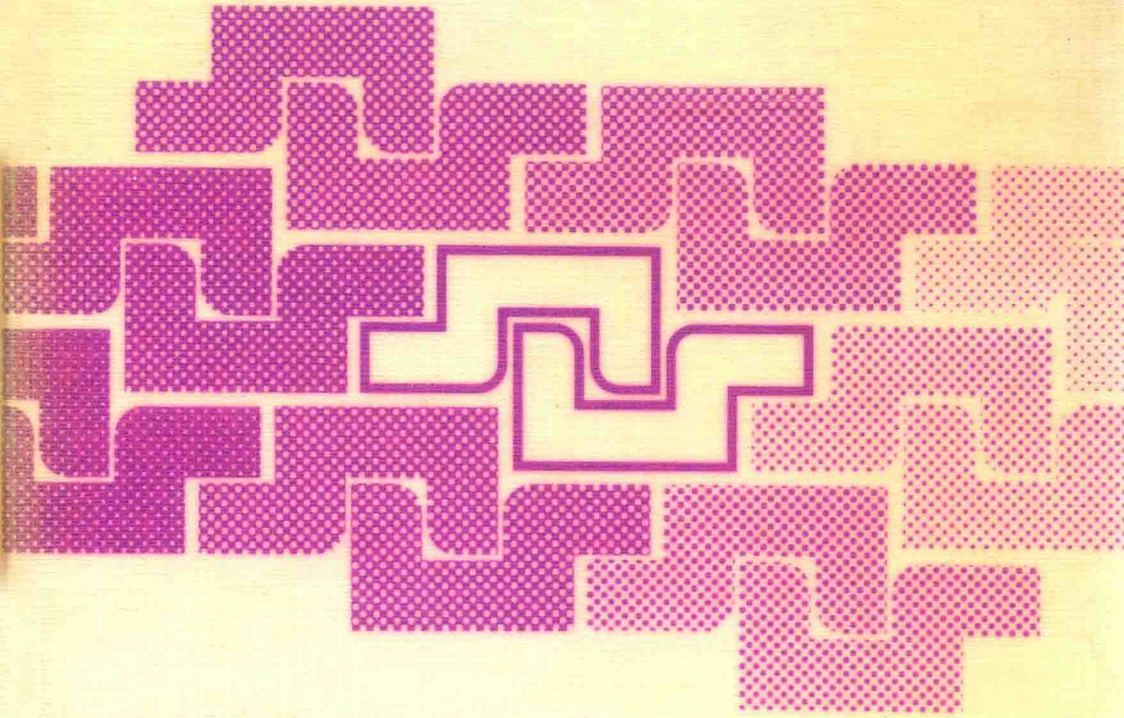

Analytical Uses of Immobilized Biological Compounds for Detection, Medical and Industrial Uses

edited by George G. Guilbault and Marco Mascini

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Analytical Uses of Immobilized Biological Compounds for Detection, Medical and Industrial Uses

edited by

George G. Guilbault

Department of Chemistry,
University of New Orleans, Louisiana, U.S.A.

and

Marco Mascini

Department of Chemistry,
University of Florence, Italy

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DEDICATION

To Susan and Mareza

PREFACE

On May 4-8, 1987, a NATO Advanced Research Workshop on the Analytical Uses of Immobilized Biological Compounds was held in Florence, Italy. The Director of the Workshop was Professor George G. Guilbault of the University of New Orleans, and the Co-Director was Professor Marco Mascini of the University of Florence

It was the purpose of this meeting to assemble scientists from all NATO Countries with an interest in immobilized biological compounds, to discuss

- methods of immobilization
- properties of immobilized compounds
- enzyme electrodes and biosensors
- optical devices utilizing immobilized enzymes
- microbial sensors and clinical uses of immobilized enzymes
- flow injection analysis using enzymes
- immobilized biological compounds in chemical defense detection
- pharmaceutical analysis
- uses in industrial analysis
- enzyme reactors
- air pollution detectors
- immunosensors
- medical uses and applications
- solid state and FET sensors

Goals to be achieved by the conference were

- to permit an exchange of views and experience in all these areas
- to review and critically assess the state-of-the-art in these fields
- to set guidelines for future research and establish collaborative projects between scientists in NATO laboratories in the above areas.

Thirty-seven lectures were given by 36 speakers in all of the above areas. Sessions were devoted to (1) methods and properties of immobilized enzymes (2) clinical and pharmaceutical analysis (3) enzyme, bio- and microbial sensors, (4) defense applications, (5) solid state/FET devices, (6) optrodes and spectroscopic applications, (7) immunosensors, (8) flow injection analysis, and (9) industrial and analytical applications. Finally, two hours were devoted to an open discussion of future status, new directions and joint projects.

This book is a publication of most of the lectures given at this workshop.

We wish to thank for their financial support of this conference: Cassa di Risparmio di Firenze, Eli Lilly (Indianapolis), Esacontrol SpA (Genova), Instrumentation Laboratory (Milano), Universal Sensors (New Orleans), Universita degli Studi di Firenza and especially NATO for the ARW grant that made the Conference possible. We also thank our organizing committee (Drs. Coulet, Patriarche, Campanella and Palleschi) for the organization and local support, and D. Moscone, R. Pillotan and S. Salleri for providing the secretarial services that made the Conference run smoothly. Finally, the typing support of Mrs. Gayle Barlow is gratefully acknowledged.

CONCLUSIONS

In the last afternoon, a round table discussion of selected speakers (see program), the Directors and audience centered on the status of the field of Analytical Uses of Immobilized Biological Compounds, cooperation between labs and the future.

Several participants expressed appreciation to the Conference for the possibility to meet and develop contacts with scientists of other NATO countries doing similar research. Collaborative projects were established between (1) the Universities of Rome, Florence and New Orleans on NAD/NADH dependent dehydrogenases, modified electrodes and immobilization techniques for substrates of clinical interest, (2) the University of Porto in Portugal and Barcelona in Spain in new electrodes for whole blood flow analysis, (3) U. S. Defense Labs in Edgewood, Md. and the University of Rouen in France on Receptor Electrodes, (4) University of Brussels, Belgium, and New Orleans on New Methods for Artificial Sweeterners and Carbohydrates, (5) University of Cincinnati and University of Lund on Modified Electrodes, and (6) Technical University of Denmark and University of New Orleans on Flow Injection Analysis of Artificial Sweeterners.

Discussion on new areas was devoted to (1) Receptor Electrodes and Methods for use in Defense and Analysis (2) Modified Electrodes as better analytical methods (3) Miniaturized solid state (FET type electrodes) for the sensors of the future (4) Immobilized Enzymes in Medical Shunts (5) Enzyme Immunoassay and Immuno Probes and (6) Optrodes - now finally getting established.

Because of the highly productive nature of the Conference, it was decided to have a second NATO Conference in 1990.

LIST OF CONTRIBUTORS

Salvador Alegret - Department of Chemistry, Universitat Autonoma de Barcelona, E08193 Bellaterra, Spain

M. A. Angellotti - Institute of Analytical Chemistry, University of Bologna, Italy

Piet Bergveld - Department of Electrical Engineering, Twente University, P. O. Box 217, 7500 AE Enschede, The Netherlands

L. J. Blum - Laboratoire de Genie Enzymatique - Atelier de Biotechnologie CNRS - Universite Claude Bernard Lyon 1-43, Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

L. Campanella - Department of Chemistry, University of Rome I, "La Sapienza", Piazzale A. Moro, 5, Rome, Italy

G. Carrea - Institute of Chemistry of Hormones, CNR Milan, Italy

Pierre R. Coulet - Laboratoire de Genie Enzymatique - Atelier de Biotechnologie, CNRS- Universite Claude Bernard, Lyon 1-43, Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

M. Alves da Silva - Department of Chemistry, University of Coimbra, 3000 Coimbra, Portugal

Christopher C. Feistel - Liston Scientific Corp., Newport Beach, CA

Larry Gayleard - Liston Scientific Corp., Newport Beach, CA

M. H. Gil - Department of Chemistry, University of Coimbra, 3000 Coimbra, Portugal

S. Girotti - Institute of Chemical Sciences, University of Bologna, Italy

Masao Gotoh - NOK Corporation, Fujisawa 251, Japan

G. G. Guilbault - Department of Chemistry, University of New Orleans, New Orleans, LA

Elizabeth Hall - The Biotechnology Centre, University of Cambridge, Downing Street, Cambridge, CB2 3EF, United Kingdom

H. B. Halsall - Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172

Elo H. Hansen - Chemistry Department A, Technical University of Denmark, Building 207, DK-2800 Lyngby, Denmark

W. R. Heineman - Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172

A. Atilla Hincal - Hacettepe University, Faculty of Pharmacy, Pharmaceutical Technology Department, 06 100 Ankara, Turkey

Isao Karube - Research Laboratory of Resources Utilization, Tokyo University of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, 227 Japan

H. Suheyla Kas - Hacettepe University, Faculty of Pharmacy, Pharmaceutical Technology Department, 06 100 Ankara, Turkey

J.-M. Kauffmann - Free University of Brussels, Institute of Pharmacy, Campus Plaine 205/6, Bd du Triomphe, Brussels, Belgium

Izumi Kubo - Research Laboratory of Resources Utilization, Tokyo University of Technology, Nagatsuta-cho, Midori-ku, Yokohama, 227 Japan

Glenn J. Lubrano - Universal Sensors, 5258 Veterans Blvd., Suite D, Metairie, LA 70006

C. D. Luther - Liston Scientific Corp., Newport Beach, CA

Adelio A. S. C. Machado - Chemistry Department, Faculty of Science, P4000 Porto, Portugal

Angelo Manzoni - Research and Development Department, Instrumentation Laboratory S.P.A., Viale Monza 338, 20128 Milan, Italy

Marco Mascini - Istituto Chemica Analitica, Universita di Firenza, Via Gino Capponi 9, 50121 Firenze, Italy

J. Ngeh-Ngwainbi - Universal Sensors, 5258 Veterans Blvd., Suite D, Metairie, LA 70006

Valerie M. Owen - Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Beds MK43 OAL, United Kingdom

G. Palleschi - Department of Chemical Science and Technology, 2nd University of Rome, Via O. Raimondo 8, 00173 Rome, Italy

Gaston J. Patriarche - Free University of Brussels (U.L.B.), Institute of Pharmacy, Campus Plaine 205/6, Bd du Triomphe Brussels, Belgium

Ayse Kevser Piskin - Hacettepe University, Faculty of Medicine, Medical Biology Department, Ankara, Turkey

Erhan Piskin - Hacettepe University, Chemical Engineering Department, Ankara, Turkey

R. Rizzoli - Institute of Chemical Sciences, University of Bologna, Italy

A. Roda - Institute of Analytical Chemistry, University of Bologna, Italy

P. Simoni - Institute of Analytical Chemistry, University of Bologna, Italy

Koji Sode - Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, 227 Japan

Charles F. Stemburg - Liston Scientific Corp., Newport Beach, CA

J. D. R. Thomas - Applied Chemistry Department, Redwood Building, UWIST, P. O. Box 13, Cardiff CF1 3XF, Wales, United Kingdom

M. Tomassetti - Department of Chemistry, University of Rome, "La Sapienza", Piazzale A. Moro, 5, Rome, Italy

Anthony P. F. Turner - Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire, MK43 OAL, United Kingdom

P. Vagdama - Department of Clinical Biochemistry, The Medical School, Newcastle University, Newcastle Upon Tyne, NE2 4HH, United Kingdom

Bart H. Van der Schoot - Department of Electrical Engineering, Twente University, P. O. Box 217, 7500 AE Enschede, The Netherlands

B. J. Vinicke' - Free University of Brussels (U.L.B.), Institute of Pharmacy, Campus Plaine 205/6, Bd du Triomphe, Brussels, Belgium

J.-C. Vire - Free University of Brussels (U.L.B.), Institute of Pharmacy, Campus Plaine 205/6, Bd du Triomphe, Brussels, Belgium

Howard H. Weetall - Cornig Glass Works, Ciba-Corning Diagnostics Corp., 63 North Street, Medfield, MA 02052

Otto S. Wolfbeis - Analytical Division, Institute of Organic Chemistry, KF-University, 8010 Graz, Austria

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ENZYMES IMMOBILIZED ON INORGANIC SUPPORTS

Howard H. Weetall
Corning Glass Works
Ciba-Corning Diagnostics Corp.
63 North Street
Medfield, MA 02052

ABSTRACT. Enzymes immobilized on inorganic supports by covalent attachment show unique characteristics with regard to pH optimum, thermal profile and kinetics. Covalent attachment of enzymes requires that the support first be treated with a silane coupling agent whereby organic functional groups can be covalently attached to the support. Following this the enzyme may be covalently coupled to the now available organic functional groups on the support.

1. INTRODUCTION

Immobilized enzymes have, over the past twenty years, moved from the laboratory to the industrial plant as a method for the commerical production of food stufs, pharmaceuticals, and fine chemicals. The advantages of enzymes, and in particular immobilized enzymes, for processing, are many. The major advantages are:

1) Specificity of reactions is high.
2) Gentleness of conditions for the chemical reactions are generally better than for most chemical reactions.
3) Temperature ranges are generally between 4^{o}-60^{o}C.
4) Immobilized enzymes are reuseable.
5) Immobilized enzymes allow closer control of the reaction.
6) Immobilized enzyme reactions can be less expensive than some chemical reactions when overall processing costs are considered.

Although this paper will deal mostly with inorganic supports, the characteristics of these supports are, in fact, similar to enzymes attached to organic matrices.

The choice of an inorganic system as a support material was based upon several perceived advantages.

1) Inorganic materials can be prepared in most pore diameters and particle sizes ranging from $30A^{o}$ - $2000A^{o}$ pore diameters and 400 U.S. mesh size to anything as large as marbles. This allows one to optimize a process in regards to diffusion limitation, pressure drop

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and other similar parameters.

2) Inorganic materials are impervious to biological attack by enzymes produced by contaminating and even non-contaminating bacterial systems, as compared to some of the more common carriers such as celluloses, polydextrans, and polyamines.

3) Inorganic materials do not change morphology under different solvent and pH conditions as do many organic polymers.

4) Inorganics can be doped with activator ions which can then be eliminated from the feed. Together these advantages increase the lase and the speed by which scale up is possible, as well as decrease many of the usual scale up and operational problems.

When choosing a support for a specific application it is always wise to know something of the new properties of the support material and the conditions that material will encounter. In the case of inorganic supports we have carried out exhaustive studies on the physical and chemical characteristics of the carriers with regard to the presence of acids, bases, chelates, organic solvents, increasing and decreasing pressure drops; and even sterilizing agents.

With this information available we can choose the carrier vest suited for operation in a higher or lower ionic strength environment of some specific pH, temperature and range of flow-rates.

2. PHYSICAL AND CHEMICAL CHAACTERISTICS

The carriers described here are all porous ceramics. Many of the carriers are similar to those developed by R.A. Messing (1). These materials can be prepared in a variety of pore morphologies and particle sizes. Table 1 gives the physical characteristics of some of these ceramic support materials.

Table 1

Physical characteristics of several ceramic support materials

Composition	Size (US mesh)	Pore diameter range (A°)	Pore diameter average (A°)	Pore volume (ml/g)
TiO_2 98%, MgO 2%	30/45	205-500	410	0.53
SiO_2 75%, Al_2O_3 25%	30/60	205-575	435	0.89
SiO_2 89.3%, ZrO_2 10.7%	30/60	110-575	235	1.30
SiO_2 100%	30/60	185-700	435	0.76
SiO_2 100%*	30/60	310-655	550	2.2
SiO_2 90%, ZrO_2 10%	30/60	185-700	435	0.76
SiO_2 75%, TiO_2 25%	30/45	875-205	465	0.76
Controlled Pore Glass	30/60	450-600	550	0.50

*Modified binder used.

The method used for determining these data is mercury intrusion. The pressure required to cause the mercury to fill the pores of a particle is proportioned to the pore diameter. The volume required determines the pore volume (Figure 1).

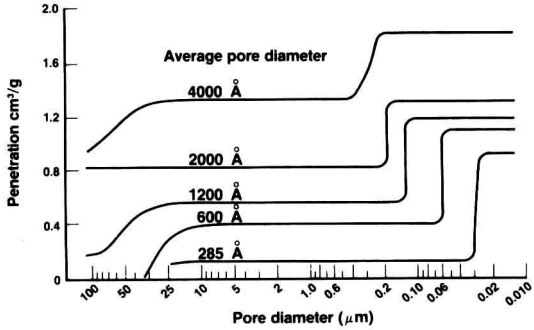

Figure 1. Mercury intrusion data for several controlled pore glasses indicating average pore diameter, pore ranges and pore volumes (penetration, volume per gram of CPG) for each material. (1.0 m = 10000A.)

Chemical durability is determined by carrying out a series of static and dynamic tests utilizing strong acids, alkalies and buffers (2). This type of study aids one in choosing a carrier for a particualr application.

2.1. Covalent Attachment

Inorganic supports can be covalently coupled ot organic materials by a variety of methods. Two of the presently used approaches involve activation with $TiCl_4$ or adsorption of some amino carrying polymer such as poly-L-lysine followed by reaction with the protein of interest.

We have developed and optimized the process which involves the use of intermediate silane coupling agents. The advantages offered by the silane is mainly stability. Since the covalent linkage is directly to the inorganic support, one find that enzyme leakage is non-existent over a pH range of about pH5-8. In addition the cost of silane is far less than poly-L-lysine, the most common aminoopolymer used and far less obnoxious than $TiCl_4$

The most common silane coupling agent used is gamma-amino propyltriethoxysilane. Silane such as this one will ract with any available oxide or hydroxide groups on the surface of glass, ceramics or metal oxides.

The general scheme is given in Figure 2.