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PREBIOTIC CHEMISTRY

The Prebiotic Provenance of Semi-Aqueous Solvents

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Abstract

The numerous and varied roles of phosphorylated organic molecules in biochemistry suggest they may have been important to the origin of life. The prominence of phosphorylated molecules presents a conundrum given that phosphorylation is a thermodynamically unfavorable, endergonic process in water, and most natural sources of phosphate are poorly soluble. We recently demonstrated that a semi-aqueous solvent consisting of urea, ammonium formate, and water (UAFW) supports the dissolution of phosphate and the phosphorylation of nucleosides. However, the prebiotic feasibility and robustness of the UAFW system are unclear. Here, we study the UAFW system as a medium in which phosphate minerals are potentially solubilized. Specifically, we conduct a series of chemical experiments alongside thermodynamic models that simulate the formation of ammonium formate from the hydrolysis of hydrogen cyanide, and demonstrate the stability of formamide in such solvents (as an aqueous mixture). The dissolution of hydroxylapatite requires a liquid medium, and we investigate whether a UAFW system is solid or liquid over varied conditions, finding that this characteristic is controlled by the molar ratios of the three components. For liquid UAFW mixtures, we also find the solubility of phosphate is higher when the quantity of ammonium formate is greater than urea. We suggest the urea within the system can lower the activity of water, help create a stable and persistent solution, and may act as a condensing agent/catalyst to improve nucleoside phosphorylation yields.

Keywords Origin of life · Condensation · Urea · HCN · Formamide · Formate · Phosphorus

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Introduction

While ubiquitous in biology, phosphorus is a comparatively minor element in terrestrial rocks. Phosphorus-containing ATP is the energy currency for biochemistry as well as a common cofactor for enzymes. Phosphate is essential for the biomineralization in of bones and teeth, and is an active component of cellular structure and RNA/DNA (Pasek et al. 2015). Given the prevalence of phosphorus in life, the question of how it was first incorporated into biomolecules has been a focus of prebiotic interest (Gulick 1955; Lohrmann and Orgel 1971; Miller and Urey 1959; Österberg et al. 1973; Ponnamperuma and Mack 1965; Schwartz 1972; Schwartz et al. 1973, 1975). In contrast to the other major elements of life—carbon, hydrogen, nitrogen, oxygen, and sulfur (CHNOS)—phosphorus does not have a major volatile phase on Earth. On the surface of Earth, phosphorus is a lithophile element associated with silicates. With few exceptions, the redox conditions on the Earth's surface favor the +5 oxidation state (Pasek 2019), and phosphorus therefore primarily exists in mineral sources as phosphate (PO_4^{3-}). These phosphate minerals are the primary source of phosphorus for the phosphorus cycle on Earth (Föllmi 1996).

Most common phosphate minerals are typically insoluble in water under common geological surface conditions, and therefore, phosphorus is often the limiting nutrient for biota. Before biology deposited more soluble phosphates in soils and liberated phosphorus into solution, phosphorus is conventionally thought to have come from mineral sources such as apatite minerals or iron phosphate minerals (Hazen 2013). The apatite mineral group is insoluble and unreactive in water under neutral conditions (Handschuh et al. 1973; Schoffstall 1976; Schwartz 1972). These issues create a dilemma in prebiotic chemistry referred to as "the Phosphate Problem" (Gulick, 1955). This problem is a subset of the "Water Problem" (Benner et al. 2012), which is a well-known paradox to prebiotic chemists and directly relates to phosphorylation, as the C–O–P linkages in biological molecules require thermodynamically disfavored condensation reactions to occur. Despite the challenges presented by the fact that water impedes condensation reactions, water is an essential component of life as we know it. Prebiotic environments likely were characterized by an abundance of water.

To overcome the challenges of the water problem, researchers have attempted a number of approaches, from utilizing reactive phosphorus compounds (Gibard et al. 2018; Gull et al. 2015; Karki et al. 2017; Kolb and Orgel 1996; Krishnamurthy et al. 1999; Ni et al. 2009; Pasek 2005), to promoting phosphorylation by driving off water through heating (Ponnamperuma and Mack 1965; Steinman et al. 1964), to substituting alternative non-aqueous solvents for water (Costanzo et al. 2007; Gull et al. 2014, 2017; Schoffstall 1976). Reactive phosphorus sources such as amidophosphates, trimetaphosphate, and the meteoric phosphide mineral schreibersite have been utilized in numerous studies (Gibard et al. 2018; Gull et al. 2015; Karki et al. 2017; Kolb and Orgel 1996; Krishnamurthy et al. 1999; Ni et al. 2009; Pasek 2005). The abundance of these reactive phosphorus sources on prebiotic Earth is questionable (Hazen 2013; Keefe and Miller 1995).

Elevating the temperature above the boiling point of water can not only supply the energy necessary to make a reaction kinetically accessible, but will also evaporate water from the system to make condensation reactions thermodynamically favored. This approach has been employed in several model prebiotic studies (Ponnamperuma and Mack 1965; Steinman et al. 1964). These experiments involved heating nucleosides and various aqueous sources of

phosphate at temperatures above the boiling point of water to drive phosphorylation reactions. Whether surface temperatures of 100 °C were frequently encountered on the surface of the early Earth is a subject of debate (Sagan and Mullen 1972; Levy and Miller 1998; Lohrmann and Orgel 1971), though local regions with high temperatures are certainly reasonable. Although adding heat can allow a reaction to proceed by overcoming its activation barrier, the reagents and products can be unstable at the temperatures required for the reaction to proceed (Bada and Lazcano 2002; Levy and Miller 1998; Miller and Lazcano 1995).

A different approach to overcome the "water problem" is the use of nonaqueous solvents. Nonaqueous solvents include formamide and urea-rich, so-called "deep eutectic" solvents (Costanzo et al. 2007; Gull et al. 2014, 2017; Schoffstall 1976). Multiple phosphorus sources were also tested these studies such as struvite, pyrophosphate, hydroxylapatite, libethenite, pseudomalachite, sodium phosphate, trimetaphosphate, monetite, and phosphoric acid. In 1976, Schoffstall published work using formamide as a nonaqueous medium, where mild heat (70 °C) resulted in the phosphorylation of adenosine to form ADP, AMP, and cyclic AMP (Schoffstall 1976). A large reservoir of pure, neat formamide on the prebiotic Earth was unlikely (Bada et al. 2016) but may have been available in microenvironments such as evaporating tidal pools (Adam et al. 2018; Vichietti et al. 2019), or as a solute. Accordingly, semi-aqueous solvents with low water activities have also been explored with respect to the origin of life such as the urea-ammonium formate mixtures of Burcar et al. 2016, Burcar et al. 2019). Another nonaqueous solvent that has been utilized is the deep eutectic system (DES) of choline chloride and urea. Gull et al. (2014) reported phosphorylation of nucleosides and glycerol within this DES utilizing soluble phosphate sources (Gull et al. 2014). This DES was revisited substituting ethanolamine for choline in the DES, again demonstrating phosphorylation in this alternative solvent (Gull et al. 2017). The prebiotic relevance of this DES has been debated as choline has not been detected in meteorites and is a more complex molecule with questionable prebiotic availability on the young Earth (Schwartz 2019).

Burcar et al. (2016) found that formamide is formed in a solvent composed of a 1:2:4 M initial ratio of urea, ammonium formate, and water (UAFW), and that these four compounds are the main species that form a persistent liquid. The UAFW solvent builds on prior work by Lohrmann and Orgel (1968, 1971), and these semi-aqueous solvents are perhaps the most promising alternative solvents for overcoming the challenges water presents to prebiotic chemistry. Not only are appreciable yields (4–58%) of phosphorylated products obtained in these solvents, but they are composed of prebiotically available molecules and do not require the complete removal of water. For example, Burcar et al. demonstrated that mild heating (between 65 and 85 °C) of hydroxylapatite, $Ca_5(PO_4)_3OH$, in the UAFW with an initial 1:2:4 M ratio and dissolved $MgSO_4$ generated a more soluble form of phosphate, the mineral struvite, $NH_4MgPO_4{\cdot}6\ H_2O$ (2016). In Burcar et al. (2016) only the 1:2:4 UAFW solvent composition was used. The effects of varying the starting compositions on P solubilization were not studied. The present work investigates the robustness of this alternative solvent, assuming that the 1:2 ratio of urea to ammonium formate is not a prerequisite of this chemistry.

Urea ($CO(NH_2)_2$) is a simple, one-carbon organic compound that has been detected in both the solid and gas phases in interplanetary objects such as comets (Bockelée-Morvan et al. 2000), meteorites (Cronin and Pizzarello 1997; Martins et al. 2008), and micrometeorites (Matrajt et al. 2004). Extraterrestrial sources of urea are hypothesized to have augmented the supply of urea on the primitive Earth (Chyba et al. 1990). Additionally, it has been demonstrated that in the presence of clay minerals such as montmorillonite, urea is shielded from ionization radiation, thus enabling the accumulation of urea in small evaporative pools

(Lohrmann 1972; Amend and McCollom 2009; Cruz-Hernández et al. 2015). Urea has been synthesized in the lab under potentially plausible prebiotic conditions by the irradiation of ammonium cyanide with ultraviolet light and electrical discharge (see Lohrmann 1972 and references therein).

Formamide ($HCONH_2$) is the least complex, naturally-occurring amide and has been identified in the interstellar medium (Rubin et al. 1971). It can be formed by UV irradiation of gaseous NH_3 and CO (Berthelot & Gaudechon, 1910). Formamide is the hydrolysis product of HCN (Marsh & Martin, 1957) and is a liquid between 3 and 210 °C at 1 atm. Ammonium formate is the hydrolysis product of formamide. These components are generally accepted as having been available on a prebiotic Earth in appreciable quantities under a variety of geological conditions, though we investigate this assumption below.

When determining the effect UAFW has on the solubility of hydroxylapatite (HAP) at various molar ratios, Burcar et al. (2016) demonstrated phosphorylation yields of 4% of adenosine utilizing HAP as the phosphate source within the solvent at the 1:2:4 ratio. In contrast, the yield improved to 58% using Na_2HPO_4, a soluble phosphate salt, minerals but it is unclear if other initial starting conditions would yield the same results (or if the UAFW would only support phosphorylation when strict controls were placed on initial conditions). To this end, here we explore the compositional space of varying UAFW ratios to examine when mixtures maintain liquid stability after heating for a set period of time and the extent of phosphate dissolution within these mixtures. We also revisit the prebiotic potential of urea/ammonium formate/formamide mixtures using a model geothermal system (the "warm little pond" model).

Materials and Methods

We purchased ammonium formate (NH_4COOH, 97%), formamide ($HCONH_2$, 97%), urea ($(NH_2)_2CO$, 97%), nitric acid (HNO_3, 98%), hydroxylapatite ($Ca_5(PO_4)_3OH$, 97%) from Fisher Scientific (New Hampshire, USA. Deionized water was obtained in-house via a Barnstead (Dubuque, IA, USA) NANO pure® Diamond Analytical system with a combined reverse osmosis-deionization system (18.2 MΩ·cm).

A. Modeling

We modeled the generation of ammonium formate in a geothermal pond (Damer and Deamer 2015) using numerical calculations. Specifically, we computed the concentration of ammonium formate that would result from the hydrolysis of HCN. We assumed a HCN flux (F_{HCN}) into a body of water of 10^{10} molecules $cm^{-2}{\cdot}s^{-1}$ (Tian et al. 2011) and coupled this with hydrolysis rates from (Miyakawa et al. 2002a-c), which are rapid on the timescale of years (t).. This flux was then related to the effective surface area (SA) to volume (V) of a pond via Avogadro's number (N_A).

$$[AF] = \frac{F_{HCN} \times t \times SA}{N_A \times V}$$

Loss terms, such as photolysis of ammonia (Ferris and Nicodem 1972; Kuhn and Atreya 1979), complexation of HCN by iron (Burcar et al. 2019), breakdown of formate (to CO and

H_2O), and HCN polymerization (generally not important at low concentrations, see Miyakawa et al. 2002a) were ignored, and hence this should be viewed as a maximum concentration with respect to a pond formed de novo, in an active prebiotic environment with HCN forming in its atmosphere.

We also investigated the thermodynamics of urea formation from CO_2 and NH_3 gases using HSC Chemistry (Outokompu Research Oy[1]) using the reaction equation module, and formamide and ammonium formate formation from HCN hydrolysis chemistry utilizing thermodynamics of formamide from Antoine's Equation (Perry and Green 1997). These calculations all assume a major fraction of water in the environment, in other words, the activity of water was assumed to be one (for solvated formate, ammonium, formamide, and HCN data). Therefore, calculated concentrations should be viewed as estimates for water-dominated solvents. In systems that are closer to equimolar in ammonium formate and water, these thermodynamic data do not apply as the system is non-ideal. The thermodynamic data used is presented in the SI.

B. Determination of Liquid State and Speciation Analysis of Mixtures

We conducted experiments to determine the robustness (where "robustness" is the ability of the UAFW system to maintain a fluid state over time under heating conditions) of the UAFW system and its evolution over time, specifically, discerning if the solvent remained a liquid over a range of various initial molar ratios. For these experiments several molar ratios of the UAFW solution were mixed on a heat-block at 75 °C until no solid particles of ammonium formate or urea were visible within the liquid to the naked eye. 10 ml of each sample were transferred to glass vials, the samples were then placed uncapped in a heated oven (74 °C) for 7 days. The persistence of the solvents was visually inspected daily and the state, whether liquid, solid or a mix, was recorded daily. These systems were open systems where water was free to evaporate.

A second set of experiments combined 1) ammonium formate and water (1:1 by moles) and 2) formamide and water (1:1) in flame-sealed 5 mm NMR tubes that were then subjected to heating at 85 °C for 15 days. In each tube a 3 mm sealed tube filled with D_2O was added for locking by the NMR. The samples were monitored by ^1H NMR over this 15 day timeframe to identify the formation of 1) formamide and 2) formate, respectively. Concentrations were calculated by integration of NMR peaks, and NMR analyses procedures followed Burcar et al. (2019). These systems were closed systems where water was not free to evaporate.

C. Solubility of Hydroxylapatite

To evaluate the robustness of the UAFW solvent, we formulated solvents with various molar ratios of the three components to simulate possible conditions of the early Earth (as informed by the experiments in section B). These mixtures were placed on a stirring heat-block (capped) at 75 °C until no solids remained, and the mixtures were a uniform liquid throughout. Once the solvents were prepared, 10 ml of each was transferred to a glass vial and 100 mg of hydroxylapatite were added. These samples were then capped and stirred on a hotplate at 75 °C for 30 min. Once the samples were mixed, the caps were removed and the samples were then transferred to a heated oven (74 °C) and left for 14 days. After 14 days, the samples were removed from the oven, reconstituted with deionized water (so that they could be analyzed by

[1] HSC is a thermodynamic calculator; details can be found at www.hsc-chemistry.net

Table 1 ICP–OES Operating Conditions

	Optima 8300	Avio 200
Plasma gas (L min^{-1})	15	10
Auxilary gas (L min^{-1})	0.2	0.4
Nebulizer gas (L min^{-1})	0.6	0.7
Power (kW)	1.4	1.5
prep*FAST* flow rate (μL min^{-1})	800	1500
Read delay (sec)	60	25
Read replicates	3	5
Viewing	Axial (P)	Axial (P)
Purge gas flow	Normal	Normal
Nebulizer	Meinhardt® Type K1	Meinhardt® Type K1
Spray chamber	Cyclonic	Cyclonic
Torch cassette position	−2	−3
Analyte wavelengths (nm)	P (213.617 and 214.914)	P (213.617), Ca (317.933)
Peak processing	Peak area	Peak area
Calibration type	Linear, calculated intercept	Linear, calculated intercept

fluid injection on the ICP-OES) and mixed for 30 min. The samples were then filtered with a 0.45-μm syringe filter.

In order to determine the solubility of apatite in these solutions, one mL of sample was diluted with 2% HNO_3 and analyzed either by a Perkin-Elmer Optima 8300 Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) equipped with a prep*FAST*™ sample/standard autodilution system from Elemental Scientific at Saint Louis University or by a Perkin Elmer Avio 200 ICP-OES coupled to an SC-DX2 FAST auto sampling system at the University of South Florida. Both instruments were controlled by the Syngistix for ICP software package. For calibration using the prep*FAST*, we prepared a 5 mg/L standard and a 200 mg/L standard for each element. The prep*FAST* system automatically diluted these solutions to prepare other calibration standards. Calibration curves were automatically constructed by this system with 8 points ranging from 0 mg/L to 100 mg/L, $R^2 > 0.999$. We measured yttrium ($\lambda = 371.029$ nm) at 10 mg/L in 2% HNO_3 as an internal standard on the

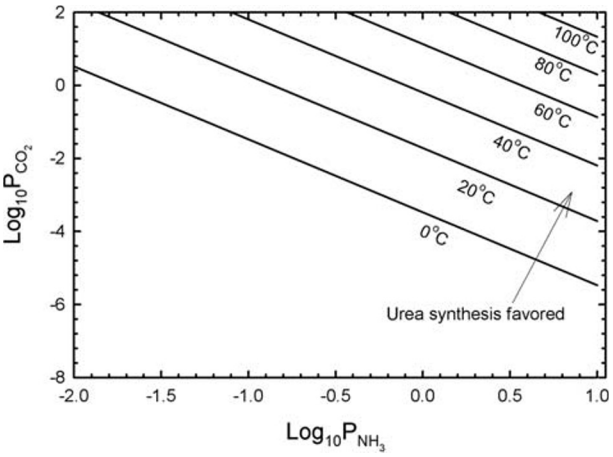


Fig. 1 Prebiotic plausibility of the formation of urea, as calculated by equations of state from Gibbs free energies (data in SI). Urea is favored under conditions above each line at the shown temperatures

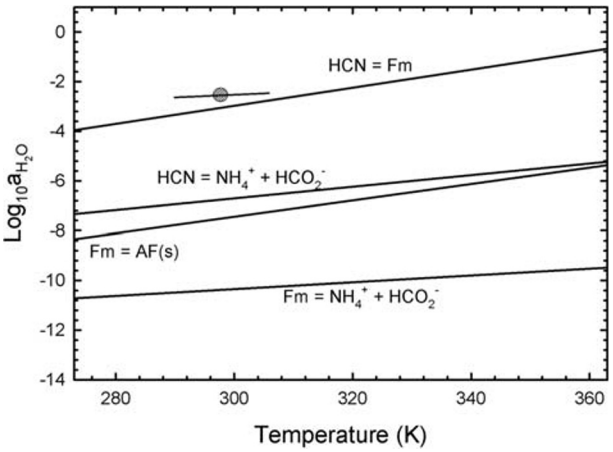


Fig. 2 Conditions of ammonium formate (AF) and formamide (Fm) formation, as calculated by equations of state from Gibbs free energies (reactants favored below line, products favored above). Thermodynamic data for species is available in the SI. Note that the ΔG values used for these experiments are based on water-solvated compounds, where the activity of water is much greater than that of the solute. Ammonium formate is favored under conditions with higher water activities. The gray circle (which is data at 298 K and 1 atm) and line (data extended using the van't Hoff equation from the enthalpy of this reaction and confidently matches reaction ΔGs for varying temperatures over this line. The line may be extensible beyond this temperature range but is not matched to measured ΔG values, and notes water activity below which AMP formation is thermodynamically favorable (e.g., from the reaction adenosine + phosphate = AMP + H_2O, using thermodynamic data from Pasek 2020 and references therein)

Optima 8300, whereas scandium (λ = 361.383 nm) at 5 mg/L in 2% HNO_3 was used as the internal standard for the Avio 200. Phosphorus standards were prepared from a 1 g/L SPEX CertiPrep P standard for ICP.

Each instrument was set up with the standard Perkin-Elmer operating conditions shown in Table 1. Axial viewing was selected for phosphorus in the case of low dissolved concentration, while radial viewing was selected for calcium. Multiple wavelengths were selected for both phosphorus and calcium as a cross–check for any potential wavelength interferences. In all cases the concentrations shown in this manuscript are the average values obtained from the two wavelengths selected for each element.

Results

Free energy calculations indicated that urea is stable under high CO_2 and NH_3 partial pressures, and lower temperatures tend to favor aqueous urea formation (Fig. 1). At 0.01 atm of NH_3, the partial pressure of CO_2 that promotes urea formation is slightly more than 1 atm at 0 °C, indicating that at a high partial pressure of CO_2 urea formation should have been thermodynamically favorable.

Furthermore, the thermodynamics of hydrolysis of HCN indicate that the formation of formamide and ammonium formate is exergonic in water at a pressure of 1 atm (Fig. 2). Ammonium formate is the end product of HCN hydrolysis, and in most cases where liquid water is present and abundant, ammonium formate is predicted to be the dominant species determined from the law of mass action (quotient K, equal to $e^{-\Delta G/RT}$, but see caveat in Discussion).

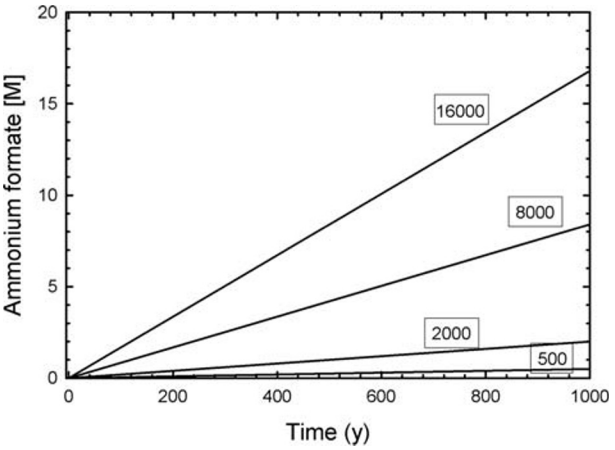


Fig. 3 The formation of ammonium formate from HCN accumulates as a function of the area to volume of a pond or lake (number in box, units in cm^{-1}). The area of the pond/lake is assumed to be equivalent to the drainage basin, and values as shown in the box would correspond to small lakes with large basins, such as those that occur in craters and/or basins that experience wet-dry cycling

On the prebiotic Earth, ammonium formate should be the major product of HCN hydrolysis in bodies of water that are capable of concentrating organics (i.e., with little outflow and high inflow from precipitation/drainage. Ammonium formate concentrations on the order of 1 M are feasible in a newly formed body of water (where t = 0 corresponds to no formate) with a high surface area to volume ratio after about 100 years (Fig. 3). Such bodies of water are those that form from effectively pure H_2O, and slowly accumulate HCN over this timescale (see caveats about losses in Methods). Furthermore, these systems describe macroscale environments, whereas minute volumes can reach molar quantities seconds such as via drying

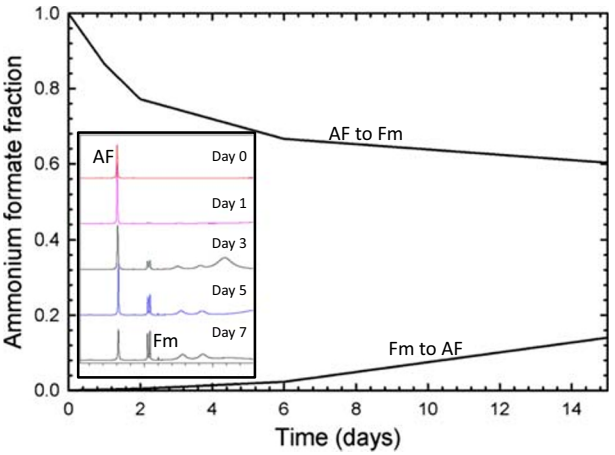


Fig. 4 The thermal evolution (85 °C) of solutions of ammonium formate (AF) turning to formamide, and of formamide (Fm) turning to ammonium formate over time in a sealed system. The inset shows an example of the NMR tracking evolution over time of formate to formamide, with changing spectra over 7 days. In open systems allowed to evaporate, the formate to formamide reaches a ratio of 1 to 1.5, stabilizing at that ratio. In this closed system, we project a ratio of formamide and water to ammonium and formate of about 1.3, similar to the open system

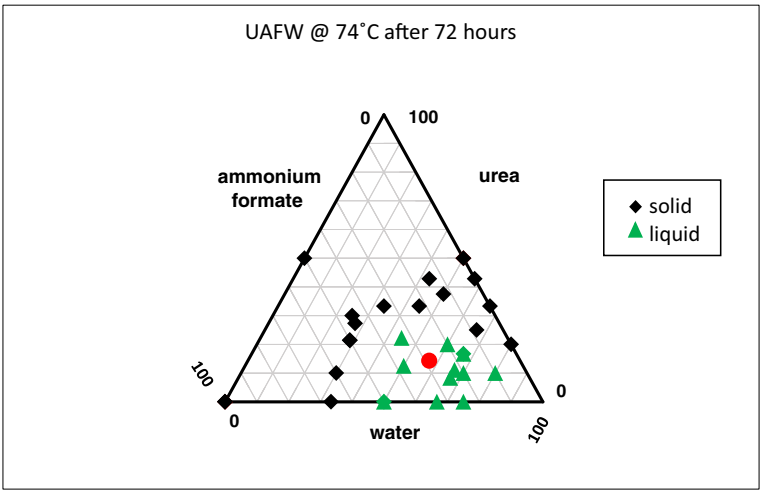


Fig. 5 The fluid stability of the UAFW system as a function of varied mole percent of individual components. Red diamond indicates 1:2:4 M ratio of UAFW

in aerosols as occurs in sea spray or adsorption onto mineral surfaces (Castañeda et al. 2019). To reach the concentration of ammonium formate used in prior experiments (~27 M, e.g., Burcar et al. 2016) it would take on the order of ~1000–10,000 years in such dry a basin environment (Fig. 3). Note that the effective surface area of a lake can be equivalent to its drainage basin if the lake regularly receives precipitation that solubilizes accumulated HCN and delivers it to the water body. However, if a pond or lake is subjected to wet/dry cycling, then higher concentrations of ammonium formate and urea may be possible. The kinetic calculations of Miyakawa et al. (2002a, 2002b, 2002c) and the thermodynamic calculations above (Fig. 2) do not apply to systems that bear high concentrations of formamide or ammonium formate as water and these compounds can form stable mixtures that do not

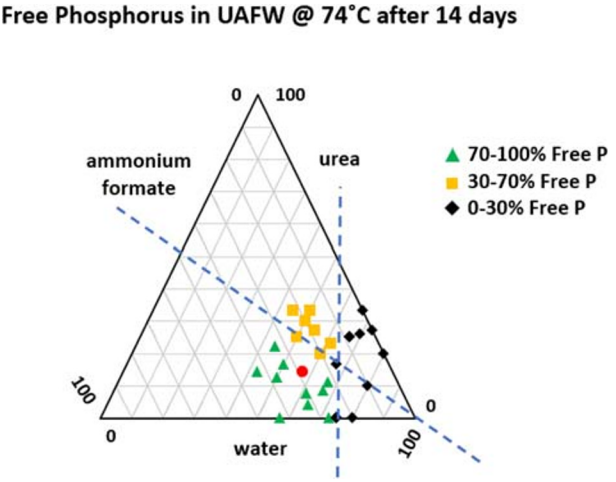


Fig. 6 The solubility of hydroxylapatite within the UAFW at varying mole percentages. Blue dashed lines depict the condition requirements for optimal yield. Red diamond indicates 1:2:4 M ratio of UAFW

hydrolyze (see below). Miyakawa et al. (2002a, 2002b, 2002c) predict low formamide concentrations at steady state under such circumstances.

The conditions of high ammonium formate activity and low water activity actually promote dehydration of ammonium formate to formamide. NMR spectra demonstrate an increasing concentration of formamide in sealed samples of ammonium formate and water (1:1 initially) subjected to heating at 85 °C. This result demonstrates that formamide is formed from ammonium formate even in the presence of water. The reverse reaction, of formamide hydrolysis to ammonium formate, competes with the dehydration reaction, and also occurs readily (see Fig. 4). Though the systems do not achieve equilibrium steady state at these experimental timescales (~2 weeks), an analog experiment with a sample containing urea and an initial formamide to formate ratio of 1.15 to 1.1 reached an apparent steady state ratio of formamide to formate of 1.5 to 1 by mole within 15 days (pH ~5.5). In the absence of urea, the formamide to formate system would likely appears reach a similar steady state ratio of ~1.3 to 1 (Fig. 4).

In order for the above systems to be prebiotically relevant (plausible without additional modification or intervention from a researcher), they must maintain a fluid state to allow diffusion of reactive substrates and promote condensation. The fluidity of the UAFW milieu is dependent on the initial molar ratios of urea to ammonium formate to water (Fig. 5), for the system at 74 °C (bracketing the experiments done by Burcar et al. (2016) at 65 and 85 °C). Urea is the main agent of solidification, and if the urea concentration is greater than 25% by mole fraction of the mixture, then solidification occurs rapidly (<3 days, Fig. 5). The initial molar fraction of water must always be greater than 50% to maintain stability of fluid within the system. Note that at other temperatures the system may remain fluid for longer times, either due to a slower rate of water evaporation (at low temperature), or promotion of the condensation of ammonium formate to formamide (potentially at higher temperature), or because of different melting temperatures associated with different ratios of solvent components.

Burcar et al. (2016) used UAFW in a 1:2:4 ratio to promote the phosphorylation of nucleosides. In this solvent phosphorylation appears to be contingent on the dissolution of phosphate minerals, with the yield of phosphorylated product corresponding to the solubility of the phosphate source mineral. Using the map of liquid stabilities (Fig. 5), we identify the UAFW compositions at which hydroxylapatite is the most soluble or least stable (Fig. 6) at 74 °C. When the water concentration in the mixture is less than 75%, and if the ammonium formate to urea ratio is greater than 1, the dissolution of hydroxylapatite is maximized.

Discussion

The addition of ammonium formate to urea/water increases phosphorylation of nucleosides and does so using prebiotically relevant phosphate minerals (Hazen 2013) at lower temperatures (Burcar et al. 2016). In Lorhmann and Orgel's prior work (1968, 1971), the phosphates used were predominately ammonium phosphates, whereas Burcar et al. (2016) and this work utilize the prebiotically plausible mineral hydroxylapatite (Pasek et al. 2017), which is insoluble in water ($K_{SP} \sim 10^{-57}$). The robustness and prebiotic plausibility of the UAFW was not investigated fully in Burcar et al. (2016). We examined here the ability of this mixture to address concerns of the robustness of the solvent (Schwartz 2019). Thermodynamic calculations predict both urea and ammonium formate should be the reaction products of the prebiotic environment assuming the prebiotic environment contained NH_3, CO_2, and HCN.

Furthermore, ammonium formate concentrations in bodies of water on the surface of the prebiotic Earth should approach those used in prebiotic phosphorylation experiments through HCN hydrolysis on geologically-short timescales (<1 million years), especially with wet-dry cycling that concentrates the HCN and formamide, promoting hydrolysis of these compounds.

In the original study of this solvent system (Burcar et al. 2016) it was not discussed if the 1:2:4 UAFW ratio was "special", and this formulation was based on prior work on diesel exhaust fluid (e.g. Solla et al. 2005). We find here that 1:2:4 ratio is within the range of liquid stabilities at 75 °C, and UAFW mixtures are liquid as long as the urea concentration is less than 25% of the mole fraction and water is greater than, or equal to 50% mole fraction. UAFW is thus robustly liquid over a wide range of molar ratios at 75 °C.

Within the range of UAFW liquid stability, there is a "sweet zone" for phosphate solubilization. Phosphate solubilization occurs under a narrower range of UAFW mixture compositions than that of UAFW mixtures are liquid, yet still occurs readily under a suite of molar ratios, mostly ammonium formate-rich fluids. We postulate that ammonium formate promotes hydroxylapatite dissolution due to a combination of pH effects (the pH of the UAFW solvent is about 5–6), and through chelation of calcium by formate (Pasek et al. 2017; Schwartz 1972; Joseph 1946). Apatite increases in solubility as pH decreases (Pasek et al. 2017).

It is important to note that the UAFW solution here produced formamide as it was heated over time in closed systems (Fig. 4). This would appear to lie in contrast to the predicted thermodynamic instability of formamide in aqueous solution (e.g., Fig. 2, Bada et al. 2016). However, the UAFW solution is initially (and continues to evolve to) a low water-activity solvent (57% mole fraction H_2O in Burcar et al. 2016). Thermodynamic models (including section 2A) of formamide stability also neglect the formation of stable water-formamide solutions, which likely reach an energy minimum that allows for the co-existence of formamide and ammonium formate in water. Furthermore, in the UAFW systems, the application of heat continues to dry this solvent via water evaporation, eventually promoting the condensation of ammonium formate to formamide, even in the presence of liquid water. These factors combined imply that the solvent favors production of formamide from ammonium formate during heating. Indeed, the UAFW solution could be viewed as evolving towards a solvent that is "buffered" with low water activity through the hydrolysis of formamide. Such low water activity solvents should be conducive to organic phosphorylation (see Fig. 2, showing AMP formation).

Conclusions

Phosphorus is a cardinal factor in the biochemistry of life as we know it. However, the most abundant phosphorus-containing mineral group on the Earth's surface today (apatite) is not soluble in typical water bodies with pH > 7 without carbonate (Toner and Catling 2020). Here, we study the robustness of the UAFW system as an alternative solvent. When studying the robustness of the UAFW system, we see that the liquid stability of the system is central to solubilizing phosphate. If the product evolved solvent system is no longer a liquid, we did not observe any free phosphorus in the system. The solubility of phosphate is controlled by the molar ratios of the UAFW and the dissolution of phosphate required a liquid medium. If the UAFW mixture remains a liquid, the solubility of phosphate will be enhanced. Greater enhancement comes when the mole ratio of ammonium formate is more than urea, and when water (in which

apatite is minimally soluble) is not greater than 75% of the molar composition. These data lead us to conclude that urea does not play a role in solubilizing phosphate, but instead lowers the solubility of hydroxylapatite and that the role of urea within the UAFW system is to help create a stable solvent in which the activity of water is lowered/buffered to a point in which the energy needed to phosphorylate nucleosides is feasible through condensation. Thus, a potential avenue in overcoming the phosphorus "water problem" is presented through the UAFW system.

This work shows that the UAFW system is a liquid that can potentially form over a range of environmental conditions. All the components of the UAFW system are simple and prebiotically plausible. Additionally, solutions approaching that of the 1:2:4 UAFW are plausible through wet-dry cycling under geological timescales, and can evolve to generate formamide. The UAFW system is liquid over a range of component ratios, and many of these actively solubilize phosphate.

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