

Extraction of vanadium(V) from seawater by tunicates: A revision of concepts¹

A number of tunicates spectacularly concentrate trace metals, among them the common tunicate *Ciona intestinalis*, which assimilates vanadium from seawater. Despite a long history of study erroneous concepts remain of vanadium chemistry and of fluid and chemical dynamics as applied to assimilation.

For example, certain investigators assume that vanadium(V) in seawater exists as a positively charged species (McIntyre 1970). Evidence for this conclusion is based on ion flotation experiments showing complexing of vanadium in seawater by negatively charged chelating agents (Kalk 1963). However, chelation of negatively charged metal-containing anions by chelating agents of like charge sign is a well known phenomenon; e.g. vanadate with EDTA (ethylenediaminetetraacetate) (Ringbom et al. 1957), molybdate and tungstate with 8-hydroxyquinolate (Diebler and Timms 1971), and tungstate with gallate (Halmekoski 1959). Thus the charge on the vanadium(V) species should not be inferred from the charge on the chelating agent.

Instead, we assume that the well described vanadium(V) equilibria in moderately high ionic strength media (Ingri and Brito 1959; Brito et al. 1964) also exist in seawater. We offer the following evidence in support of this assumption. Oxidation states of vanadium lower than (V) are not important in aerated aqueous solutions, since any vanadium(IV) in seawater is immediately oxidized to vanadium(V) (Dean and Herringshaw 1963). The only relevant cationic vanadium(V) species, VO_2^+ , is unstable above pH 2 (Cotton and Wilkinson 1972).

In addition, the experimental evidence collected and analyzed by Brito et al. has shown that the important equilibria in 0.5 M NaCl involve anionic monomeric and

polymeric vanadate species. Evaluation of their equations at pH 8 and a total (in seawater) vanadium(V) concentration of 5×10^{-8} M (Ladd 1974) shows that monomeric HVO_4^{2-} and H_2VO_4^- species predominate, being essentially equimolar. The approximate concentration of vanadate polymers is 10^{-12} M, or less. As the pH is gradually shifted to lower values, the species H_2VO_4^- tends to predominate, until quite acid solutions are reached. These considerations also rule out a previous suggestion that the reactive form of vanadium between pH 5 and 6 is $\text{V}_4\text{O}_{13}^{6-}$ (Kovalski and Rezayeva [cited in Carlisle 1968]). Since vanadium is at least an order of magnitude less in the particulate than in the aqueous phase and since *C. intestinalis* has been observed to extract vanadium from filtered seawater, the extraction primarily is from the aqueous phase. We conclude therefore, that the tunicate extracts monomeric vanadate species.

The extraction of vanadium(V) from seawater is inhibited by a variety of oxyanions according to Goldberg et al. (1951) and Rummel et al. (1967). These authors concluded that phosphate competes with vanadate for the appropriate binding site on the tunicate. However phosphate and vanadate can react to form a complex (Russel et al. 1961). Therefore in experiments where seawater is enriched with added phosphate, a significant fraction of the total vanadate is tied up by the phosphate. At the phosphate level naturally present in seawater— $5 \mu\text{M}$ —complex formation is relatively insignificant. (This explanation may also be applied to other oxyanions and to other inhibitors.)

Another misconception relates to pumping, assimilation, and feeding by tunicates. We believe that the flow rates previously measured often reflect experimental artifacts rather than the true pumping rate of the tunicate. Methods using tubes inserted into the animal's siphons (especially the incurrent, cilia bearing siphon) traumatize

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the organism; these methods and those measuring the volume of pumped seawater do not rigorously eliminate hydrostatic pressure heads. Methods involving the rate of depletion of suspended particulate matter filtered out by the tunicate are less subject to error, but may produce erratic results due to variations in particle retention and its effect on the organism. Direct measurements of the rate of pumping of dyed seawater through the tunicate avoid these errors (Hamwi and Haskin 1969; Holmes 1973). Our measurements (Kustin et al. 1974) utilizing all these methods, particularly the direct method, indicate that healthy specimens pump at rates varying between 2 and 185 ml h⁻¹ g wet wt⁻¹; the average value based on clearance and direct methods is 50 ml h⁻¹ g wet wt⁻¹.

Assimilation of vanadium from the inhaled current is independent of the pumping rate. The reason is that measured radiovanadium exchange rates are slower than even the lower limit of the pumping rate (Kustin et al. 1975; Ladd 1974; Rumel et al. 1967). Since pumping and extraction are sequential processes, the slower of the two is rate limiting. Thus, the "efficiency" of extraction cannot be determined by substituting the pumping rate into the radiovanadium exchange equation to calculate an effective clearance rate.

The most reasonable framework in which to view the assimilation of vanadium by tunicates is therefore as follows. Tunicates remove vanadium from the aqueous phase as monomeric vanadate. As the seawater circulates through the tunicate, whether for feeding or respiration, assimilation of vanadate may take place. The time constant for extraction is inherently smaller than the pumping time constant, making extraction out of seawater into the tunicate's body the slow step in the overall process. Inhibition of vanadium uptake by phosphate or arsenate may be the result of reaction with vanadate, as the product may not bind at all, or as well, to the appropriate site on the tunicate.

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References

- BRITO, F., N. INGRI, AND L. G. SILLÉN. 1964. Are aqueous metavanadate species trinuclear, tetranuclear, or both? *Acta Chem. Scand.* **18**: 1557-1558.
- CARLISLE, D. B. 1968. Vanadium and other metals in ascidians. *Proc. R. Soc. Lond. Ser. B* **171**: 31-42.
- COTTON, F. A., AND G. WILKINSON. 1972. *Advanced inorganic chemistry*, 3rd ed. Interscience.
- DEAN, G. A., AND J. F. HERRINGSHAW. 1963. The air-oxidation of vanadium(IV) in alkaline solution. *Talanta* **10**: 793-799.
- DIEBLER, H., AND R. E. TIMMS. 1971. Kinetics of complex formation of molybdate and tungstate with 8-hydroxyquinolines. *J. Chem. Soc. Ser. A* **1971**: 273-277.
- GOLDBERG, E. D., W. McBLAIR, AND K. M. TAYLOR. 1951. The uptake of vanadium by tunicates. *Biol. Bull.* **101**: 84-94.
- HALMEKOSKI, J. 1959. A study of the chelate forming reaction between some phenolic compounds and anions formed by Mo(VI), W(VI), V(V), Sn(IV) and Bi(III). *Ann. Acad. Sci. Fenn. A2* **96**: 1-64.
- HAMWI, A., AND H. H. HASKIN. 1969. Oxygen consumption and pumping rates in the hard clam *Mercenaria mercenaria*: A direct method. *Science* **163**: 823-824.
- HOLMES, N. 1973. Water transport in the ascidians *Styela clava* Herdman and *Ascidella aspersa* (Müller). *J. Exp. Mar. Biol. Ecol.* **11**: 1-13.
- INGRI, N., AND F. BRITO. 1959. Equilibrium studies of polyanions. 6. Polyvanadates in alkaline (NaCl) medium. *Acta. Chem. Scand.* **13**: 1971-1996.
- KALK, M. 1963. Absorption of vanadium by tunicates. *Nature (Lond.)* **198**: 1010-1011.
- KUSTIN, K., K. V. LADD, AND G. C. McLEOD. 1975. The site and rate of vanadium assimilation in the tunicate *Ciona intestinalis*. *J. Gen. Physiol.* **65**: 315-328.
- _____, _____, AND D. L. TOPPEN. 1974. Water transport rates of the tunicate *Ciona intestinalis*. *Biol. Bull.* **147**: 608-617.
- LADD, K. V. 1974. The distribution and assimilation of vanadium in the tunicate *Ciona intestinalis*. *Biol. Bull.* **147**: 618-624.

- lation of vanadium with respect to the tunicate *Ciona intestinalis*. Ph.D. thesis, Brandeis Univ. 108 p.
- McINTYRE, F. 1970. Why the sea is salt. *Sci. Am.* **223**: 104–115.
- RINGBOM, A., S. SITONEN, AND B. SKRIFVARS. 1957. The ethylenediaminetetraacetate complexes of vanadium(V). *Acta Chem. Scand.* **11**: 551–554.
- RUMMEL, W., AND OTHERS. 1967. Absorption and accumulation of vanadium by tunicates. *Protides Biol. Fluids Proc. Colloq. Bruges* **14**: 205–210.
- RUSSEL, R. U., J. E. SALMON, AND H. R. TIETZE. 1961. Condensed ions in aqueous solutions. Part 2. Compounds of quinquevalent vanadium with orthophosphate. *J. Chem. Soc.* **1961**: 3211–3214.

Carbon dioxide and metabolism in marine environments¹

Investigators of community metabolism in aquatic environments have frequently followed changes in the O₂ content of the water. Changes in the carbon dioxide content provide an alternative metabolic record which has also been used. Previous biological applications have often simplified treatment of the marine CO₂ system to the point of losing valuable information, while more rigorous chemical discussions of the marine CO₂ system are often so laborious that they obscure relevant biological interpretations. This comment is meant to focus attention on those aspects of the marine CO₂ system directly relevant to studies of community metabolism. Detailed examples of the use of CO₂ in such studies are reported elsewhere (Smith 1973; Smith and Pesret 1974), as are more rigorous and detailed descriptions of the chemistry of CO₂ in seawater (Skirrow 1965; Park 1969).

Photosynthesis (or respiration) and calcification (or solution) within a closed body of water induce a variety of changes in the absolute and relative concentrations of the CO₂ species present. Photosynthesis and calcification both lower the CO₂ content of the water, while respiration and CaCO₃ solution raise it. Only the precipitation or solution of CaCO₃ significantly alters the total alkalinity of the water; consequently, alkalinity changes can be used to calculate the effects of CaCO₃ reactions on total CO₂ changes. Changes in CO₂ not directly attributable to CaCO₃ reactions represent photosynthesis or respiration and

can be related to organic carbon production or consumption. Both the organic carbon reactions and the CaCO₃-C reactions are biologically important processes in a variety of marine environments.

The two most convenient parameters to describe the CO₂ content of seawater are pH and total alkalinity. The only major instrumentation required to measure these parameters is a good pH meter. With reasonable care, one can achieve a precision (standard error on duplicate analyses) of about ±0.005 pH units and ±0.005 meq liter⁻¹; this precision allows calculation of the total CO₂ content of the water to a precision of about ±0.005 mM (or about 0.25% of the total CO₂ content of seawater). The calculation of CO₂ from these parameters is sufficiently tedious to be best accomplished by computer.

To determine the CO₂ changes as the sample incubates in the system of interest, one calculates the total CO₂ content of the water as described above. The molar change in CO₂ from CaCO₃ reactions equals half the equivalents of total alkalinity change; that CaCO₃-induced CO₂ change is subtracted from the total CO₂ change, and the remainder equals the net CO₂ change that may be attributed to photosynthesis or respiration.

Alkalinity is the capacity of water to neutralize hydrogen ions. Carbonate alkalinity is the neutralizing capacity of the ionic CO₂ species alone, while total alkalinity includes not only the carbonate alkalinity but also other species (almost entirely boron species) that neutralize hydrogen ions, *minus* the offsetting effect of hydrogen ions present in the solution. Changes in

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