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ADVANCED COMPOSITE APPLICATIONS FOR SUB-MICRON BIOLOGICALLY DERIVED MICROSTRUCTURES

J. M. Schnur, R.R. Price, P.E. Schoen
Center for Bio/Molecular Science and Engineering
Code 6090, Naval Research Laboratory
Washington, D.C. 20375-5000

Joseph Bonanventura
Director, Marine Biomedical Center
Duke University Marine Laboratory
Pivers Island
Beaufort, North Carolina

Douglas Kirkpatrick
Scientist
Scientific Applications International Co.
McLean, Virginia

ABSTRACT

Advanced materials of the 21st century require development of new materials with significantly improved properties. These new materials may lead to solutions of existing problems, and additionally open the door to entirely new technologies. A major thrust of advanced material development has been the area of self assembled ultra-fine particulate based composites (micro composites). We report on the application of biologically derived, self-assembled microstructures to form advanced composite materials. Hollow 0.5 micron diameter cylindrical shaped microcylinders self assemble from diacetylenic lipids. These microstructures have a multiplicity of potential applications in the material sciences. Exploratory development is proceeding in application areas such as controlled release for drug delivery, wound repair, and biofouling as well as composites for electronic and magnetic applications, and high power microwave cathodes.

INTRODUCTION

Recent advances in self assembly and nanotechnology suggest a number of difficult material problems faced by the engineers of the 20th century are likely to be solved early in the 21st. Opportunities abound for new products based upon "smart" materials or materials with significantly improved properties.

NRL's Center for Bio/Molecular Science and Engineering (CBSE) has focused its initial efforts in self assembly and bio/molecular materials on fabrication of submicron structures. Much of our effort has been aimed at modification of lipids to enable the formation of technologically interesting microstructures. Our ability to fabricate these structures represents an exciting synthesis of biotechnology and molecular engineering.

This paper will focus on one particular type of self assembled micro structure that was discovered at NRL. By inserting diacetylene groups into phosphocholine lipids, we have been able to form sub micron diameter hollow cylinders called tubules. These cylinders are very much like micron sized soda straws with outer diameters from 0.05 microns to 0.7 microns or so and lengths from 10 microns to over 1 millimeter[1,2,3,4,5,6] (See Figure 1).

BACKGROUND

Lipids are the basic building block of biological membranes. The bilayers of lipid self assemble to form either two dimensional sheets, or liposomes which are spherical structures composed of a lipid bilayer enclosing an aqueous space. A number of important applications, most involving targeted release, have been identified for liposomes. Our laboratory has been one of the pioneering centers in the development of an artificial blood surrogate utilizing liposomes as the encapsulant for hemoglobin. While the spherical geometry has a number of important benefits, other geometries may also be of interest. For instance, a hollow cylinder can provide a narrow channel for diffusion leading to zero order diffusion rates (i.e. a constant rate of release that is independent of time) for controlled release applications. A suitably conducting hollow cylinder provides opportunities for the development of advanced high dielectric materials[7,8,9,10]. Other geometries may such as template formed tubule channels or euctectic devices also have their own particular technological niche.

CONTROLLED RELEASE

Since man first set sail on the oceans of the world, sailors have been plagued by biofouling and deterioration of their vessels caused by both plant and animal species. One early solution was copper sheathing chosen to protect wooden hulls against the ravages of the teredo worm. Today the modern equivalent of copper sheathing is a polymeric ablative or self polishing paint, using copper powder or cuprous oxide as the primary toxicant. Although copper as an antifoulant has stood the test of time, it is often more effective against animal species than plant growth. In order to improve on the performance of copper the addition of many other metal species have been employed, such as mercury, arsenic, cadmium, lead and tin. Currently, in more enlightened times, these heavy metals have been abandoned as persistent toxicants that have adverse effect on the environment and more directly on man's health as a consumer of fish and shellfish.

In addition to environmental concerns, as fuels become more expensive and fossil fuel reserves begin to shrink the economic costs associated with biofouling begin to rise. Marine biofouling growth on underwater ship hulls increases hydrodynamic drag and hull weight due to the increasing biomass. If reasonable service speeds for the ship are to be maintained, power output must be increased, resulting in higher consumption of fuel and increased wear on the machinery. Increases in fuel consumption exceeding 10% are common, as are decreases in operational hull speeds of up to 16%.

In an effort to ease the economic pressures that continue to mount on governments, industry and individuals, service cycles are being increased. The U.S. Navy has lengthened operational cycles to 5 years, with 7-10 year cycles under consideration to further reduce maintenance costs. When antifouling paints fail early on in the service cycle, it may not be possible to haul and paint the ship ahead of schedule due to lack of funds or facilities availability. This situation would result in higher fuel costs and decreased performance associated with a fouled hull for a greater portion of the maintenance cycle, but would have to be tolerated unless extended antifouling performance can be attained.

In order to achieve the goal of less polluting yet effective antifouling paints, strict control of the release rates in copper based paints in addition to the entrapment and controlled release of alternate compounds must be accomplished. These alternate compounds are often active at levels far lower than those needed for copper. Thus proper control of release rates is necessary to prevent these alternate compounds from being discharged from the coating in excess of actual requirements. Conservative, i.e. low release rates led directly to long service lifetimes for the paints.

Not only must an antifouling paint offer performance in service; it must meet increasing demands from governmental regulators concerning water pollution standards (< 18 ug/l maximum), air quality standards for volatile organic solvents (VOC) and the occupational and health regulations governing the application and disposal of antifouling coatings.

The small pore size and long cylindrical shape of the tubule offer significant opportunities for control of long term control of release. By controlling the viscosity of the polymer, the length and diameter of the tubule, and the permeability of the paint matrix, variations of the release rate of several orders of magnitude have been demonstrated. The tubules isolate the encapsulant from the environment thus providing a mechanism for enhanced chemical stability of the encapsulants. The tubules are quite compatible with existing paints and may even offer some improvements to the ultimate mechanical properties of the composite [11,12,13,14].

Methods for Environmental Exposure Testing:

The metallic microcylinders utilized in the study averaged 0.5 microns in diameter and ranged from 10-250 microns in length with interior diameters ranging from 0.25-0.4 microns. Once dry the microcylinders act as microcapillary tubes entrapping and retaining a range of liquid materials. Release rates are linear and dependent on the encapsulant and the molecular weight and cross linking of the carrier vehicle.

Twenty, 11 cm fiberglass rods, which had been cut from 0.35 mm diameter stock, were coated by dipping with the desired formulation of antifouling coating. Following air drying for at least 48 hr., the rods were mounted in a floating array consisting of a rectangular float of PVC pipe to which a diamond polypropylene fish impound netting was attached (Figure.2). The rods were attached to the net with rubber grommets and the entire array was attached to a raft in the field at Coconut Island, Hawaii, or Taylors Creek, Beaufort, North Carolina. At the first sign of fouling rods were withdrawn and examined to determine the composition and relative percentage cover of fouling organisms.

Tetracycline was used in the initial studies of release rates from coatings and microstructures as it is easily quantified by spectrophotometric analysis in water. In addition tetracycline is a registered antifouling agent and therefore was considered safe in this application. Initial findings indicated tetracycline could be released at linear rates from both epoxy resin films, and from vinyl based paints. It is interesting to note in Figure 3 that when not encapsulated the tetracycline is found to release in a matter of a few hours from VYHH coatings; however, when in an encapsulated sample, tetracycline continues to release after 500 days of use.

In order to explore the possibility of lowering the need for antibiotics or persistent copper toxicants, two further approaches were tested. First was the use of isothiazolone which is an experimental antifouling agent which has been shown to be non-persistent in the marine environment (Harrington, 1989). When encapsulated in both copper and iron microcylinders and added to a vinyl matrix this additive was shown to be effective at repelling fouling marine species in testing (Figure 4).

One other approach to the development of non-toxic or biodegradable coatings is the use of extracts from the Sea Pansy (Renilla reniformis), and structural analogs of these compounds. Figure 5 illustrates a pair of test rods which were taken from a sample exposed at Coconut Island, Hawaii for six months. It can be clearly seen that the experimental rod has successfully repelled fouling during the test period with concentrations of 2% by weight active agent.

Figure 6 is a comparison of the fouling characteristics of four coatings which consisted of a control baseline self polishing coating consisting of 1) methacrylate paint base on the test rods, 2) copper microtubules in the paint base, 3) encapsulated isothiazolone, and 4) encapsulated isothiazolone and a crude extract of renilla. The paint base is observed to become heavily covered by fouling (both micro and macro fouling are considered on an equal basis) by day 20, after which the fouling is reduced somewhat. This may be a result of predation of the fouling community during testing, as the coverage is again observed to increase following 90 days exposure to greater than 90% of the test surface. In the case of the paint base containing 5% by weight copper tubules the fouling is observed to increase to 50% coverage at day 40 and then is reduced to levels less than 40% until day 120. Again this may be due to the effect of predation or the settlement rates of larva as the test progressed. Test paint with encapsulated isothiazolone was observed to offer more consistent performance as compared to paint containing only copper tubules. Maximum coverage for all fouling species was less than 40% at day 40 and was observed to decrease during the period of testing. In contrast the addition of renilla extract to this mixture

provided the best initial performance of the formulations tested; however, by day 120 the average settlement had exceeded that of the isothiazolone only. As the loading of microtubules remained constant by weight, the isothiazolone in this last sample was reduced by half, and thus it may be that the renilla which was encapsulated had either become depleted or become ineffective against the fouling species settling at the end of the testing period.

Controlled Release: Summary

A number of major issues must be resolved before the ultimate utility of this approach can be determined. The variable controlling release rate must be quantified and optimized for each particular application. Important variables are the molecular weight of the antifouling agent, diameter and length of the tubules, permeability of the incorporating polymer as well as the matrix paint. While we have been able to make gallon quantities of test paints in our laboratory, the question of scale up has still to be seriously addressed. Cost is always an important issue. We had thought that lipid costs (up to $4000 per pound) would be a serious problem for technology transfer. However, we have had a recent success at recovering the lipid for metalized tubules and recycling that lipid to make more tubules which were subsequently metalized.[1] In addition, as the commercial production of this class of phospholipid compounds is undertaken the economies of scale should (as with other high technology chemical systems such as teflon) be reduced to a reasonable level. Further reductions of cost will be realized with large batch processing which would reduce labor costs, thus production costs of microstructures should not be barrier to use. Costs of the metal, biofouling control agent, and matrix, coupled with the processing costs will determine the commercial viability of this approach.

The system holds promise as a means of providing a controlled release mechanism for antifouling paints with significant advantages over existing formulations. The inclusion of microencapsulated toxicants is effective in short term assays designed for the study of larval settlement, and appears to be a means of providing controlled release independent of the paint vehicle.

In addition, the use of microcylinders instead of rosin allows for a more robust coating which is better able to resist erosion in high flow areas, and which will be better able to survive long periods of immersion in seawater.

While antifouling has been our first attempt at utilizing tubules for controlled release, several other possible applications are currently being evaluated. These include wound healing, enzyme storage and delivery, and long term antimicrobial and antifungal coatings. As a controlled delivery system microcylinders offer advantages in antimicrobial control systems for metal working fluids and coolants, prevention of deterioration in paper processing plants, and stabilization of kerosene and diesel fuels in storage.

ELECTRONIC AND MAGNETIC MATERIALS

When we first began to assess the potential applications for these micron sized "soda straws", the physicists in our research team were struck with the possibilities for enhanced electronic material applications. This is due to their large and adjustable aspect ratio, their cylindrical geometry, and very thin walls of the tubule.

Experiments and detailed calculations on the interaction of electromagnetic radiation with conducting hollow cylinders in composites have shown significant improvements[7,8] in temperature and frequency stability of the dielectric constant, both real and imaginary, as well possibilities for exploiting their highly anisotropic properties.[3,4]

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In order for these calculations to be confirmed and the potential applications assessed a process for rendering the tubules conductive had to be developed. This process[5] has now been refined so a coating can be placed on the inside and outside of the tubule. The thickness of this coating can be varied within a 150Å tolerance limit within the thickness range of 200Å to 1200Å. Copper was the first metal to be electrolessly deposited onto the lipid tubules, but now they are coated with nickel, nickel-iron-boron (a permalloy), gold, or palladium. The direct current conductivity of our current coatings appears to be about 2-3 orders of magnitude below the values expected for the bulk metal. Improving the metallization process is currently a subject of intense interest in our laboratory. Nevertheless the current coatings have demonstrated that tubule based templates offer significant advantages for the development of improved electronic materials. Behroozi et al[7] have reported that at 10% volume loading of permalloy coated tubules in an epoxy matrix a real dielectric constant approximately equal to 50 along the alignment axis at a frequency of 9.5 Ghz has been achieved for tubules of average length of 30 microns with a lipid diameter of 0.5 micron and a 1000Å thick metal coating. This result agrees with their electrodynamic calculations. The paper suggests that far higher values may be achievable with longer tubules and improved metal coatings. Such results suggest the possibility for applications such as high density packaging for microelectronic applications, high dielectric materials for miniaturized microwave device applications, and phased arrays and waveguides for radar applications. Tubules may also serve as templates for fabricating field emitting arrays for microwave cathode applications. For any of these applications to be introduced into actual use the electroless metal deposition process will have to be improved and appropriate matrix materials identified, e.g. ceramics or polymers etc.

Field Emission Applications

Recently vacuum field emission was realized from a cathode fabricated of metallized microtubules. The surface micro-morphology of sharp cylindrical emitting tips is produced by using tubules, as the template for metal deposition, and subsequently forming an aligned composite of these metal microstructures[10].

Limitations of present electron source technology are manifested in microwave devices high energy particle accelerators, laser pumping, and other fields which utilize electron beams as a means of energy transfer. Presently available electron sources are divided into three categories: thermionic emitters, laser-activated photo-emitters and field emitters. Included in this last category of field emitters are both exploding field emitters, sometimes termed plasma cathodes, and vacuum field emitters which do not form an intermediate plasma. The structures we discuss here may provide a means to achieve electron beam brightness in excess of 10^6A/cm2-rad2 from an unsaturated field emission cathode, by using the electrostatic lensing produced near the tip of the hollow emitter micro structures. The generation of macroscopic electron beam currents through vacuum field emission from a large number of emission sites requires a surface with a complex micro structure . To date, fabrication of surfaces suitable to this task has been dominated by micro lithographic techniques. In these processes, masks are used in conjunction with etching or deposition techniques to produce arrays of micron scale cones or wedges recessed only a few microns from apertures in a gate structure[15,16]. The micro structure composite cathode materials described here do not use such an electrode configuration with gate, and are similar in this respect to more conventional electron source materials. The structure of an array of hollow cylinders protruding a uniform distance from a base electrode is preferred over an array of pyramids or cones because of the larger available emission area. As additional advantages, these materials appear to be relatively insensitive to the background vacuum pressure, and operate at DC.

The necessary local enhancement of applied electric field is produced by the geometry of the exposed tubules: their height, inner and outer radius, the average spacing between nearest neighbors, the radius of curvature of the metal wall at the edge of the exposed hollow cylinder, and the character of the surface in the vicinity of the exposed edge. Detailed numerical simulations of the electrostatic field in the vicinity of the hollow cylindrical structure have shown that field enhancement factors in the range of 150--250 are readily achieved with the 0.5 micron diameter tubules protruding a height of 10 to 15 microns above the base surface. The intrinsic surface roughness of electrolessly deposited metal film that makes up the outer tubule surface would probably increases this nominal enhancement factor by an additional factor of 2-4, yielding an expected range in the enhancement factor of B= 300--1000.

The hollow nature of the protruding tubule micro structures (Figure 7) that make up field emitter arrays is of particular interest, because it provides an electrostatic lensing effect for the emitted electrons. Strength of this electrostatic lensing depends on the inner and outer radii of the tubule, and the position of the emission area on the end of the tubule structure. The thinner the metallic wall, the greater is the self focussing effect of the structure, and the more collimated the emitted micro beamlet becomes. For suitably fabricated structures, with thin wall thickness near the emission tip, the previously cited numerical simulations have indicated that normalized electron beam brightness well in excess of 10^9 A/cm2-rad2 can be achieved[17].

In conclusion, we have described measurements of vacuum field emission from an unsaturated field emitter array fabricated from a composite of self-assembling biomolecular micro structures. Micro structure composite materials offer an interesting alternative to micro lithographic techniques for the achievement of complex surface micromorphologies. Complex biological systems and organic molecules, in particular self-assembling biomolecular micro structures, offer a wide variety of micro structure geometries potentially useful for application in physical systems. The hollow, thin walled , high-aspect ratio tubule micro structures discussed here might provide a surface micro morphology suited to the generation of high current, high brightness electron beams. An identical structure would be difficult to generate using existing micro lithographic techniques.

SUMMARY AND CONCLUSION

The ability to on a molecular level engineer sub micron particles represents a seminal advance in the development of advanced composite materials leading to a number of new composite materials with significantly enhanced mechanical and electronic properties. We believe this to be true as we have substituted natures ability to self-assemble complex molecular structures rather than to rely on often expensive and sensitive technological equipment to achieve the same result.

We are now actively pursuing applications for the lipid tubule microstructures. These applications range from antifouling to the development of advanced electronic materials. Antifouling controlled release paint systems have been successfully developed and tested. Other applications such as wound healing, antifungal paints, and bioremdiation systems are currently under evaluation. Prototype fabrication of tubule based high dielectric and field emitting materials suggest possible commercial applications for high resolution displays and advanced microwave devices. It is clear that advanced materials research and development is entering a new stage due to recent advances in "self assembly" and possibility of the design of molecules for the "engineering" of supermolecular micron sized structures for specific applications.

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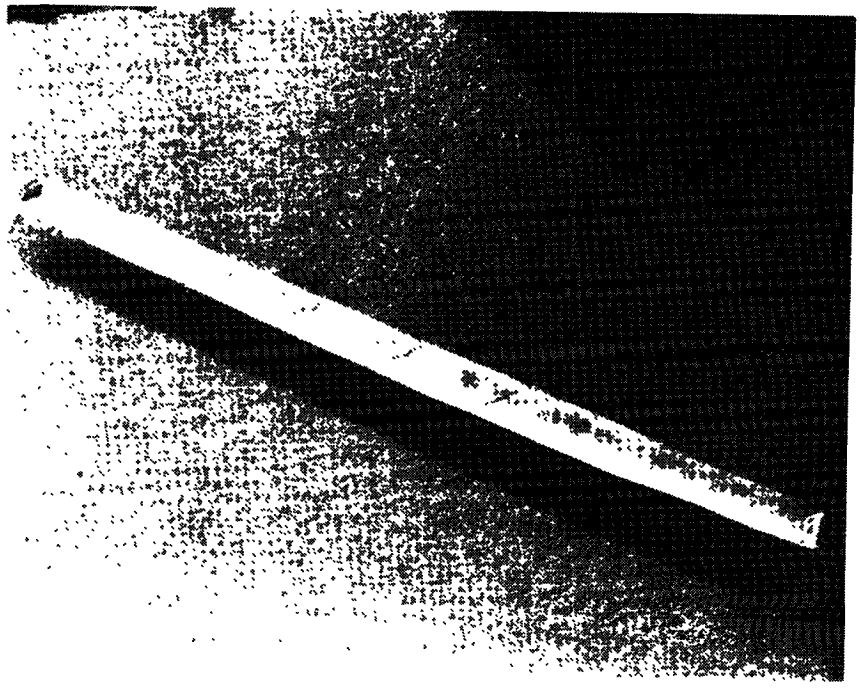

Figure 1 Electron micrograph of a typical lipid tubule.

Figure 2 Photograph of rods were mounted in a floating array.

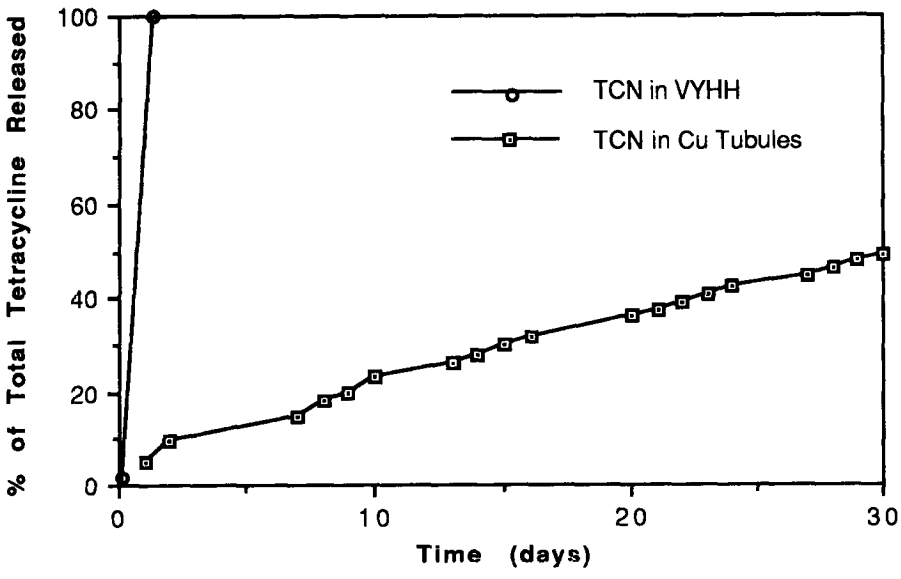

Figure 3 Encapsulated vs. non-encapsulated release rates for tetracycline in a vinyl matrix.

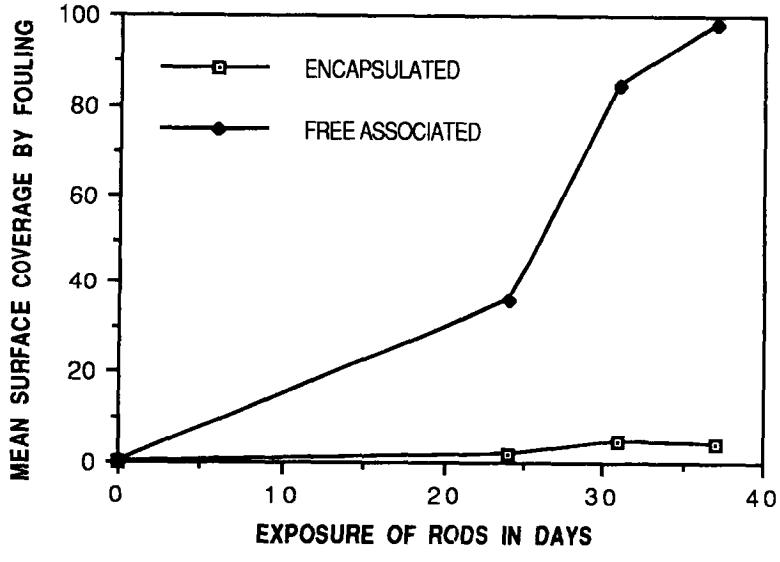

Figure 4 Comparison of effectiveness of encapsulated vs. non-encapsulated isothiazolone on marine fouling.

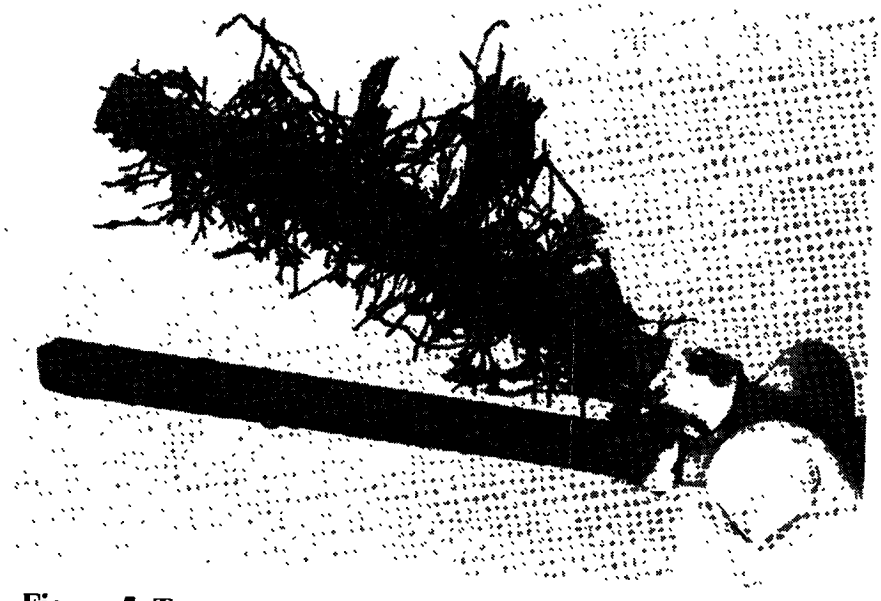

Figure 5 Test rods from Beaufort, N.C. effectiveness test, illustrates effectiveness of microencapsulated natural product.

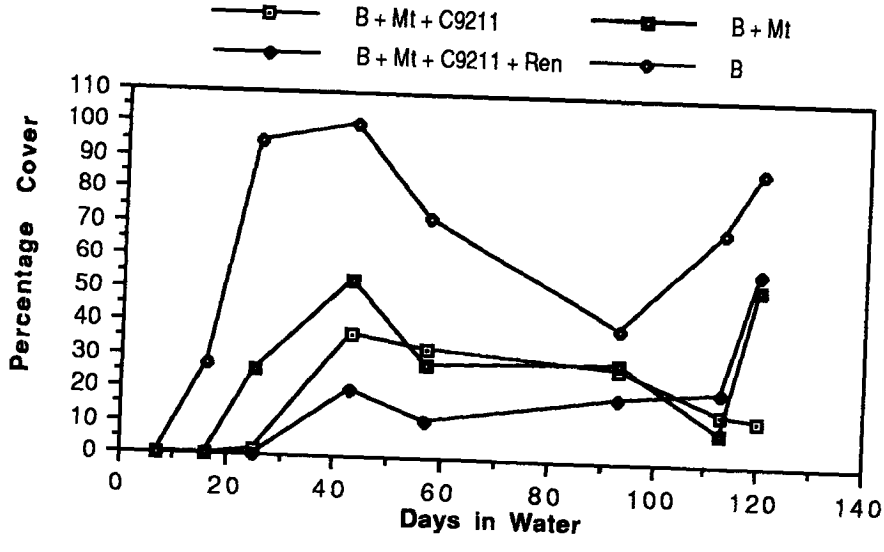

Figure 6 Comparison of fouling characteristics in four coatings consisting of 1) copolymer methacrylate paint, 2) Copper microtubules in paint, 3) Isothiazolone encapsulted, 4) isothiazolone and extract of renilla.

Ni tubules in epoxy, plasma etched. SEM

Figure 7 **Electron micrograph of a tubule based cathode array.**