

## THREE MODEL SPACE EXPERIMENTS ON CHEMICAL REACTIONS

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## ABSTRACT

Three simple science demonstrations conducted aboard Skylab IV and Apollo-Soyuz involved phenomena that are of interest to the biochemistry community. The three experiments are identified here as the Formaldehyde Clock Reaction, the Equilibrium Shift Reaction, and the Electrodeposition Reaction. The Formaldehyde Clock Reaction and the Equilibrium Shift Reaction experiments conducted aboard Apollo-Soyuz demonstrated the effect of low-g foams or air/liquid dispersions on reaction rate and chemical equilibrium. The Electrodeposition Reaction experiment conducted aboard Skylab IV demonstrated the effect of a low-g environment on an electrochemical displacement reaction.

In a formaldehyde clock reaction, a number of chemical reactions occur simultaneously and at such rates that the end of the reaction, signaled by a change of color from colorless to red, does not occur until about 20 seconds after the reactant solutions have been mixed. A clock reaction embodies some of the features of periodic chemical reactions which are of great interest at present because they suggest a relevancy to mechanisms controlling biological rhythms. In exploratory ground experiments, the purpose of which was to identify a good space demonstration experiment, it was discovered that a formaldehyde clock reaction displays effects that can be attributed to Gibbs (or van der Waal) adsorption of polymeric formaldehyde solution species. Also discovered were internal effects that are caused either by internal shear as the result of residual fluid flow or by formation of three-dimensional formaldehyde species networks. The various noted behaviors of the Formaldehyde Clock Reaction in ground tests and in the low-g tests are described. It is concluded that the unique behaviors observed in low-g are the result of the presence of many more small air bubbles than were present in the one-g cases.

In the Chemical Shift Reaction a reversible chemical equilibrium is caused to shift by means of foam formation. Evidence of the chemical shift is given by the color of the foam (pink) which is different from the color of the bulk solution (amber brown). In low-g the pink foam was many times more stable than under one-g conditions.

In the Skylab Electrodeposition Experiment, a chemical displacement reaction caused silver crystals to be deposited. The silver crystals obtained in low-g were quite different than those obtained in one-g because of the differing convection currents generated in the two situations. This experiment is not discussed in detail. Only the implications of the conclusions for biochemical type reactions are considered.

The implications of the three space experiments for various applications are considered.

## THE FORMALDEHYDE CLOCK REACTION

It is well known that the rates of many, if not most, chemical reactions are heavily dependent on the concentrations of the reacting species. Thus, if reacting chemical species are not uniformly distributed throughout the solution, a reaction can occur faster in one part of the solution than in another. At constant temperature and pressure, a non-uniform distribution of solute species in a well mixed solution can occur as the result either of adsorption at a liquid/gas or a liquid/solid interface or of a hydrostatic pressure



effect on chemical potential. The first of these effects is the one of interest here. For the sake of clarity, it may be well to note that the type of adsorption we are considering here is Gibbs adsorption, i. e. , a solution becomes more or less concentrated (positive and negative adsorption) in solute species in the liquid-gas or liquid/solid interface zones but no change in phase occurs nor is there any chemical reaction between solute species and species in the gas or solid phases.

It is generally well known that Gibbs adsorption can cause the surface tension of a solvent to be either increased or decreased when solute is added. The effect of Gibbs adsorption on rates of chemical reaction, however, has not, to the best of our knowledge, been directly demonstrated. A number of previous investigators have conducted chemical reaction experiments, however, in which either insoluble monomolecular films were involved or chemisorption had occurred. In some cases where Gibbs adsorption was undoubtedly involved, the evidence was indirect. These various cases as well as some prior, reported speculations are briefly reviewed in the following paragraphs.

E. K. Rideal was a very active investigator in this area and published a number of works on chemical reactions involving monolayers of one reactant (Refs. 1-4). Among some of the systems investigated by Rideal and others are (Refs. 1-9):

<u>Type Reaction</u>	<u>Monolayer Film</u>	<u>Bulk Reactant</u>
oxidation	oleic acid	permanganate
hydrolysis	ethyl butyrate	pancreatin enzyme
hydrolysis	ethyl palmitate	aqueous alkali
hydrolysis	lecithin	snake venom enzymes
photolysis	stearanilide	
	benzylstearylamine	
	$\beta$ -phenylethylstearyl-amine	
	proteins	
	barium stearate-stearic acid	
polymerization	maleic anhydride compound of eleostearin	
chemical reaction	amines and aldehydes	
chemical reaction	egg albumin	trypsin enzyme
	caseinogen	
chemical reaction	sterol	sodium cetyl sulfate
	carcinogenic hydrocarbons and sterols	
lactonization	$\gamma$ -hydroxystearic acid	
complex formation	stearic acid	heavy metal ions

The rates of chemical reaction were shown to be strongly affected by species adsorption at charcoal interfaces in the hydrolysis of bromoethylamine and in the conversion of dimethyleneimine hydrobromide into bromoethylamine in hydrobromic acid solution (Ref. 10). Foaming was found to promote the lipolysis of milk (Ref. 11). The cited study concluded that a foam promotes lipolysis by providing optimum conditions as follows (i) greatly increased liquid surface, (ii) selective concentration of enzyme at the air-liquid interface, (iii) activation of the substrate by surface denaturation of the membrane materials surrounding fat globules, and (iv) intimate contact of enzyme and activated substrate.

In ground studies conducted to identify a good space demonstration experiment, the discovery was made that a formaldehyde clock reaction can display both a Gibbs adsorption and an internal structure or a shear effect on the rate of the reaction. A report of the observed evidences of Gibbs adsorption and internal shear or three-dimensional structure



effects in a formaldehyde clock reaction has not yet appeared in the open literature although a submission is currently being reviewed for publication (Ref. 12). A brief summary, therefore, is given here.

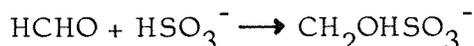
The surface and internal effects to be described were observed with solutions and procedures which are as follows:

Stock solutions: (a) 3.3% formaldehyde (9.0 ml 37.7% reagent grade formaldehyde diluted to 100 ml with distilled water), (b) 1 gm phenolphthalein dissolved and diluted to 500 ml with 50% ethanol-water, and (c) 10.0 gms sodium metabisulfite ( $\text{Na}_2\text{S}_2\text{O}_5$ ) and 1.5 gms of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ), reagent or certified grades, diluted to 100 ml with distilled water. Procedures: One ml of formaldehyde stock and 0.5 ml of phenolphthalein stock are added to 8 ml of distilled water in a test tube. A 0.5 ml portion of the bisulfite/sulfite stock solution is then added rapidly, the test tube capped, and shaken vigorously for about 5 sec. The mixed solution remains colorless for about 20 sec at the end of which time a sudden appearance of a red color occurs. The time interval between the time of bisulfite/sulfite addition to the time of red color appearance can be varied by adjustment of solution concentrations (Ref. 13). Plastic syringes bought in drugstores for a few cents make handy devices for measuring and adding the small amounts of reagents involved. The stock solutions of formaldehyde and phenolphthalein are stable indefinitely. The sulfite/bisulfite solution, however, deteriorates. The deterioration rate, however, is fairly slow if oxygen and light exposure are kept to a minimum.

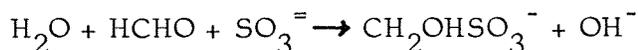
The chemical reactions involved are as follows (Ref. 13):

#### Rate Constants

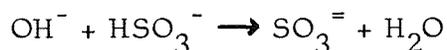
2.8 l/mol sec



0.14 l/mol sec



instantaneous



Thus, excess hydroxide ion becomes available to react with phenolphthalein indicator only when all of the bisulfite ion is used up.

The surface and internal structure effects observed are briefly as follows: In plastic (Lexan) test tubes the color change is most frequently seen to occur first in the small drops that cling to the sides of the tube. Or the color change will start at a point in the liquid/vapor/solid interface or in the bulk of the solution and then spread out as a wave into the remainder of the solution. If the reaction is allowed to occur in contact with a polystyrene surface, color spots appear at the solid/liquid interface sites, the color change then proceeding into the bulk of the solution. In chilled solutions the surface effects are greatly enhanced. In addition to the described surface effects, internal structure effects, as evidenced by complex colored shapes in the bulk of the solution, are also seen. The internal structure effects may be caused either by shear as the result of residual fluid flow or by a three-dimensional formaldehyde species network. In stirred solutions the color changes outline vividly fluid flow phenomena such as vortices. Various size drops of reacting solution placed on various surfaces change color most frequently in the order of large drops first, medium-sized drops next, and small drops last. Further evidences of the Gibbs and internal structure effects are given in the not-yet-published report (Ref. 12) and in film strips of the various ground tests. In the Apollo-Soyuz experiment Astronaut D. K. Slayton performed in earth orbit the formaldehyde clock reaction in capped Lexan centrifuge tubes. The objective of the space experiment was to determine the effect of a



water solution  
red

benzene solution  
yellow

on quartz powder  
red

#### Silver Eosinate

aqueous solution containing silver nitrate  
pinkish-yellow with green fluorescence

adsorbed on surface of silver halide  
red

"

solution + benzene emulsion  
red precipitate at interface

"

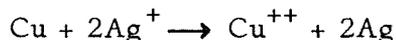
shaken in air  
red precipitate at interface

Probably not all of the preceding reactions are strictly reversible. The illustrative point, however, is not affected.

In the Apollo-Soyuz experiment an aqueous solution of thymol blue was shaken. The pink foam lasted a great deal longer than one generated on the ground. Times for foam collapse, however, were not measured.

### THE ELECTRODEPOSITION REACTION

The Electrodeposition Reaction experiment conducted aboard Skylab IV was designed primarily to study metal crystal growth in a low-g environment. However, because the reaction involved an electrochemical displacement reaction, certain features of it should be of interest to the biochemical community. The experiment consisted of the astronaut inserting a copper wire into a 5 wt % silver nitrate solution. The following electrochemical displacement reaction occurred:



The silver crystals grown in space are quite different from those grown on the ground because of the differing convection currents generated in each case. Full details will be found in a report which will appear shortly (Ref. 14) and in a paper which is currently being reviewed for publication (Ref. 15). The experiment, while not immediately relevant to biochemistry, does raise some common questions. For example, what are the gravity effects on e.m.f. and what is the nature of low-g convection in cases where electric fields are involved. These common aspects are covered in more detail in the following discussions.

Gravity effects on e.m.f.: The fact that gravity can affect e.m.f. values has been long known (Ref. 16). The gravity dependency of e.m.f. in ordinary cells is the result of the change of chemical potential of the ions with change in gravity level. This dependency is given by:

$$d\mu_i = (M_i - \bar{V}_i \rho) g dh$$

where  $\mu_i$  is the chemical potential of the ion in question,  $M_i$  its molecular weight,  $\bar{V}_i$  the partial molal volume,  $\rho$  the density of the solution,  $g$  the gravitational acceleration, and  $h$  the height measured from some reference height. One early worker found that e.m.f. changed about  $0.510 \times 10^{-8}$  volt per cm of height for a cell employing a 2.71 molal potassium chloride solution. Thus, on earth an e.m.f. may be generated just by having one half cell higher than another identical half cell. In living systems gravity has been shown to electrically polarize an organ placed horizontally. For example, after shoots and leaf



stalks had been placed horizontally, their upper sides assumed a potential several millivolts more negative than the lower (Ref. 17). The explanation given for this effect is that the diffusion potentials already existing across membranes becomes modified as the result of gravity. The diffusion potentials can be modified by a change of ion mobilities due to gravity and by displacement of growth hormone which raises the ionic selectivity of the membranes concerned in the region of its enrichment (Ref. 17).

Low-g electro- and other types of convection: Various types of relatively ill-understood convections undoubtedly play important roles in biochemical processes. For example, Dr. W. Dorst, Amsterdam, believes that convective processes within a millipore membrane are the major factors in his experiments dealing with the effect of gravity on the permeability of a synthetic membrane (Ref. 18). Adding substance to this belief is the demonstration of free convection in electrolysis cells whose total volume was 0.25 ml and which contained solution of depths of less than 2 mm (Ref. 19). Convective processes have undoubtedly also played roles in experiments on anomalous and thermo-osmosis which gave puzzling results (Ref. 20). Recently Dr. J. R. Melcher of MIT conducted some preliminary tests on electrically-driven convections in aqueous solutions (Ref. 21). It might be added that the electro-convection Dr. Melcher was concerned with had to do with electrical forces on zones of concentration inhomogeneities within the bulk of the solution and not with electro-osmosis type convection. It is interesting to speculate what role electro-convection might play in various electrolysis and electrophoretic processes, but speculations would be fruitless at present when so little is known about the basic phenomenon. The literature is so scanty on convective phenomena in biological processes and processing that we expect that a whole new exciting era will be initiated once serious attention is turned in this direction. It may be well to add that gravity and electric fields are not the only forces that can drive fluid flow. Surface and interfacial tensions, phase changes, thermally induced volume changes, vibrations or g-jitter, magnetic fields are also possible driving forces. In addition, care must be taken when analyzing data from low-g environments not to tacitly assume that gravity-driven convection was absent. Even in low-g, gravity can be a major driving force. It all depends on the particulars of a given situation. The general nature of convection in low-g environments was recently reviewed (Ref. 21).

## POSSIBLE APPLICATIONS

The implications of the space experiments with regard to possible applications fall into two areas. One area is concerned with directions for basic research on biophysical processes and the other with directions for processing applications in low-g. In the area of basic research, the Formaldehyde Clock Reaction would appear to have relevancy to phenomena such as the clotting of blood and the formation of cataracts. For example, the following description of how a lobster's blood clots on a glass slide sounds much like a description of how a formaldehyde clock reaction occurs in a plastic tube:

"A wave of changes must start at the interface between the glass and blood, and progress through the latter, involving these sensitive corpuscles in its path. . . The two impressive features of this phenomenon are (1) that a chemical change of catastrophic character can be started at an interface, and (2) that the change can be propagated apparently indefinitely through one of the phases." (Ref. 22)

A description of the processes involved in the formation of cataracts (Ref. 23) also sounds as if processes similar to those found in the Formaldehyde Clock Reaction are involved. For example, the formation of regions of cortical opacities within a cataractous lens can be compared to the formation of red spots within the solution in the Formaldehyde Clock Reaction because an opacity is caused by an abrupt or irregular change in protein concentration. Also interesting is the fact that shear or internal structure effects appear to play a role both in the Formaldehyde Clock Reaction and in the formation of cataracts.

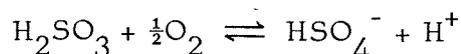


Also solution size or volume effects are notable in both phenomena. The hints that the two phenomena may be related are tantalizing and a number of experiments immediately suggest themselves. With regard to electrical phenomena in low-g, it would appear that space experiments could help a great deal in explaining the role of convection in a number of membrane transport phenomena.

In the area of processing applications, the demonstration of a formaldehyde clock reaction and an equilibrium shift reaction in low-g indicates that low-g forms can be unique environments for conducting biochemical reactions. Also the demonstration of a longer lasting foam in low-g indicates foam separation processes that cannot be done on earth because of the long times required for adsorption, i. e., the foam on earth does not last long enough for adsorption to occur. The demonstration of an electrodeposition in space points towards organic syntheses and separations utilizing electrolysis.

#### ADDENDUM

In the discussion that occurred after this paper was given at the Bioprocessing Colloquium, a couple of questions were raised on which we should like to elaborate. The first concerns the role of oxygen in the surface effects noted in the Formaldehyde Clock Reaction. Oxygen may affect significantly only the sulfite or bisulfite species through the reaction



The reaction is slow in the absence of catalysts. Even if it did occur to any appreciable extent at the liquid/air interface, however, the effect would be to make the interface solution more acid. Thus the onset of red color would be expected to be considerably delayed at air/liquid interfaces, not accelerated as is actually observed. It may, therefore, be concluded that oxygen is not a significant variable in the reaction. To verify this conclusion, a test was run which included a nitrogen purge in a closed test tube prior to performance of the reaction. The reaction was observed to proceed in all respects the same as it does when no purging is employed.

It was also mentioned after the talk that the red color is seen to form first at a negatively charged platinum electrode. The question was raised whether the red color was due to the clock reaction or to electrolysis of hydrogen ions. A check of our notes verified that no red color develops at the negative electrode if formaldehyde is left out of the reaction mixture, i. e., no or insufficient electrolysis occurs with the  $1\frac{1}{2}$  volt system used to change the color of the phenolphthalein indicator. The accelerating effect of the negative electrode on the Formaldehyde Clock Reaction, therefore, is real.



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