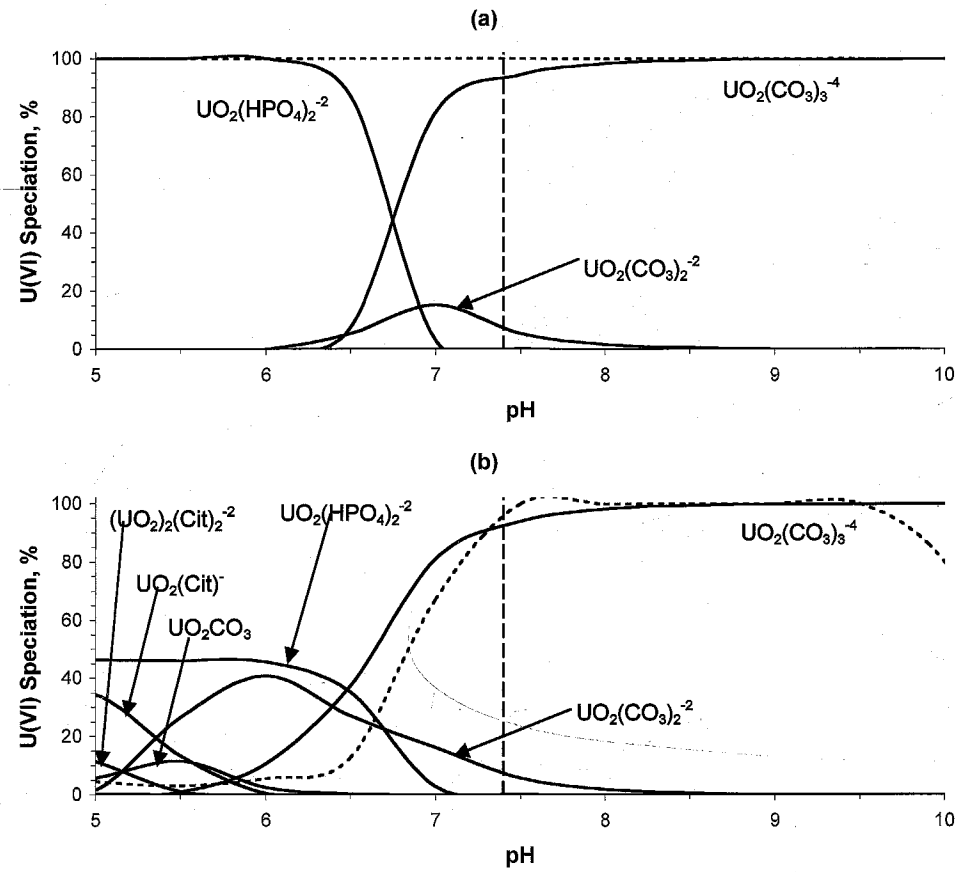

Figure 9. Uranium speciation and solubility with varying pH in SLF at [U] = (a) 1 μM and (b) 1 mM. A dashed curved line depicts solubility.

complexes are the dominant species in many of the biological fluids. The range of phosphate concentrations in ASFs dictates the uranium solubility through the formation of insoluble autunites. The phosphate concentrations are highly dependent on the lung condition of the patient. Simulation of lung fluid showed that protein analogues such as citrate and acetate showed no significant uranium binding at the correct pH for such a fluid.

Further work will be performed to experimentally verify the data obtained by thermodynamic modeling. The authors believe that a more complete characterization of uranium interactions with albumin, transferrin, and erythrocytes is required before they can be included in a critically reviewed chemical thermodynamic model. We recognize the uranium-transferrin studies by Scapolan et al. and encourage their continued research. As the underlying thermodynamic biochemistry of uranium is better characterized, these biological components can be added to a model such as that presented here. When combined with experimental data, such resulting models can be applied to the understanding of uranium metabolism and toxicology, biological monitoring, and therapeutic treatment.

In conclusion, chemical thermodynamic models can make unique and valuable contributions to the understanding of uranium speciation, solubility, and distribution in the body. A better understanding of the impact of uranium exposures on human health and improved bioassays for uranium overexposure can result from further combination of these models with results from experimental toxicology. These results are also important in the development of specific chelators to remove uranium body burden in heavily exposed persons.

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