AGAR GELATION SPECTROPHOTOMETRIC ASSAY OF CHAO- AND KOSMO-TROPICITY OF INORGANIC SALTS, AND IMPLICATIONS FOR LIFE IN TERRESTRIAL AND MARTIAN BRINES.

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Introduction: The presence of solutes in aqueous solution has a general effect on microbial behavior, as the ionic strength of a solution impacts the activity of water in the solution. However, there is also a specific effect associated with the presence of solutes upon microbial behavior, which can be considered to be either chaotropic (inhibiting microbial activity) or kosmotropic (facilitating microbial activity) [1]. These chao- vs. kosmo-tropic effects have been studied by performing microbial culture experiments in the presence of various solutes [e.g. 2-5], but have also been quantified using an empirical agar gelation spectrophotometric assay technique [2, 4-7], which has the advantage that it can produce a quantified, universal scale of chao- vs. kosmo-tropic activity (CKA). A large number of pure solutes have been assayed previously, although assays of solute mixtures are relatively uncommon. Moreover, assays of some pure salts pertinent to hyper-arid terrestrial and Martian environments are currently unavailable (e.g. alkali metal nitrates, bicarbonates, perchlorates).

This study has performed agar gelation spectrophotometric assays to quantify CKA values for various pure inorganic salts, as well as binary, ternary and quaternary mixtures of inorganic salts. In addition to quantifying CKA values for solutions relevant to possible terrestrial and Martian brines, this study attempts to identify if the chao-/kosmo-tropic effects of a salt mixture follows conservative behavior (i.e. simple additive/subtractive combination of the chao-/kosmo-tropic effects of the component pure salts). Recommendations for future CKA experimental procedures are also provided.

Methods: Gelation experiments were performed with a procedure similar to that used previously [6]. Experiments used extra pure reagent-grade agar, gel strength 600-700 g/cm^3 (Nacalai Tesque, Kyoto, Japan, code 01028-85, lot # M0G2724) at a concentration of 1.5% w/v. All inorganic salts were of ACS grade or better. Ca-salts were not used as $CaCl_2$ has a specific interaction with agar [6], and Ca-rich brines are relatively rare in nature due to the low solubility of Ca-carbonate and Ca-sulfate salts. Agar/salt solutions were prepared at salt concentrations up to concentrations that induced agar precipitation, as determined visually. Spectrophotometric assays were performed at a wavelength of 500 nm using optical glass cuvettes (Fisher Scientific, cat. #14-958-120) in an Evolution 260 Bio UV-vis spectrophotometer equipped with an 8-cell Peltier system with thermoelectric temperature control. Initial solution temperature for experiments was set approx. 7°C above the anticipated gel point, and then decreased at a rate of 0.5°C/2.5 mins., with absorbance readings taken every 2.5 mins. Gel point was identified at the temperature when the absorbance value was measured at 0.03 absorbance units greater than the initial absorbance value at gel point +7°C. Gel point ΔT = solution gel point T – agar-only gel point (41.5°C). All experiments were performed in replicate. Calculation of CKA values used a gel solution heat capacity of 4.15 kJ/kg.°C [2, 6].

Results: All pure salts showed a linear effect of gel point ΔT vs. salt concentration ($R^2 > 0.98$), except for $NaHCO_3$ and $MgCl_2$. Values of CKA for a 1°C change of gel point are plotted in Fig. 1.

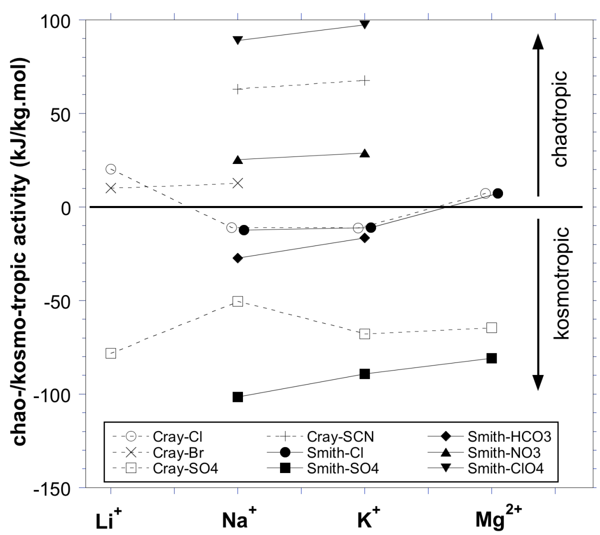

Figure 1. Values of chao-/kosmo-tropic activities for a 1°C change of gel point for pure salts. Data from this study, [6], and [7] for $MgCl_2$.

Figure 1 shows generally good agreement between results of this study and previous studies [4-6]. Perchlorate salts were previously considered to be very chaotropic [8], and Fig. 1 confirms this to be correct. Nitrate salts are also chaotropic, while bicarbonate salts are kosmotropic. In general, K-salts are consistently more chaotropic (or less kosmotropic) than the corresponding Na-salts.

Values of gel point ΔT vs. salt concentration (M) were calculated for each individual salt in salt mixtures, and these values of ΔT were combined conservatively (i.e. simple additive/subtractive behavior), to generate a calculated ΔT. Values of Measured ΔT – Calculated ΔT vs. Calculated ΔT for all salt mixtures are plotted in Fig. 2. Figure 2 shows that Measured ΔT - Calculated ΔT = 0 ± 2 °C for most salt mixtures, i.e. that salt mixtures generally show conservative chao-/kosmo-tropic behavior for mixing of their component salts. There does not appear to be any systematic explanation for the mixtures that show larger values of Measured ΔT – Calculated ΔT, although such mixtures tend to have significant concentrations of $MgCl_2$.

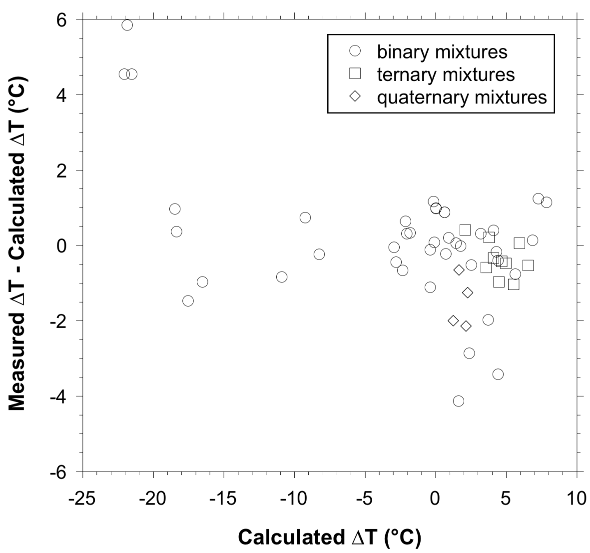

Figure 2. Measured ΔT – Calculated ΔT vs. Calculated ΔT for gel points of salt mixtures (55 experiments).

Discussion: The values of CKA for nitrates, perchlorates, and bicarbonates are generally consistent with the relative order of anions in the Hofmeister series [9]. The chaotropic nature of nitrate and perchlorate salts is particularly relevant to microbial habitability in hyper-arid terrestrial environments such as the Atacama Desert, Chile [10], but also in locations such as Gale Crater, Mars [11]. However, the common co-occurrence of kosmotropic bicarbonate and sulfate salts may help to ameliorate the chaotropic effects of nitrate and perchlorate salts.

Chao-/kosmo-tropic behavior of salt mixtures. The generally conservative chao-/kosmo-tropic behavior of salt mixtures enables prediction of the CKA value of a range of naturally-occurring brine compositions with a reasonable degree of confidence, and may allow for a more targeted approach to search for possible past or present microbial life in hyper-saline environments on Earth and on Mars. However, future work to determine CKA behavior for more salt mixtures (esp. quaternary mixtures) would be valuable, as would further study of the non-conservative CKA behavior observed for some salt mixtures.

Recommendations for future agar gelation assays. Agar gelation assays are a valuable experimental technique to measure CKA values, but standardization of an analytical methodology would facilitate comparison of measurements between different studies. Recommendations include: use of a consistent brand and chemical specifications for the agar gel (different brands and specifications of agar gel may provide different experimental results); the use of optical glass cuvettes (with better thermal conductivity than plastic cuvettes); and a standard thermal program when performing spectrophotometric measurements (i.e. initial starting temperature, cooling rate).

Conclusions: The chao-/kosmo-tropic activity (CKA) of various pure salts and salt mixtures have been performed using an agar gel spectrophotometric assay technique. Standardization of agar gelation assay procedures would facilitate comparison between results of future agar gelation assay studies. Results show that nitrates are chaotropic, perchlorates are very chaotropic, while bicarbonates are kosmotropic. Measured values of CKA for salt mixtures indicate that CKA behavior is generally conservative with respect to the salt mixture components. Results enable the prediction of CKA values for a range of natural brine compositions, and have implications for the feasibility of microbial life in hyper-arid environments on Earth and on Mars.

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