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ONR

Annual Progress Report

GRANT# N00014-90-J-1986 R&T CODE: 441S009

PRINCIPAL INVESTIGATORS: Prof. K. N. Raymond
Prof. C. J. Hawkins
Dr. L. R. Gahan

INSTITUTIONS: The University of California, Berkeley, and
The University of Queensland, Australia

GRANT TITLE: Piezoelectric Metal Biosensors

REPORTING PERIOD: 1 April 1990 - 30 June 1991 [Note that funds were not awarded until early August 1990. The reporting period therefore represents the 11 month period for which funds have been available.]

AWARD PERIOD: 1 April 1990 - 31 March 1993

OBJECTIVES: To investigate:

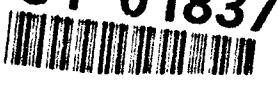
1. the effects of solutions on the vibration frequency of quartz piezoelectric transducers;
2. the covalent attachment of ligands with designs based on marine metal sequestering agents to the surface of quartz piezoelectric crystals;
3. the surface modification of quartz crystals using polymeric materials, particularly those that would enhance the selective binding of cations;
4. the effects of group IIB and d transition metal ions on the vibration frequency of modified quartz crystals; and
5. the application of surface-modified quartz piezoelectric transducers as analytical devices for the detection of transition metal ions in oceanic waters.

ACCOMPLISHMENTS - University of Queensland: (since August 1990):

1) We have investigated the response of 10 MHz AT-cut Ag-electroded piezoelectric crystals to aqueous NaCl solutions (concentration 0.5 to 1.0 mM) and found that the frequency change is linear in this region. We have also investigated the response of these crystals to 0.40 mM solutions of LiCl, NaCl, KCl, RbCl, and CsCl. The alteration of the alkali cation has an effect on the Δf such that the greatest change is for the RbCl and CsCl. Our results suggest that the response of the crystal may be a function of the size of the hydrated cation.

2) We have begun our investigation of the synthesis of cation selective receptor species suitable for attachment to the surface of a piezoelectric crystal. We have prepared three macrocyclic precursors which possess a secondary amine group suitable for further synthetic elaboration. The attachment of long alkyl chains ($C_{22}H_{45}$-) has been achieved for two of these macrocycles and studies to investigate the deposition of thin films (monolayers) of the receptors using Langmuir-Blodgett techniques are in progress. Silane derivatives of these macrocycles have been prepared by attaching C_{16} chains possessing a pendant $-HC{=}CH_2$ moiety. The alkane can be readily hydrosilated to give a terminal $-Si(OEt)_3$ moiety suitable for reaction with the crystal surface. The result will be a covalently attached macrocyclic species.

3) Our work on the isolation and identification of cyclic peptides and catecholate chelators from marine organisms continues. We are interested in the presence in the oceans of metal complexes of biologically active cyclic peptides from the didemnid ascidians. ICP-MS



has been used to study 13 aplousobranchs to see which metals, beside iron and vanadium, are accumulated, and to see if there is a relationship between the metals and the type of chelates isolated from the animals. A number of hydroxylated compounds have been isolated from ascidians, some of which are proteins. These compounds are strong chelators of metal ions; structural investigations are underway.
4) We are continuing to pursue the design of the crystal devices. Recently, we have commenced an investigation of "electrodeless piezoelectric devices" following a report by Nomura et. al. (*Anal. Chim. Acta.* 1991, *243*, 273-8). Our previous studies have highlighted the sensitivity of the silver electrodes to chemical disruption. The "electrodeless" piezoelectric crystals offer the possibility of using a device with no attached electrode. We have built a device in which a blank 10 MHz quartz plate is attached to a glass cylinder of the same diameter. The cylinder is filled with electrolyte solution (0.1 M KCl) and a platinum electrode immersed in the solution. Attached to the outside of the cylinder is another platinum electrode. Attachment of the oscillator circuit and immersion of the device in electrolyte solution results in the oscillation of the crystal (~9.9 MHz). The device is robust and simple. This aspect of the work is very new and our results are preliminary, but the implications of this accomplishment for future work are very positive.

ACCOMPLISHMENTS - University of California: (since August 1990):

We are currently investigating ways to attach catecholate-based metal-specific ligands to SiO_2. The ligands are based upon those already prepared in this group for complexing Fe^{3+} and VO_2^+. As part of a related project, we are designing octadentate actinide sequestering agents for attachment to silica. We have made some progress in functionalizing the silica, and some of this chemistry can be utilized for attachment of these ligands to quartz surfaces. We have recently begun the synthesis of catecholate ligands that can be attached to SiO_2 in a variety of ways. One method, for example, involves the attachment of an allyl group to the aromatic ring of a substituted catechol by way of a Claisen rearrangement of the allyloxy precursor. The allyl substituent can later be converted to a $-CH_2CH_2CH_2Si(OEt)_3$ group for attachment to silica or quartz.

In May 1991 Dr. Peter Bonnesen initiated the first of our direct collaborative studies by traveling from Berkeley to spend three weeks in Dr. Gahan's laboratory at the University of Queensland. The work focused on experiments with both the silver electroded and "electrodeless" crystals in solution. As a result of the collaboration, improvements on the design of the "electrodeless" crystal device have been made, and a crystal oscillator setup is being constructed at Berkeley, so that the catecholate derivatized crystals may be tested directly.

SIGNIFICANCE: As we outlined in our proposal, cation selective receptors bound to a sensitive piezoelectric device are potentially a new, rapid and highly selective way to analyze for trace metals in a complex medium such as the ocean. We are producing cation selective receptors for attachment to piezoelectric crystals in resonating circuits. The compounds are modeled on those observed in biological systems and within these biological systems we have observed a diversity of metal ion complexation. Our most recent results with the "electrodeless" crystals point to the production of a robust, almost chemically inert, device which operates in the presence of a background

electrolyte, perhaps akin to the conditions in seawater. Ligand synthesis for metal-selective complexing agents is proceeding well both at Berkeley and Queensland. The ability to selectively target metal ions using a matrix array remains a significant, if still distant, goal.

WORK PLAN: Over the next 12 months we will pursue all aspects of the project. Our Work Plan is almost unchanged from that proposed initially, since we have just finished the first 11 months of experimental work and setting up of the project. Dr. Gahan will plan to visit Berkeley during the end of next year as part of our planned exchange of personnel. We view such exchange as essential to the effective collaboration that is a central feature of this project.

PUBLICATIONS AND REPORTS: There are four papers in preparation concerning the complexation properties of the macrocycles 1,4,7,10-tetraoxa-13-azacyclo-pentadecane (A15C5), and 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (A18C6).

Annual Report Questionnaire

PRINCIPAL INVESTIGATORS: Prof. K. N. Raymond, Prof. C. J. Hawkins, Dr. L. R. Gahan

INSTITUTIONS: The University of California, Berkeley (Raymond)
The University of Queensland (Gahan and Hawkins)

GRANT TITLE: Piezoelectric Metal Biosensors

REPORTING PERIOD: 1 April 1990 - 30 June 1991 [Note that funds were not awarded until early August 1990. The reporting period therefore represents the period for which funds have been available.]

University of Queensland

Number of ONR supported:
- Papers published in refereed journals: none
- Technical reports or non-refereed papers: none
- Books or chapters published: none

Number of presentations:
- Invited: none
- Contributed: none

	Total	Female	Minority	Non-U.S. Citizen
Number of Graduate students	5	1		5
Number of postdoctoral fellows:	1			1
Number of research assistants:	1	1		1

Awards/Honors to PI and/or members of PI's research group: none

Equipment purchased:

Two Hewlett-Packard Frequency Counters HP 5316B	$4800
Personal Computer IBM Clone XT Turbo 12 MHz	$1500

[Note: these prices are in Australian dollars.]

University of California Berkeley

Number of ONR supported:
- Papers published in refereed journals: none
- Technical reports or non-refereed papers: none
- Books or chapters published: none

Number of presentations:
- Invited: none
- Contributed: none

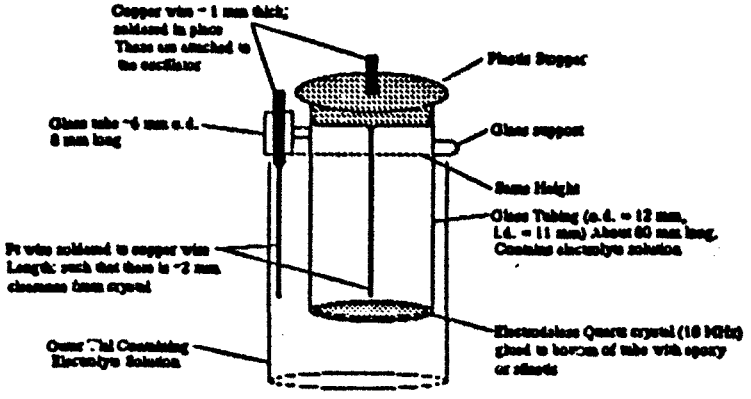
	Total	Female	Minority	Non-U.S. Citizen
Number of Graduate students	1			
Number of postdoctoral fellows:	2			1
Number of research assistants:				

Awards/Honors to PI and/or members of PI's research group: none

Equipment purchased: none

Highlight Page

Electrodeless Piezoelectric Crystal Device



Objectives

- To probe the application of piezoelectric quartz transducers as sensitive probes for metal ions in solution.
- To model the naturally occurring cyclic peptides as receptors for the metal ions on the surfaces of piezoelectric crystals.

Accomplishments

- Preparation of macrocyclic ligands with the potential for synthetic elaboration for attachment to the surface of piezoelectric crystals.
- Synthesis of the Pb^{2+}, Hg^{2+}, and Cd^{2+} complexes of these macrocyclic species.
- Isolation and identification of cyclic peptides and catecholate chelators from marine organisms and investigation of the metal binding properties of these peptides.
- Applications of the "electrodeless" piezoelectric quartz crystals.

Significance

- Production of cation selective receptor sites modelled on naturally occurring macrocyclic systems.
- Increased understanding of the diversity of metal ion complexation properties of naturally occurring ligands.
- The development of robust, chemically inert devices, based on electrodeless quartz crystals - capable of operating in the presence of background electrolyte.