OFFICE OF NAVAL RESEARCH

Contract N00014-82K-0612

Task No. NR 627-838

TECHNICAL REPORT NO. 48

Electroreleasing Composite Membranes for Delivery of Insulin And Other Biomacromolecules

by

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Prepared for publication

in

The Journal of the Electrochemical Society

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April 5, 1990

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: APPROVED FOR PUBLIC DISTRIBUTION, DISTRIBUTION UNLIMITED.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): ONR TECHNICAL REPORT #48	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Dr. Charles R. Martin, Department of Chemistry; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Texas A&M University, College Station, TX 77843-3255	7b. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, VA 22217
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract # N00014-82K-0612
8c. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, VA 22217	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification): Electroreleasing Composite Membranes for Delivery of Insulin and Other Biomacromolecules

12. PERSONAL AUTHOR(S): Michael J. Tierney and Charles R. Martin*

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM _____ TO _____	(90,04,05) April 5, 1990	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Electroreleasing Composite Membrane; Microporous Al_2O_3 Molecule; Biomolecules; Rate of Diffusion

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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Previous electrorelease systems have entailed entrapment of the target molecule within a polymer membrane. It would be difficult to use such systems to electrorelease large biomolecules or proteins, because of the slow rate of diffusion of such large molecules in the polymer phase. The electrorelease system described here is ideally suited for electrorelease of large biomolecules and proteins. Electrorelease of insulin and vitamin B-12 is demonstrated.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL: Dr. Robert Nowak	22b. TELEPHONE (Include Area Code): (202) 696-4410 — 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

Electroreleasing Composite Membranes for Delivery of Insulin and Other Biomacromolecules

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ABSTRACT

A new electroreleasing composite membrane is described. This membrane is prepared by coating a microporous Al_2O_3 support membrane with a thin polymer film. This composite membrane separates a solution of the molecule to be released (i.e. the target molecule) from a receiver solution. Electrorelease is accomplished by electrochemically rupturing the polymer film; this allows the solution of the target molecule to flow through the pores of the host membrane into the receiver solution.

Previous electrorelease systems have entailed entrapment of the target molecule within a polymer membrane. It would be difficult to use such systems to electrorelease large biomolecules or proteins, because of the slow rate of diffusion of such large molecules in the polymer phase. The electrorelease system described here is ideally suited for electrorelease of large biomolecules and proteins. Electrorelease of insulin and vitamin B-12 is demonstrated.

Electrorelease is the use of electrochemistry to control the delivery of a chemical or drug (1, 2). The major advantage of electroreleasing systems (over conventional diffusional drug-release matrices (3)), is the ability to control the delivery process. For example, electrorelease systems can be switched on and off and the rate of release can often be adjusted (1, 2).

In the systems described in the literature to date, the species to be electroreleased (i.e. the target molecule) is either covalently or ionically bound within a polymer membrane (1, 2). Electrorelease of large molecules (greater than ca. MW=300) is difficult because of the slow rate of diffusion of such large molecules in the polymer films used.

We have recently developed an electroreleasing composite membrane which is specifically designed for the electrorelease of macromolecules. This composite is prepared by coating a microporous support membrane with a thin polymer film. Electrorelease is accomplished by electrochemically breaching the polymer film; this allows the solution of the target molecule to flow through the pores of the membrane into a receiver solution. We demonstrate in this correspondence that this new electrorelease membrane can be used to deliver insulin and vitamin B-12.

The composite membrane fabrication procedure is shown schematically in Figure 1. An Anopore (Alltech) Al_2O_3 filter is used as the support membrane. Anopore contains a high density of cylindrical, 0.2 mm-dia. pores. One face of the Anopore membrane was sputtered with a 50 nm layer of Au. Ag epoxy was then used to attach an electrical lead to the Au surface.

A Nafion (4) ion exchange polymer film was then cast onto the Au surface using a high temperature (175°) solution-processing procedure (5). This high temperature casting method prevents the Nafion solution from filling the pores in the support membrane, and yields a continuous 1 to 2 mm-thick Nafion film across the Au-coated Anopore surface.

Insulin solutions were prepared by dissolving 500 mg of bovine insulin (Sigma, MW=5700) in 50 ml of a pH 9.3 ammonium buffer electrolyte made by adding 2.4 g of NH_4Cl and 4 ml of conc. NH_4OH to 500 ml of distilled water; this solution was also 0.5 M in NaCl. To allow for visible spectrophotometric detection, biuret reagent was added to the insulin solution, yielding the characteristic violet Cu(II)-insulin complex (6). Vitamin B-12 solutions were prepared by dissolving 20 mg of cyanocobalamin (Sigma, MW=1355) in 50 ml of 1 M KNO_3.

The spectroelectrochemical cell used to conduct the preliminary electrorelease experiments described here consists of two halves of a U-tube separated by the Anopore/Au/Nafion composite membrane. The left half is an electrochemical cell containing supporting electrolyte (vide supra), which serves as the receiver solution, and a Pt flag counter electrode. The Au film in the composite membrane serves as the working electrode. The right half of the cell contains a reservoir of the biomolecule to be electroreleased. The Nafion film on the composite membrane faces this solution. The Nafion film prevents release of the large biomolecule into the receiver solution. Electrorelease is initiated by passing a cathodic current (105 mA cm^{-2}) through the Au-film working electrode. This cathodic current

causes H_2 to be evolved at the Au/Nafion interface, rupturing the Nafion film. With the Nafion film breached, the reservoir solution can flow through the membrane into the receiver solution. A thinner Nafion film, such as would be used in an *in vivo* device, would be much more easily perforated, and much smaller currents could be passed.

The U-tube cell was placed in a Perkin-Elmer Lambda 4B UV-Visible spectrophotometer. The electroreleased biomolecules were detected by monitoring the absorbance (insulin, 530 nm; vitamin B-12, 360 nm) of the receiver solution. The level of the receiver solution was placed 2 to 3 cm below the level of the reservoir solution to enhance the flow rate of the biomolecule through the breached film. In the insulin experiments, a hypodermic syringe was used to maintain a positive pressure on the reservoir solution; this further enhanced the flow rate through the ruptured film.

Curve a in Figure 2 shows a plot of absorbance, in the receiver solution, vs. time for a vitamin B-12 electrorelease experiment. No current was passed during the first 30 minutes; thus, the film was not breached, and vitamin B-12 was not released. The film was breached at 30 minutes, as indicated by the abrupt rise in absorbance in the receiver solution. Results of an analogous insulin electrorelease experiment are shown in curve b. The data in Figure 2 clearly demonstrate that these new composite membranes can electrochemically deliver large biomolecules and proteins.

More sophisticated electrorelease cells can easily be designed. For example, we have shown (7) that the Au film/electrode can be divided into individually-addressable segments. Breaching the film

over one of the electrode segments establishes a base delivery rate; breaching the films over additional electrode segments causes the delivery rate to increase. Thus, the rate of drug delivery from these composite membranes can be varied electrochemically. Such electrorelease systems may have applications as implantable insulin delivery devices (8).

ACKNOWLEDGEMENTS

The authors acknowledge valuable discussions with Drs. Deborah S. Greco and M. Reagan Adams. This work was supported by Air Force Office of Scientific Research and the Office of Naval Research.

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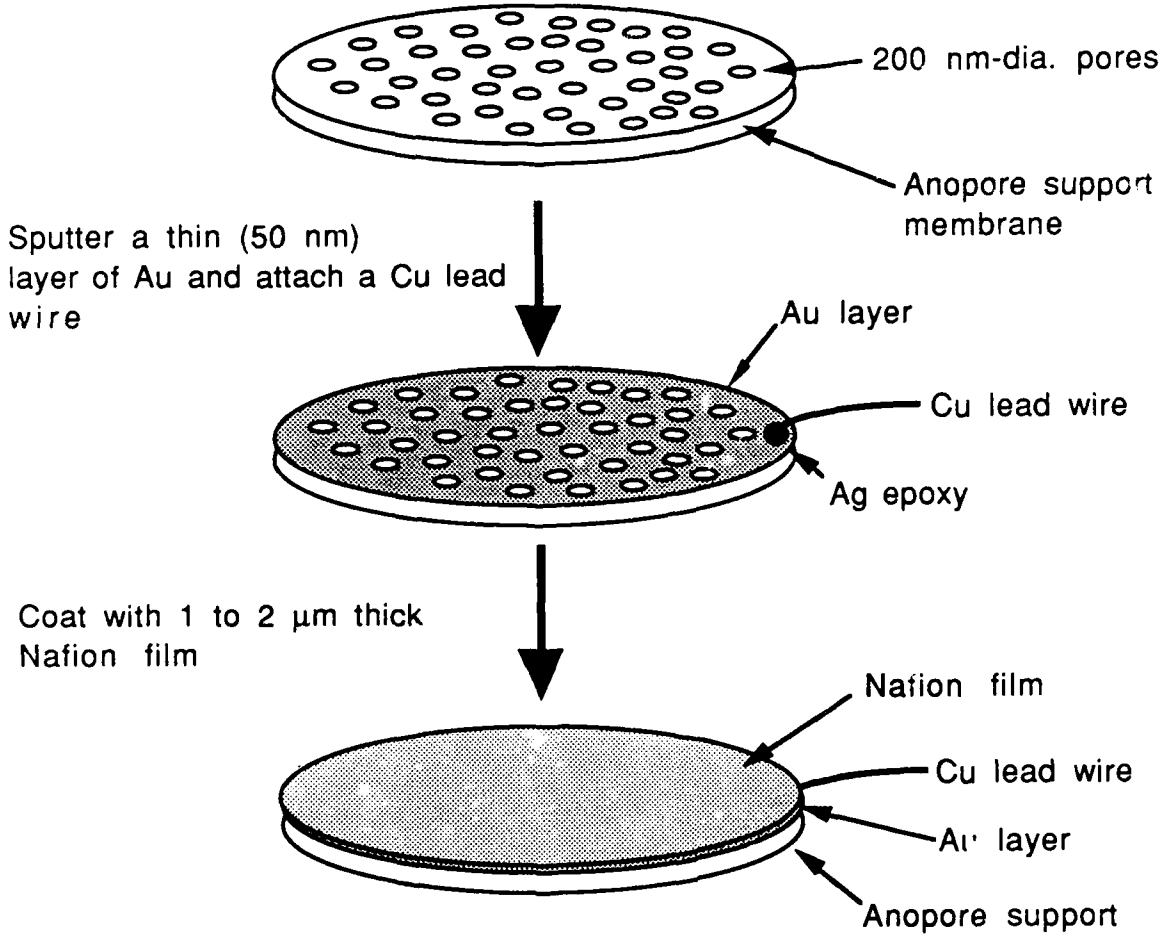
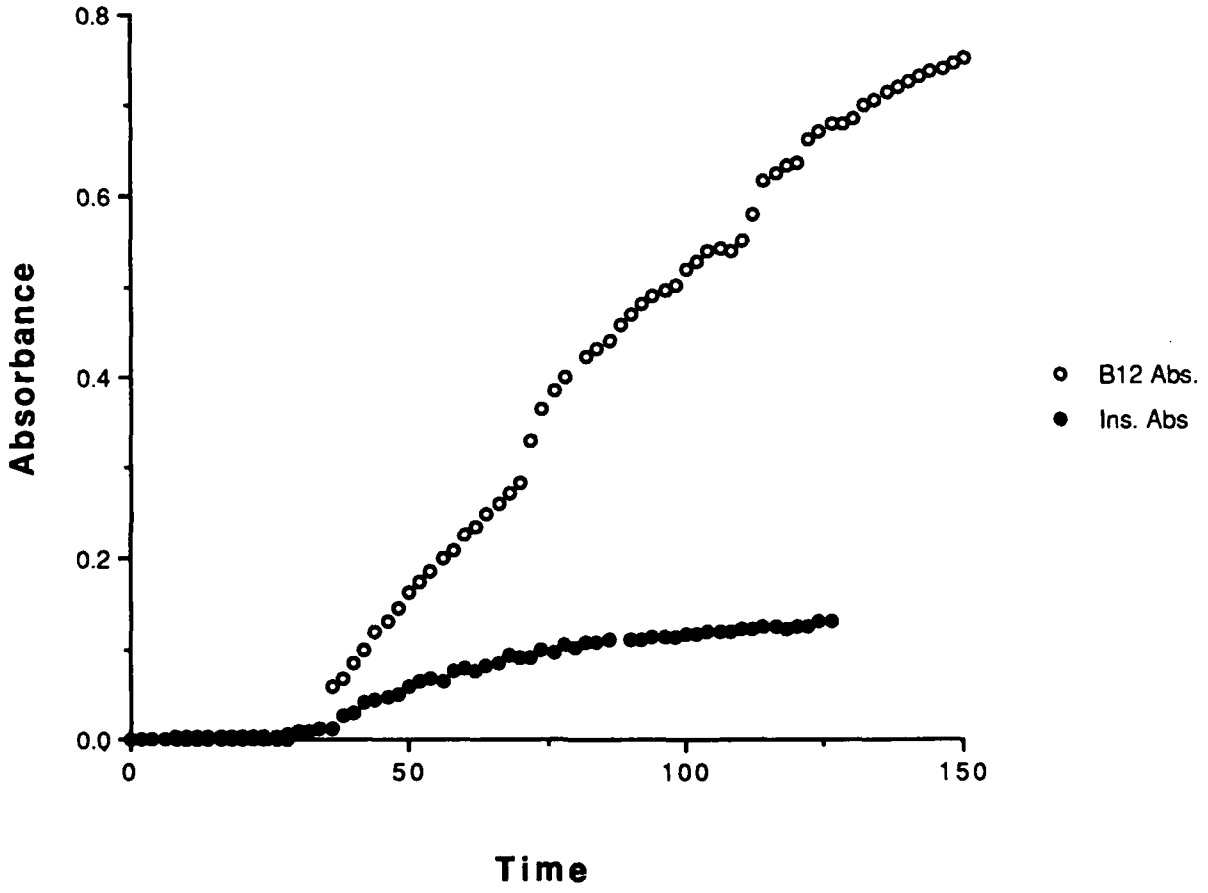
FIGURE CAPTIONS

Figure 1. Schematic diagram of composite membrane fabrication procedure.

Figure 2. Absorbance in the receiver solution vs. time during electrorelease experiments. a. Electrorelease of vitamin B-12 monitored at 360 nm; b. Electrorelease of insulin monitored at 530 nm. In both cases, electrorelease was started at 30 min.


200 nm-dia. pores
Anopore support membrane
Sputter a thin (50 nm) layer of Au and attach a Cu lead wire
Au layer
Cu lead wire
Ag epoxy
Coat with 1 to 2 μm thick Nafion film
Nafion film
Cu lead wire
Au layer
Anopore support


0.8
0.6
0.4
0.2
0.0
Absorbance
0
50
100
150
Time
B12 Abs.
Ins. Abs