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H4.SMR/916 -18

SEVENTH COLLEGE ON BIOPHYSICS:

Structure and Function of Biopolymers: Experimental and Theoretical Techniques.

4 - 29 March 1996

Self-Assembled Lipid Tubules

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20375-5348 Washington D.C.
U.S.A.

Self-Assembled Lipid Tubules

Presented by

Joel M. Schnur

Center for Bio/Molecular Science & Engineering
Naval Research Laboratory

Self-Assembly

- Self-assembly is the aggregation of molecules to form microstructures, films, or patterned bulk material; excludes crystallization.

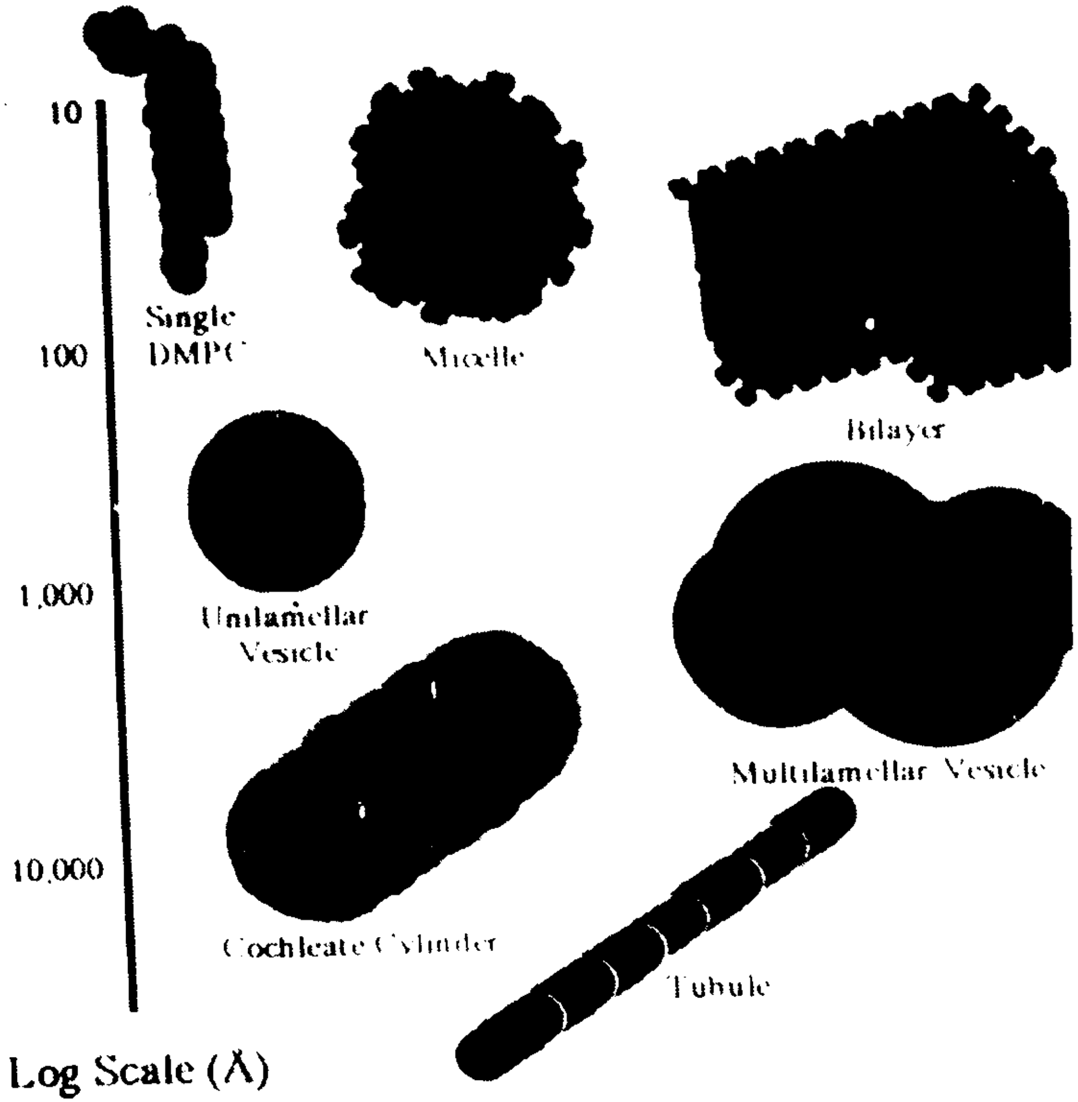
- By taking advantage of thermodynamics, we can fabricate, using self-assembly, low defect, stable, structures that could not be easily made by other techniques.

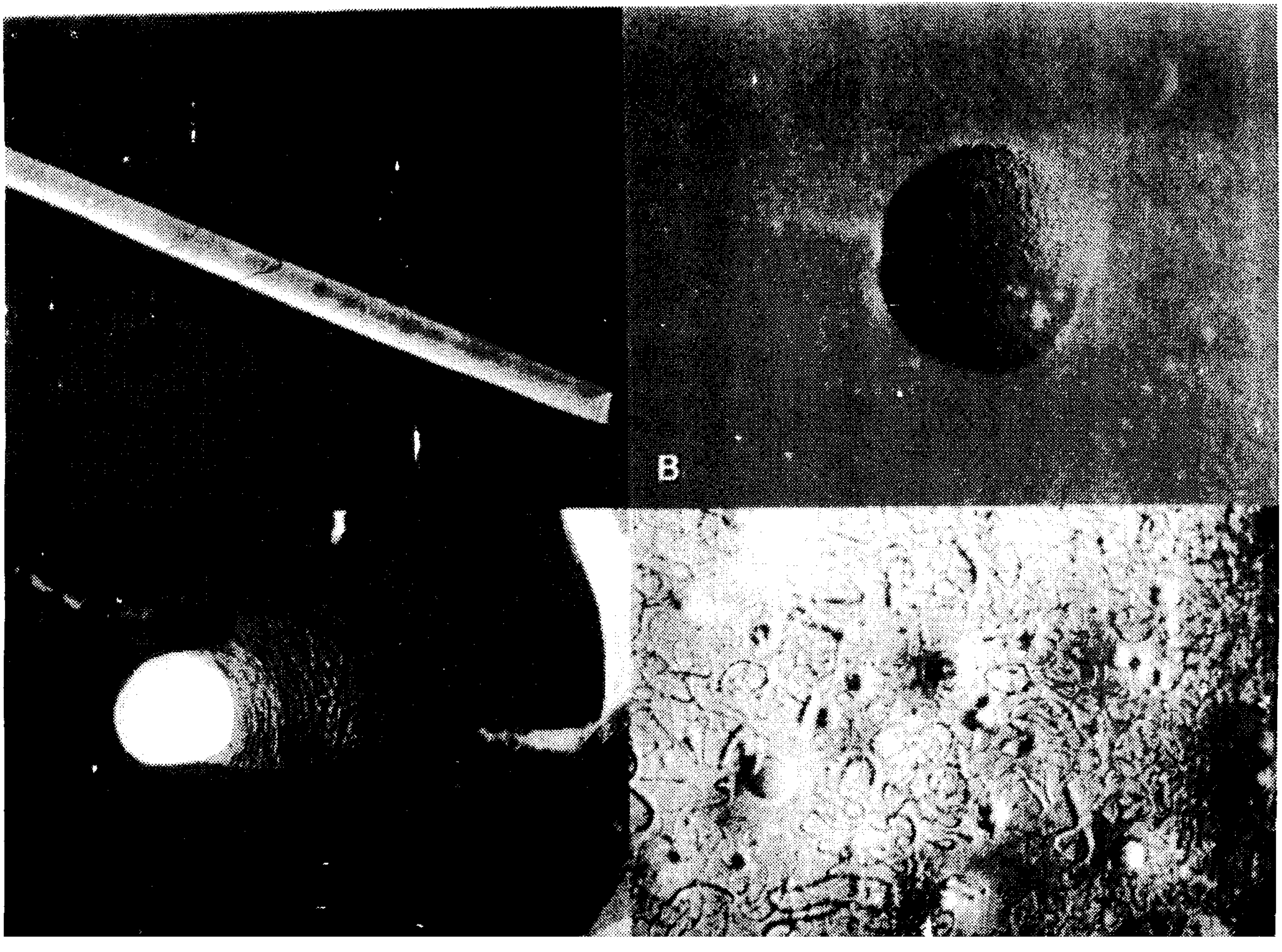
- Goal: Control of assembled structures.

- Implication: We must understand the self-assembly process.

Some Types of Self Assembly

- Lipids ✔
- Liquid Crystals
- Polymers
- DNA
- Chemical Adsorption
- Colloids

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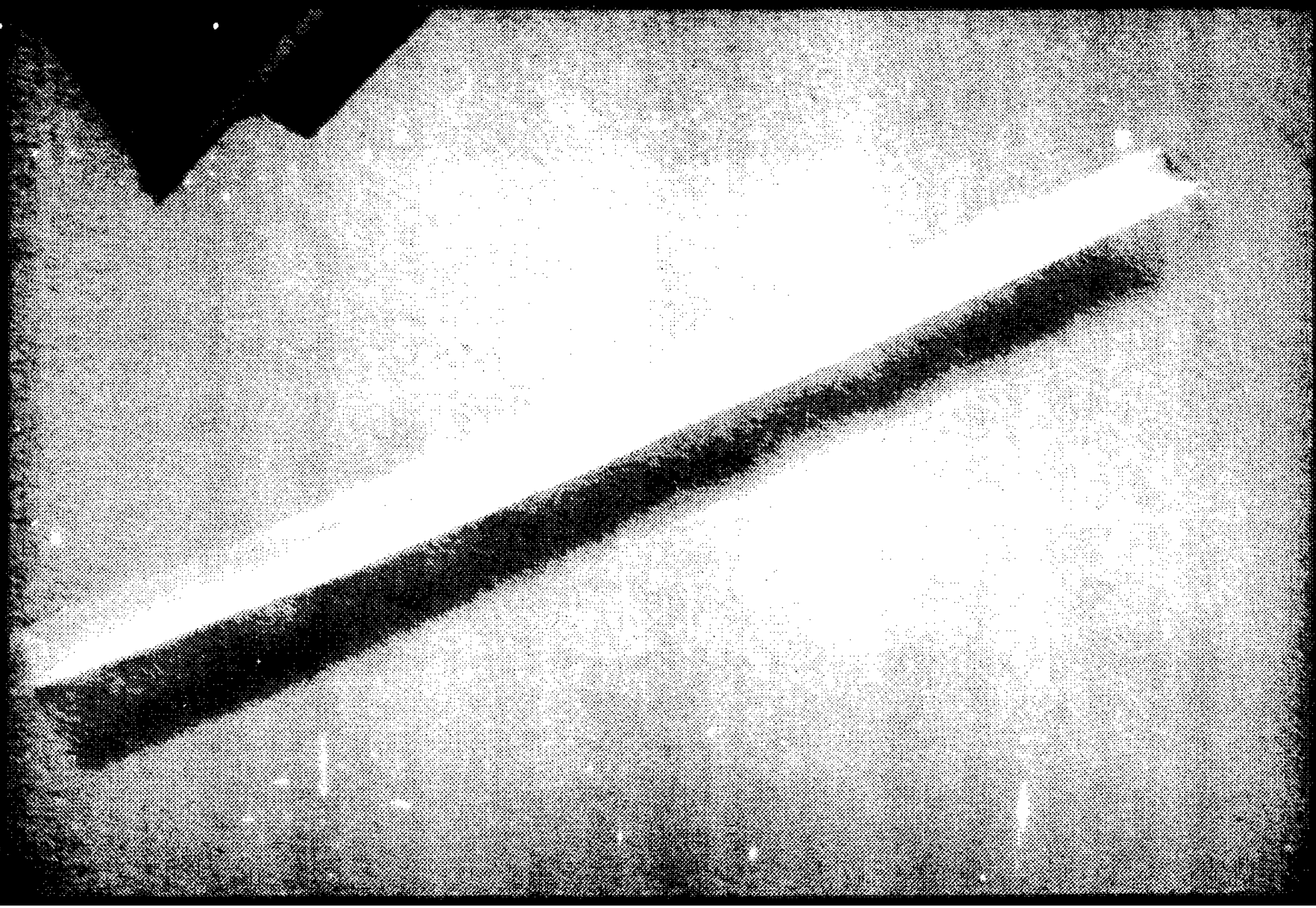

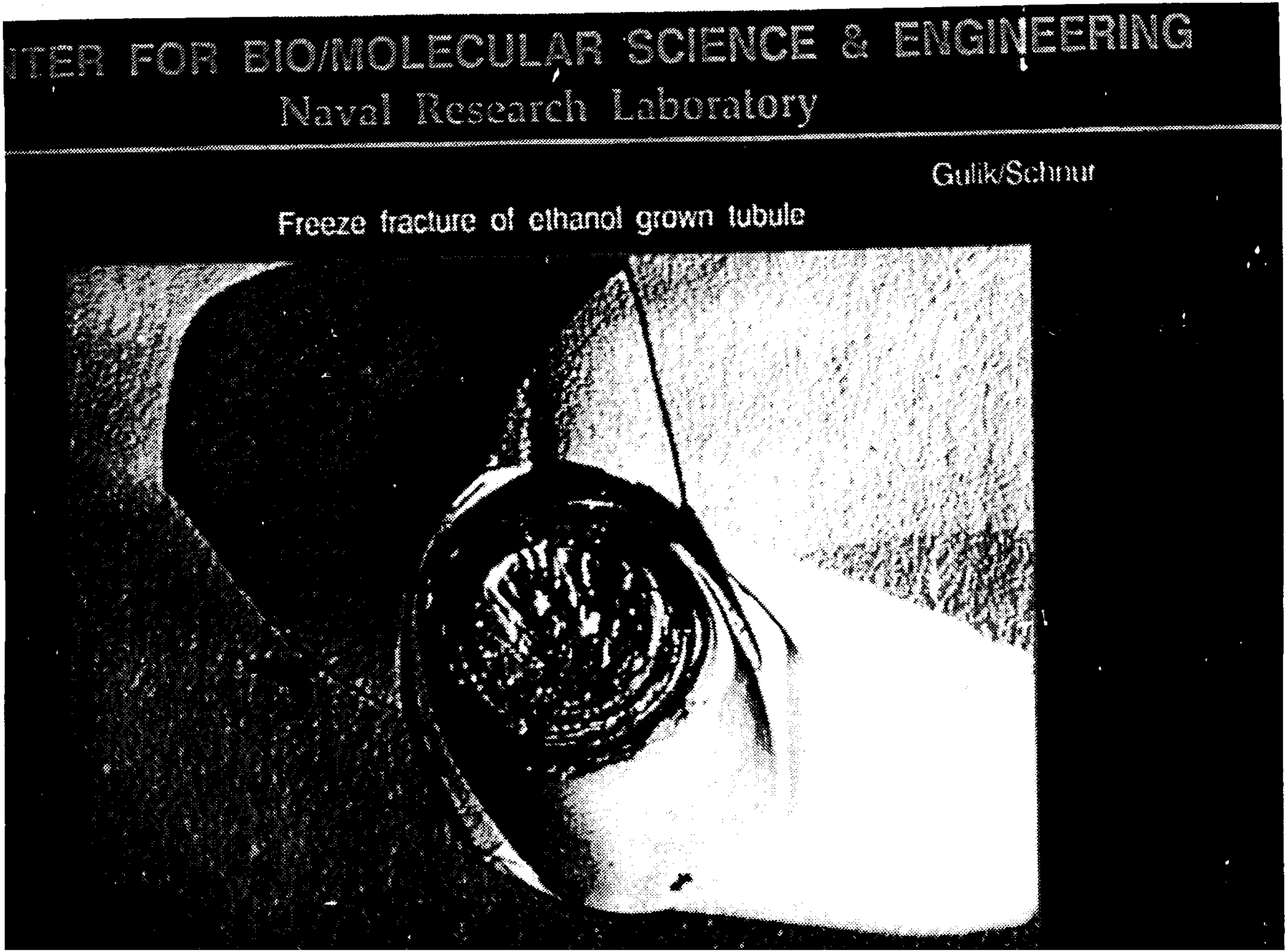
TER FOR BIO/MOLECULAR SCIENCE & ENGINEERING
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Gulik/Schnur
Freeze fracture of ethanol grown tubule

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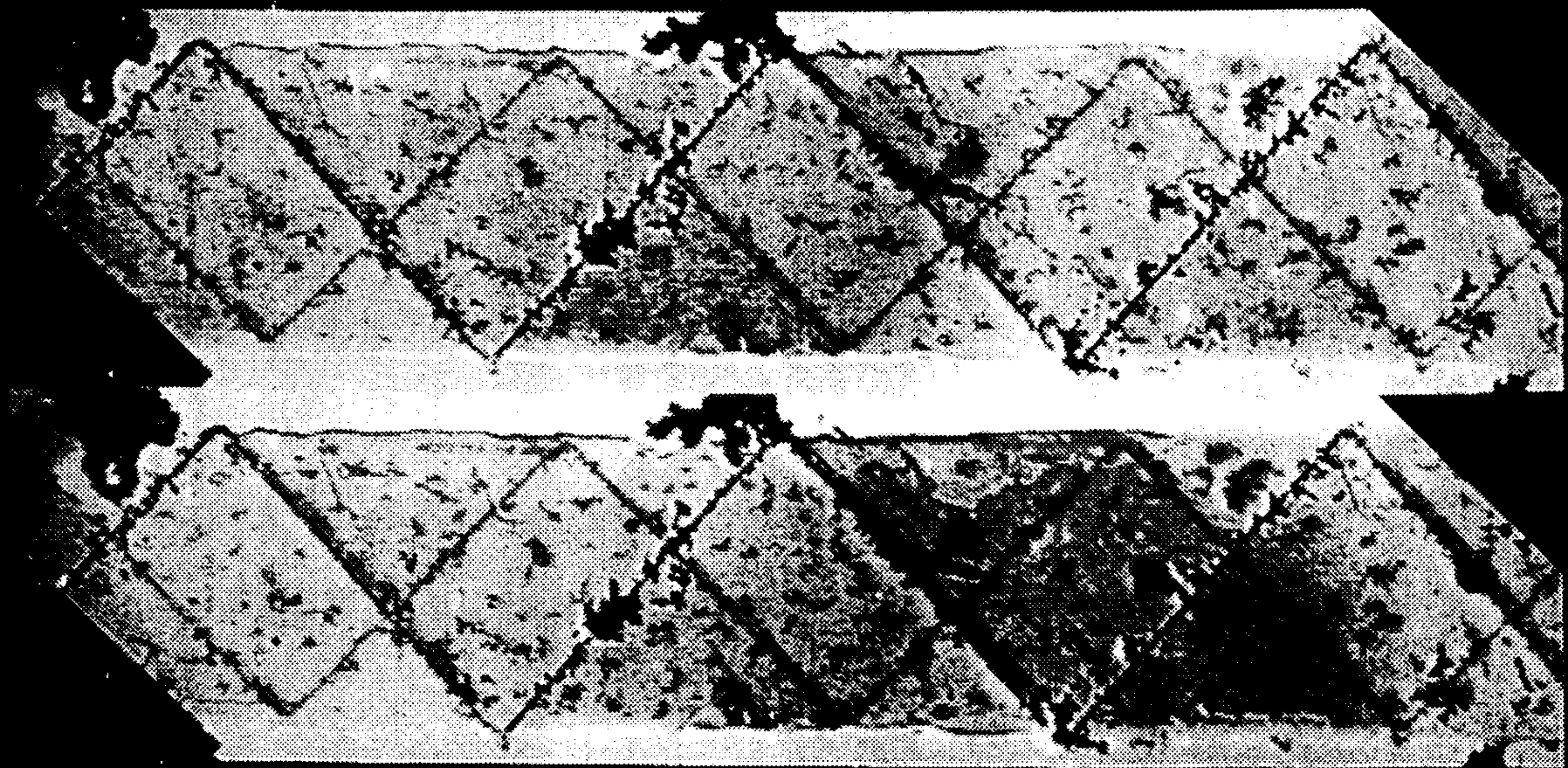
Gulik/Schnur

Tubules with the adsorbed PD/NI catalyst particles on surface



Self-Assembled Lipid Tubules

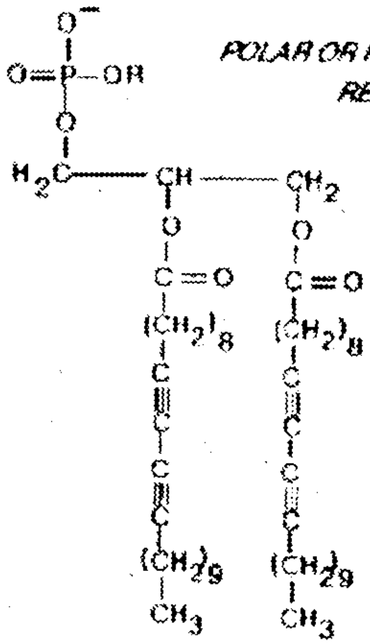
Objective:

- **Rational Design and Control of Ultra-Small Particles; e.g. Cylinders on the Submicron Scale.**

Development Process for Self-Assembled Materials

- Control Self-Assembly of the Molecules.
- Transform "FAT" Cylinders to Metal.
- Test for Potential Applications.

FACTORS INFLUENCING SELF-ASSEMBLY

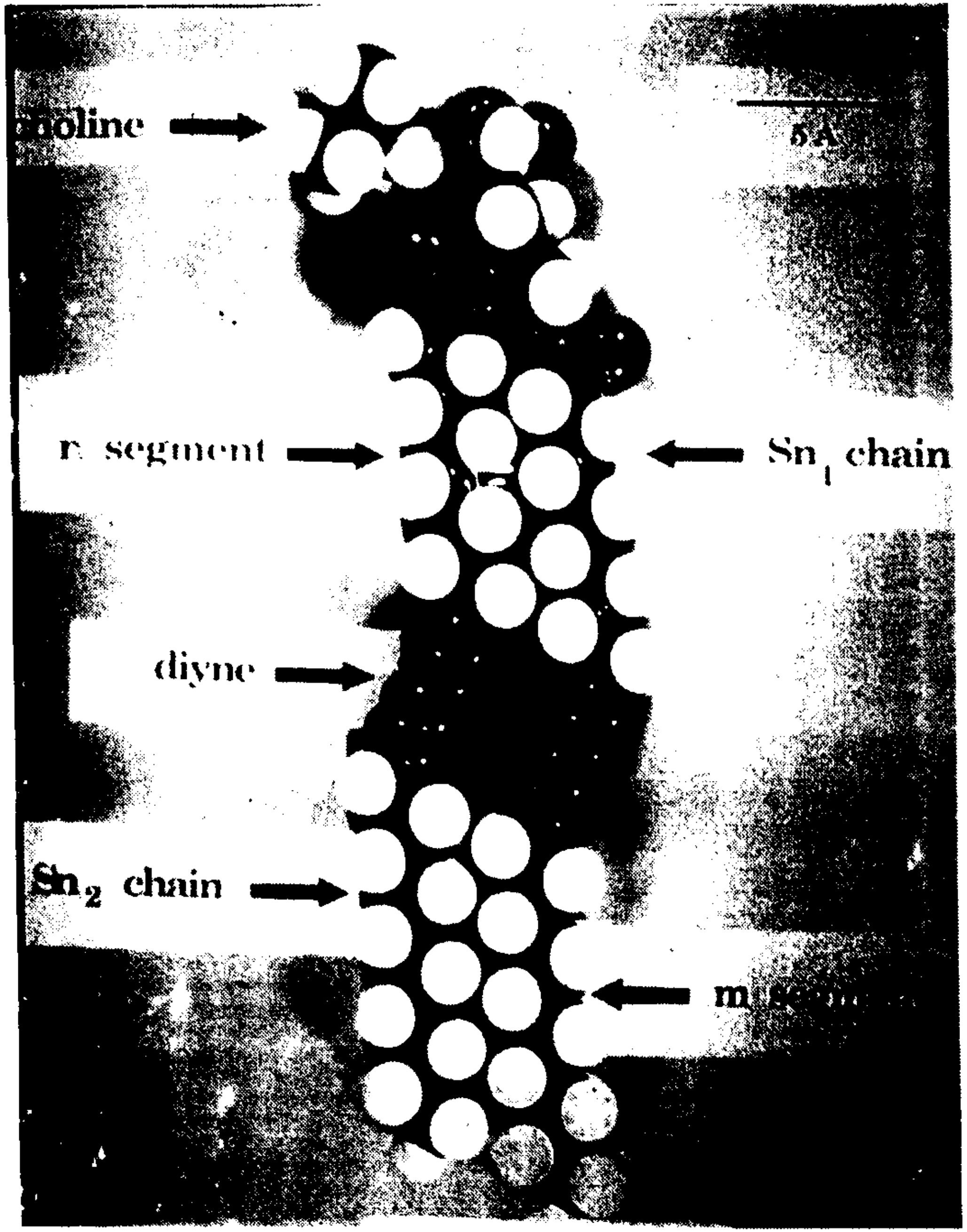


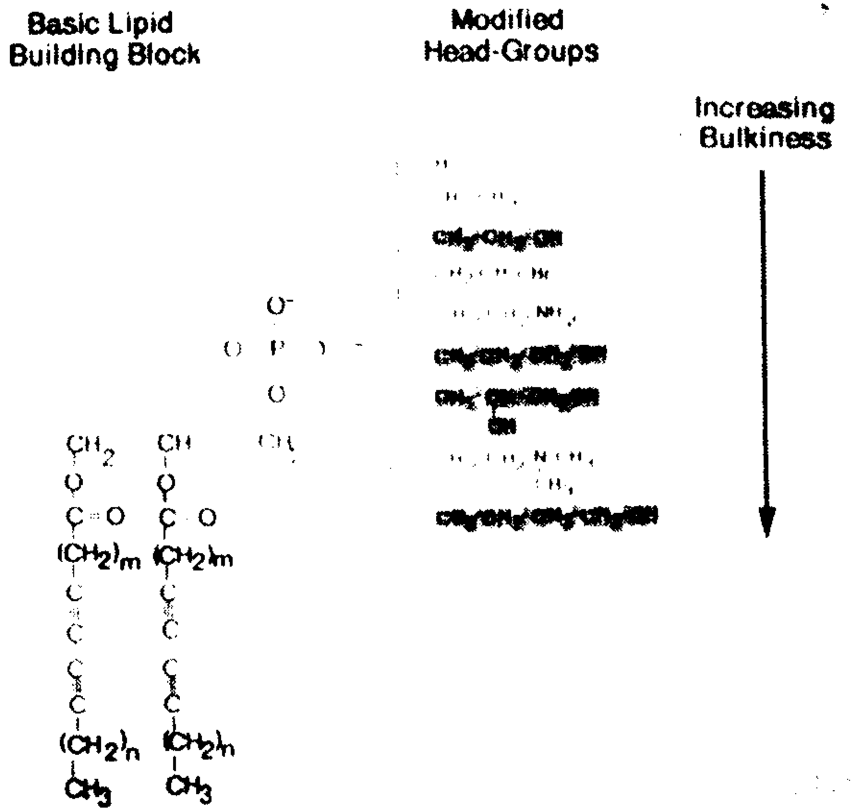
POLAR OR HEAD GROUP REGION

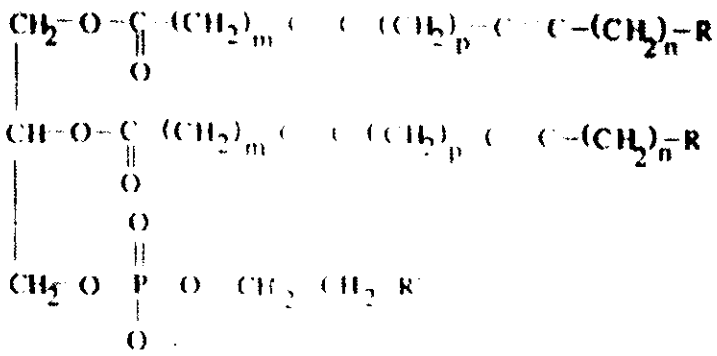
(CHARGE, ION, DISPERSION MEDIUM ETC.)

ACYL CHAIN REGION

LENGTH, UNSTURATION, PLACEMENT OF UNSTURATION, ATTACHMENT TO BACKBONE


segment
Sn_1 chain
diyne
Sn_2 chain


Basic Lipid Building Block
Modified Head-Groups
Increasing Bulkiness



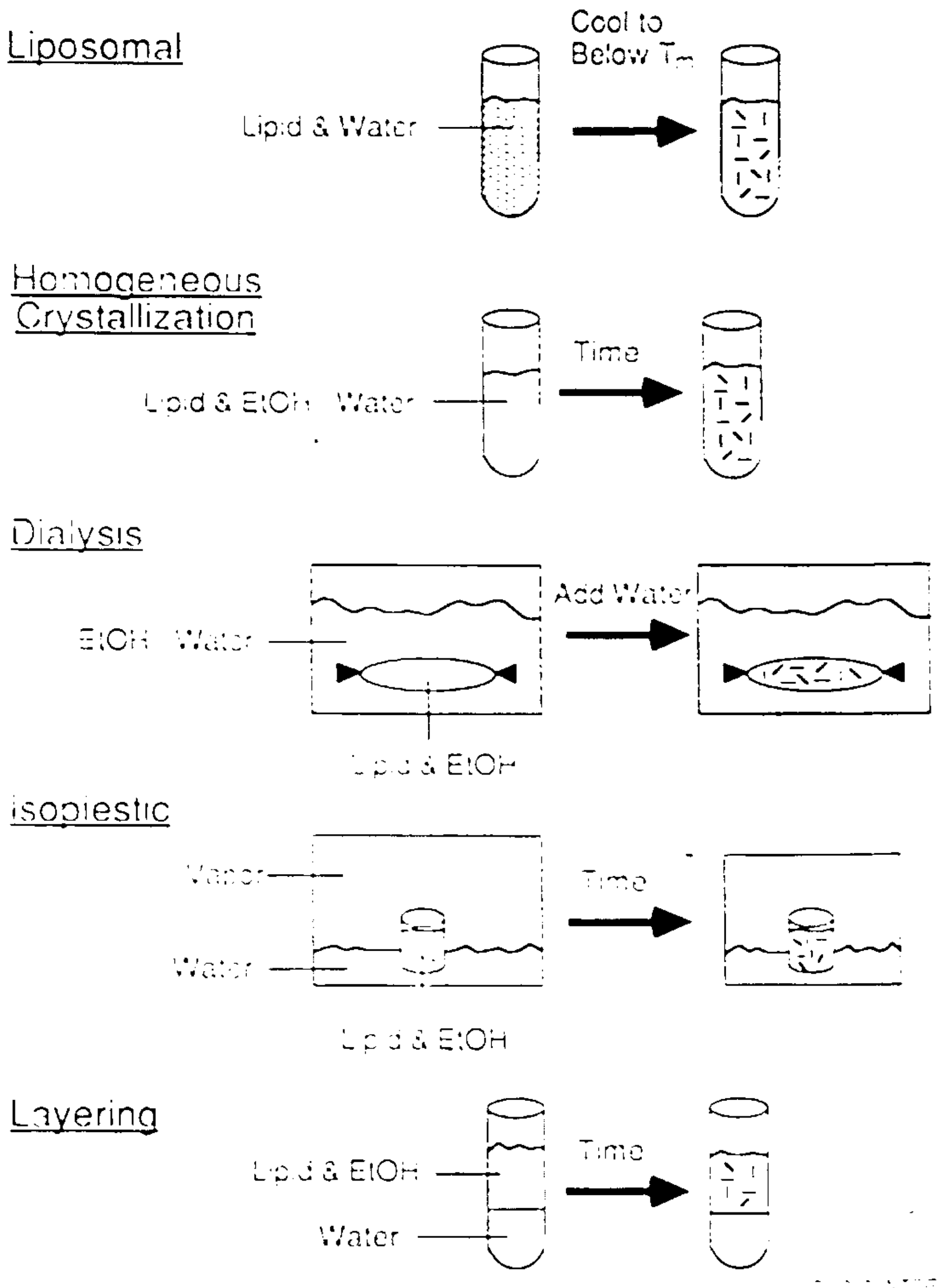
m	n	p	R	R'
5–15	6–16	0	CH_3	$\overset{+}{N}Me_3$
4–8	13–9	1	CH_3	$\overset{+}{N}Me_3$
8	8	0	$CH(CH_3)CH_3$	$\overset{+}{N}Me_3$
8	11	0	$OC(O)C(CH_3)=CH_2$	$\overset{+}{N}Me_3$
8	6	0	$C\equiv CH$	$\overset{+}{N}Me_3$
8	9	0	CH_3	OH, $\overset{+}{N}Me$, $\overset{+}{N}H_2$

DIACETYLENIC PHOSPHOLIPIDS

SUMMARY OF ACYL CHAIN MODIFICATIONS

MODIFICATION	HEADGROUP	MORPHOLOGY
isomeric diacetylenes	choline	vesicles, tubules
skipped diacetylene	choline	unusual vesicles
didiacetylene	choline	ribbons
diacetylene & methacrylate	choline	vesicles, tubules
diacetylene & olefin	choline	tubules
diacetylene & acetylene	choline	tubules
mesogen azomethine	choline	gel

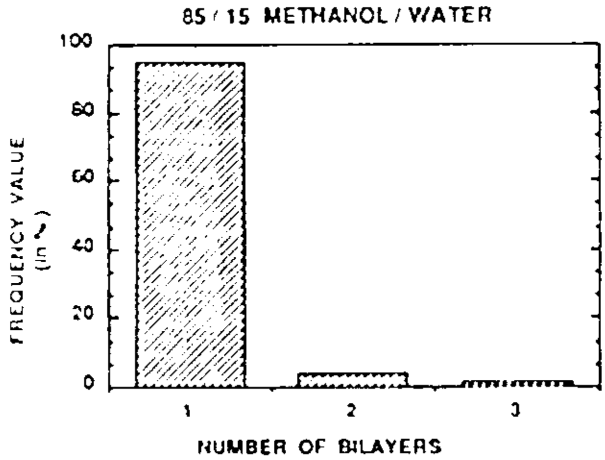
CONDITIONS FOR TUBULE FORMATION

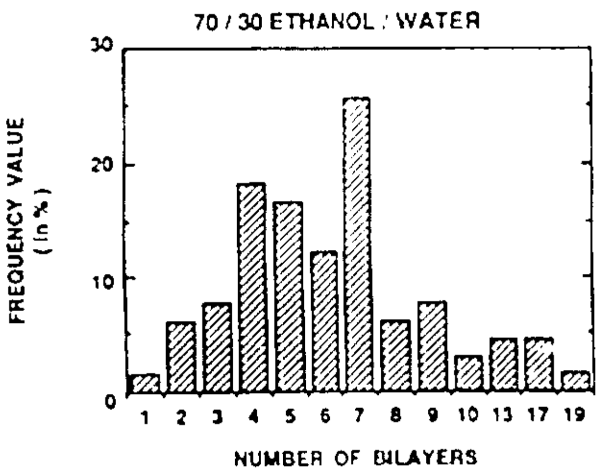


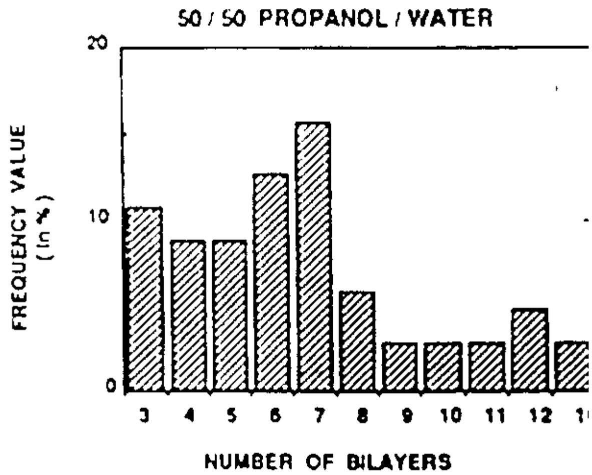
Liposomal
Lipid & Water
Cool to Below T_m
Homogeneous Crystallization
Lipid & EtOH : Water
Time
Dialysis
EtOH : Water
Lipid & EtOH
Add Water
Isopiestic
Water
Water
Lipid & EtOH
Time
Layering
Lipid & EtOH
Water
Time

Result 2 / RATNA

NUMBER OF BILAYERS IN TUBULES GROWN FROM DIFFERENT ALCOHOLS







TUBULES GROWN FROM METHANOL / WATER

Result 1 / RATNA

STRUCTURE OF TUBULES

RAMAN SPECTROSCOPY:

* CHAINS ARE HIGHLY ORDERED (NRL GROUP, 1986)

X-RAY DIFFRACTION STUDIES:
(LOW RESOLUTION STUDIES AND POWDER DIFFRACTION)

* LAYERS ARE VERY WELL DEFINED, 12 - 16 ORDERS OF DIFFRACTION SEEN
  (D. Rhodes et. al., 1989; M. Caffrey et. al., 1991)

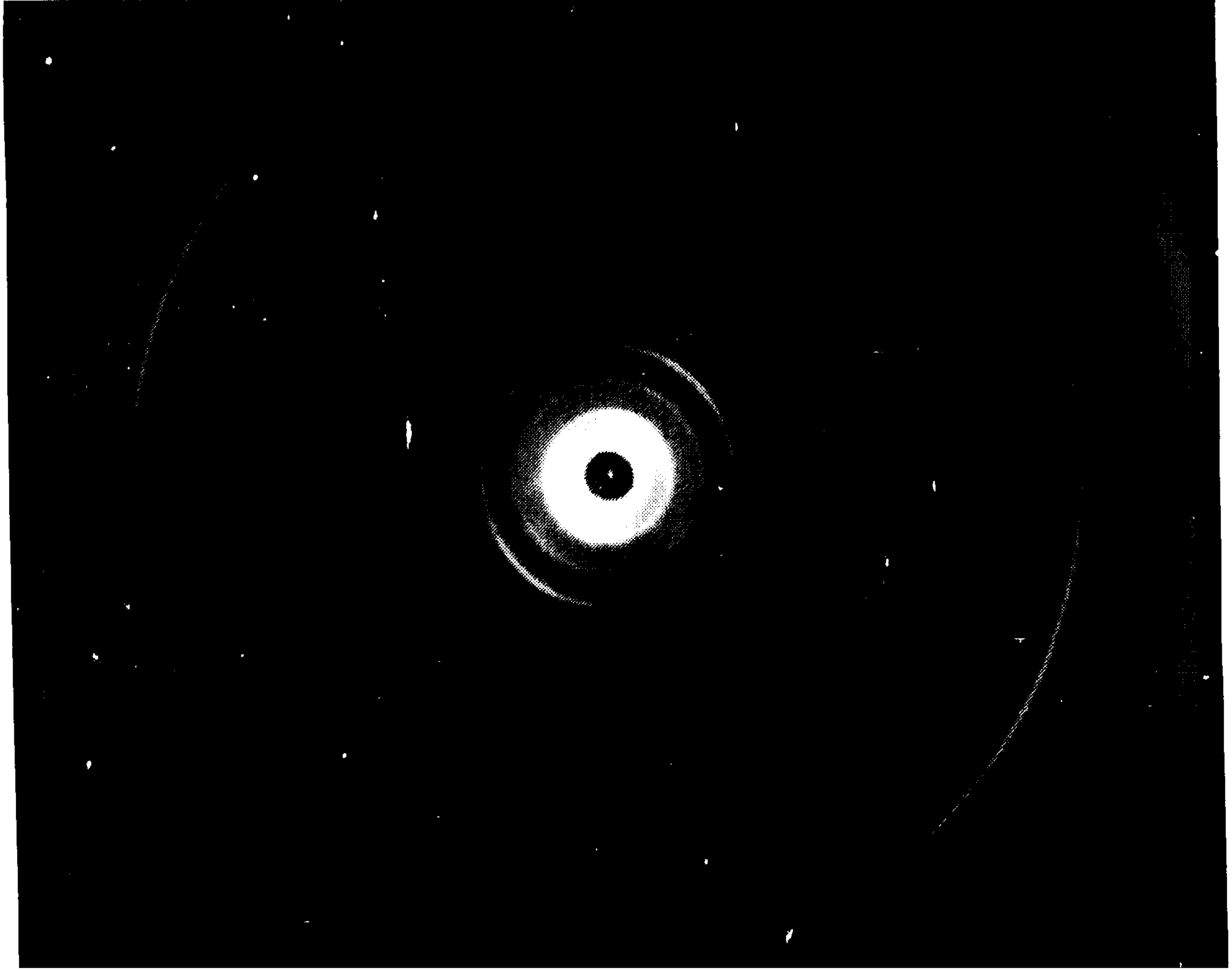
* THE DIACETYLENIC UNITS ARE TILTED WITH RESPECT TO THE LAYER NORMAL, NO ONTERDIGITATION OF TAILS
  (D. Rhodes et. al., 1989, 1990)

ELECTRON DIFFRACTION STUDIES:

* STUDIES CONDUCTED ON "STACKED" TUBULES AND ON 6-LAYER LB FILMS

* BOTH GIVE THE SAME UNIT CELL (MONOCLINIC) WITH THE SAME UNIT CELL DIMENSIONS
  (Sudiwala and Lando, 1991)

X-RAY DIFFRACTION INFORMATION



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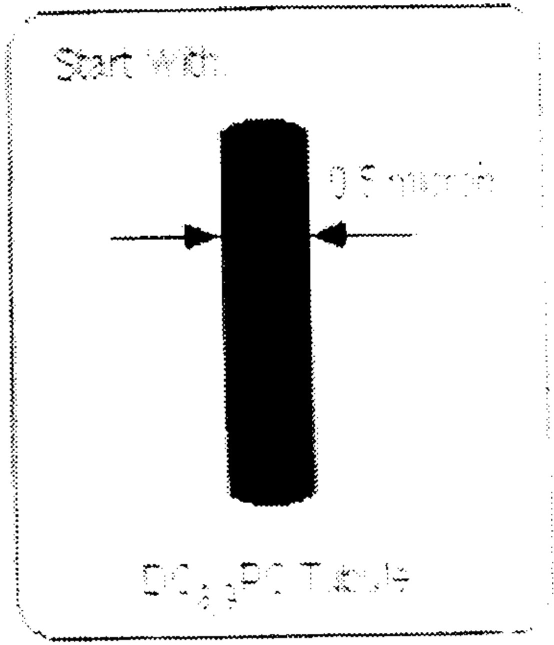
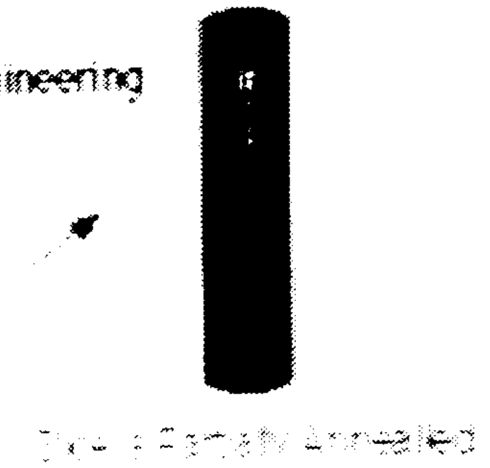
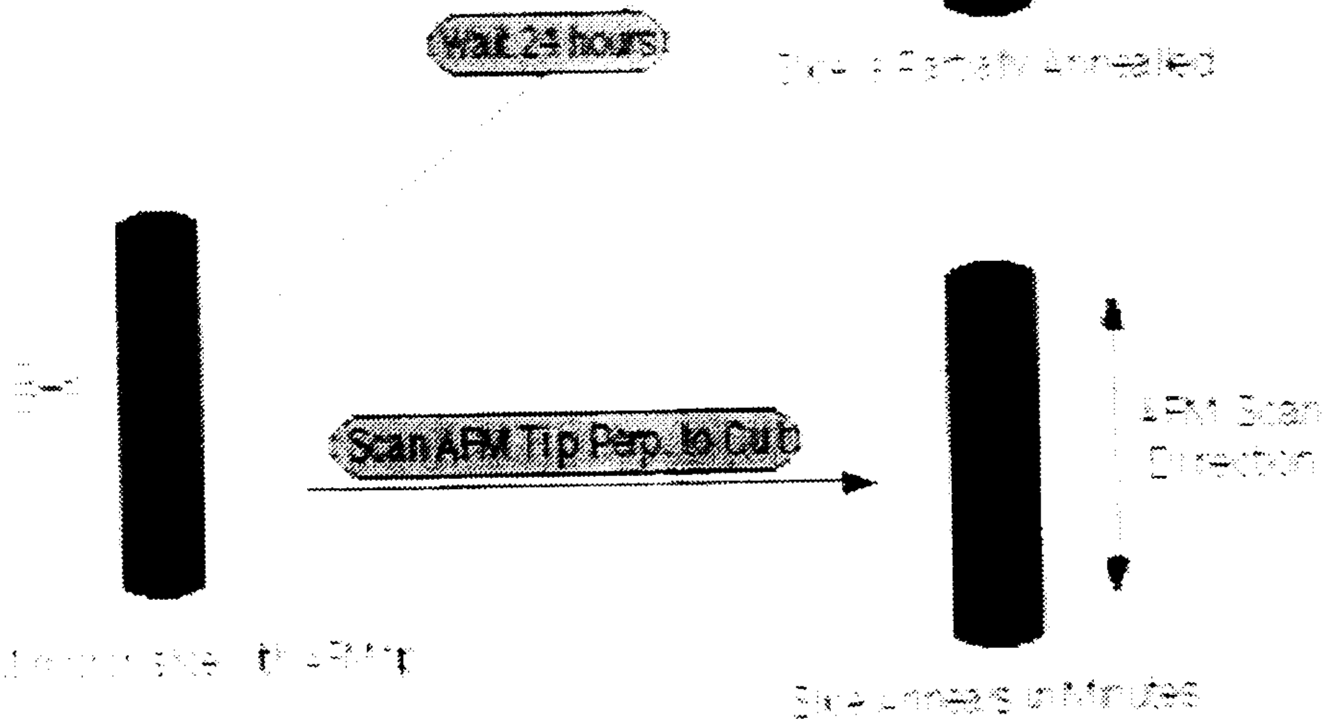
NanoMachining a Lipid Tubule

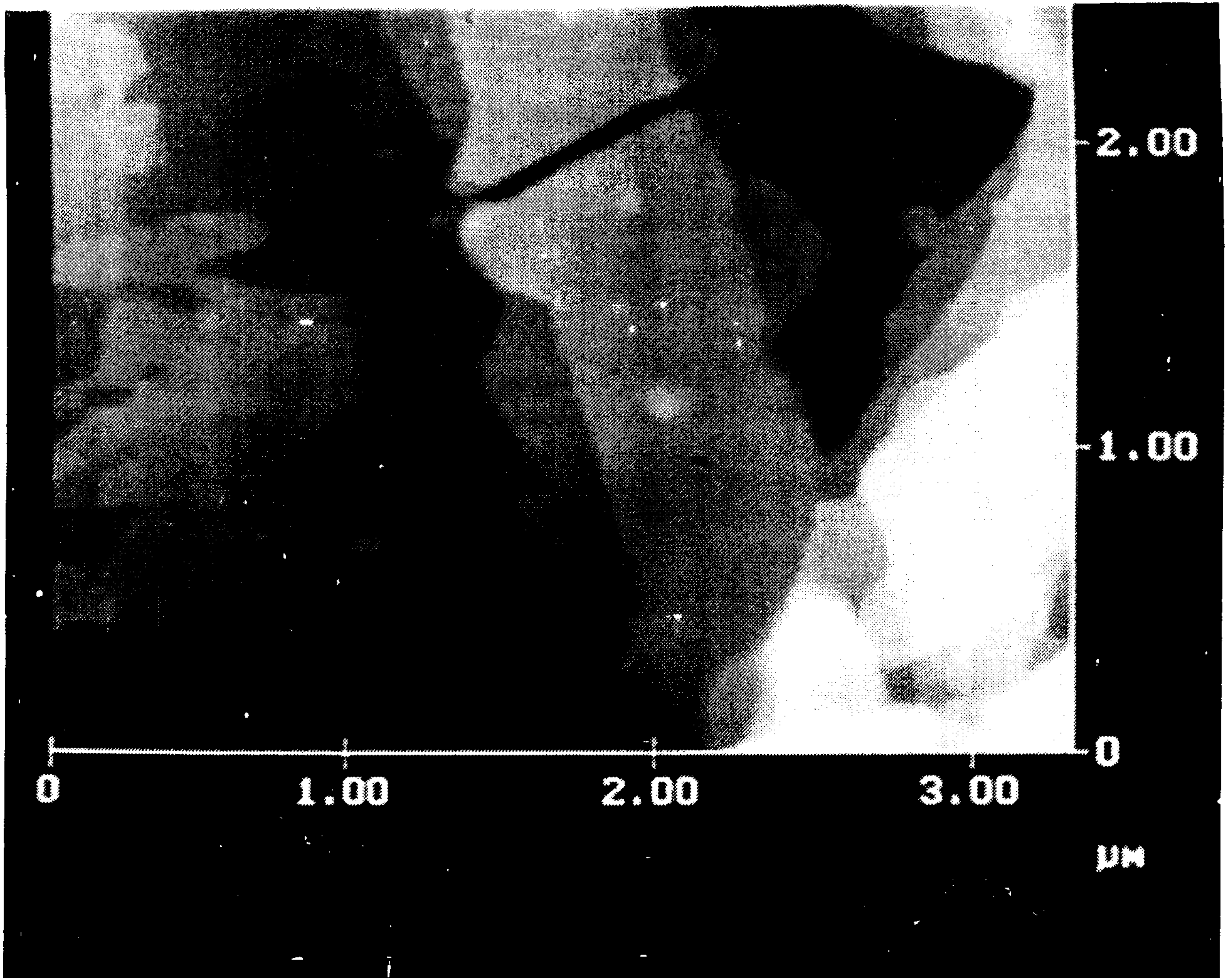


Susan L. Brandow
David Turner
Bruce Paul Gaber

Center for BioMolecular Science and Engineering






2.00
1.00
0
0
1.00
2.00
3.00
μm

Fabrication Process is Controlled - Scale Up Feasible - Need to Understand Properties of Tubules to Explore Applications

Theory of Chiral Lipid Tubules

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Naval Research Laboratory, Code 6900
4555 Overlook Avenue, SW
Washington, DC 20375-5348

Cylindrical microstructures:

Typical diameter of 0.5 μm. Typical length of 50-200 μm.

Characteristic helical "barber-pole" pattern on the cylinder.

Bilayers or multilayers of diacetylenic phosphocholine lipids: chiral molecules.

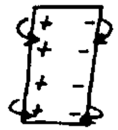
Applications:

Encapsulation of antifouling agents for controlled release.

Templates for metallic or magnetic particles.

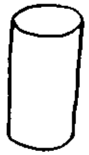
Theories of tubules:

I. Electrostatic interaction vs. curvature energy

de Gennes  $r \propto \text{const.}$

Modified de Genne  $r \propto L^{1/3}$

II. Line energy vs. curvature energy

Lubensky and Prost  $r \propto L^{1/2}$

III. Intrinsic bending force in tilted chiral bilayer

Helfrich and Prost
Ou-Yang and Liu
P. Nelson and Powers  $r \propto \text{const.}$

Experiments:

I. Lack of electrolyte effect

II. Polydispersity in radius vs. polydispersity in length

III. Helical winding

Chirality:

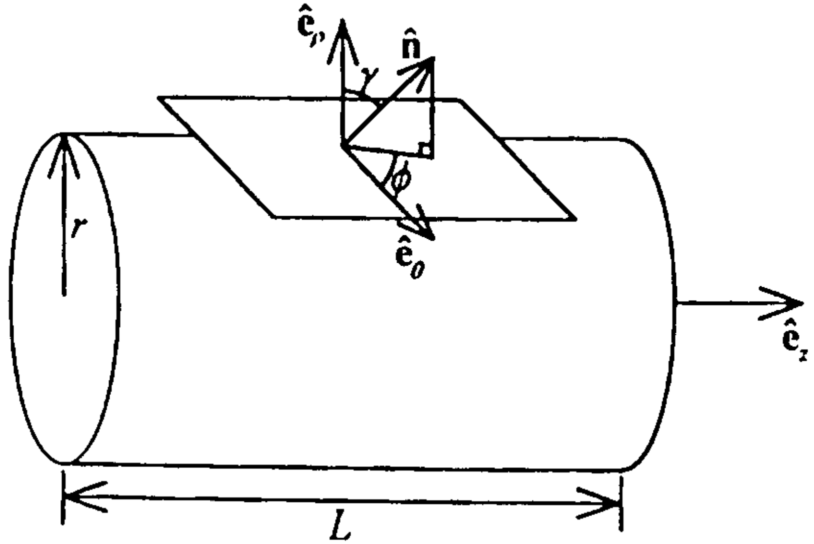
Chiral molecules: Do not have any reflection or inversion symmetry; cannot be superimposed on their mirror images. Right and left-handed forms.

Fundamental connection between chirality and microstructure formation: Long chiral molecules do not pack parallel to neighboring molecules.

Chirality parameter:

$$q = \frac{\text{angle between neighboring molecules}}{\text{distance between neighboring molecules}}$$

Geometry:



(1) Assume molecules form bilayer in any tilted fluid phase (smectic-C* or tilted hexatic).

(2) Assume bilayer forms cylinder with arbitrary radius r.

(3) Assume γ is constant, while $\phi(\theta, z)$ may vary.

Free energy:

$$F = F_{\text{Frank}} + F_{\text{tilt}} + F_{\text{curvature}}$$

Frank:

$$F_{\text{Frank}} = \int d^2x \left[\tfrac{1}{2} K_1 |\nabla \cdot \hat{\mathbf{n}}|^2 + \tfrac{1}{2} K_2 |\hat{\mathbf{n}} \cdot \nabla \times \hat{\mathbf{n}} - q|^2 + \tfrac{1}{2} K_3 |\hat{\mathbf{n}} \times \nabla \times \hat{\mathbf{n}}|^2 \right]$$

(factor of bilayer thickness absorbed into K_1, K_2, K_3)

Tilt:

$$F_{\text{tilt}} = \int d^2x \left[-\tfrac{1}{2} a\gamma^2 + \tfrac{1}{4} b\gamma^4 \right]$$

Curvature:

$$F_{\text{curvature}} = \int d^2x \left[\tfrac{1}{2} \kappa \left(\tfrac{1}{r}\right)^2 \right]$$

Optimum radius of tubule:

$$r = \frac{1}{q}\left\{\frac{1}{2}\left(A^2-1\right)^{1/2}\left[A+\left(A^2-1\right)^{1/2}\right]\right\}^{1/2}$$

where

$$A = \frac{2\kappa + K + K\cos^2\gamma}{K\sin^2\gamma}$$

Note:

(1) Chirality parameter $1/q$ sets scale of r.

(2) Limiting case: untilted (smectic-A) phase

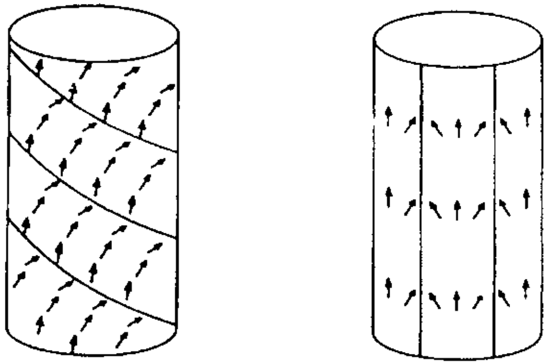
$$\gamma \to 0 \quad \Rightarrow \quad r \propto \gamma^{-2} \to \infty \quad \text{flat}$$

∴ Both chirality and tilt are needed for cylinder formation.

Stripes in director field:

Limiting cases:

Low curvature	Extreme curvature
$qr \to \infty$	$qr \to 0$



Stripes of bend and twist	Stripes of splay

Current work:

Modulations in curvature--"ripples" on the surface of the cylinders (with Fred MacKintosh, University of Michigan, and Tom Lubensky, University of Pennsylvania).

Monte Carlo simulations of the self-assembly of chiral lipid microstructures (with Bob Sinkovits, NRL Laboratory for Computational Physics).

POTENTIAL APPLICATIONS OF TUBULES

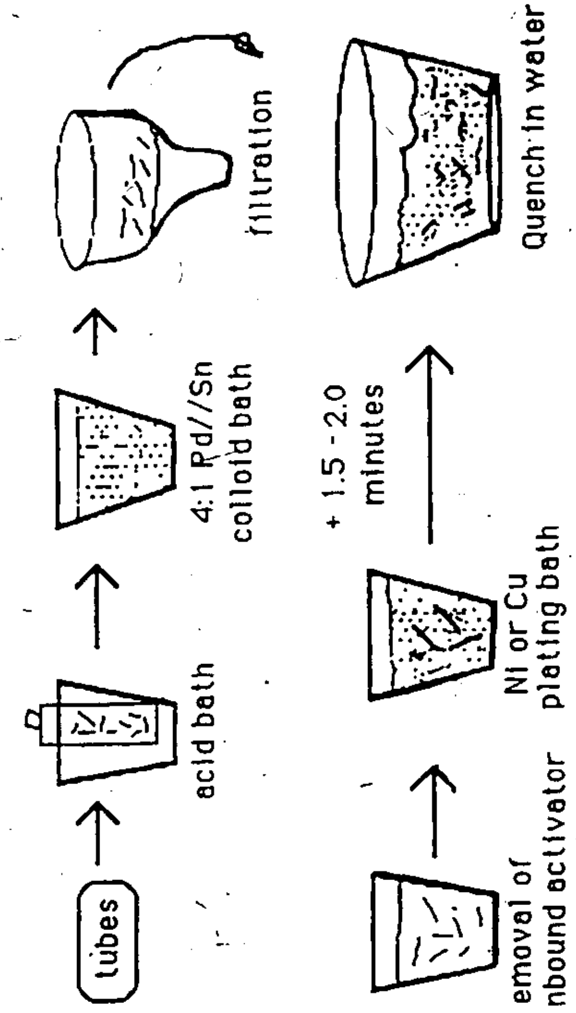
1. METALLIZATION

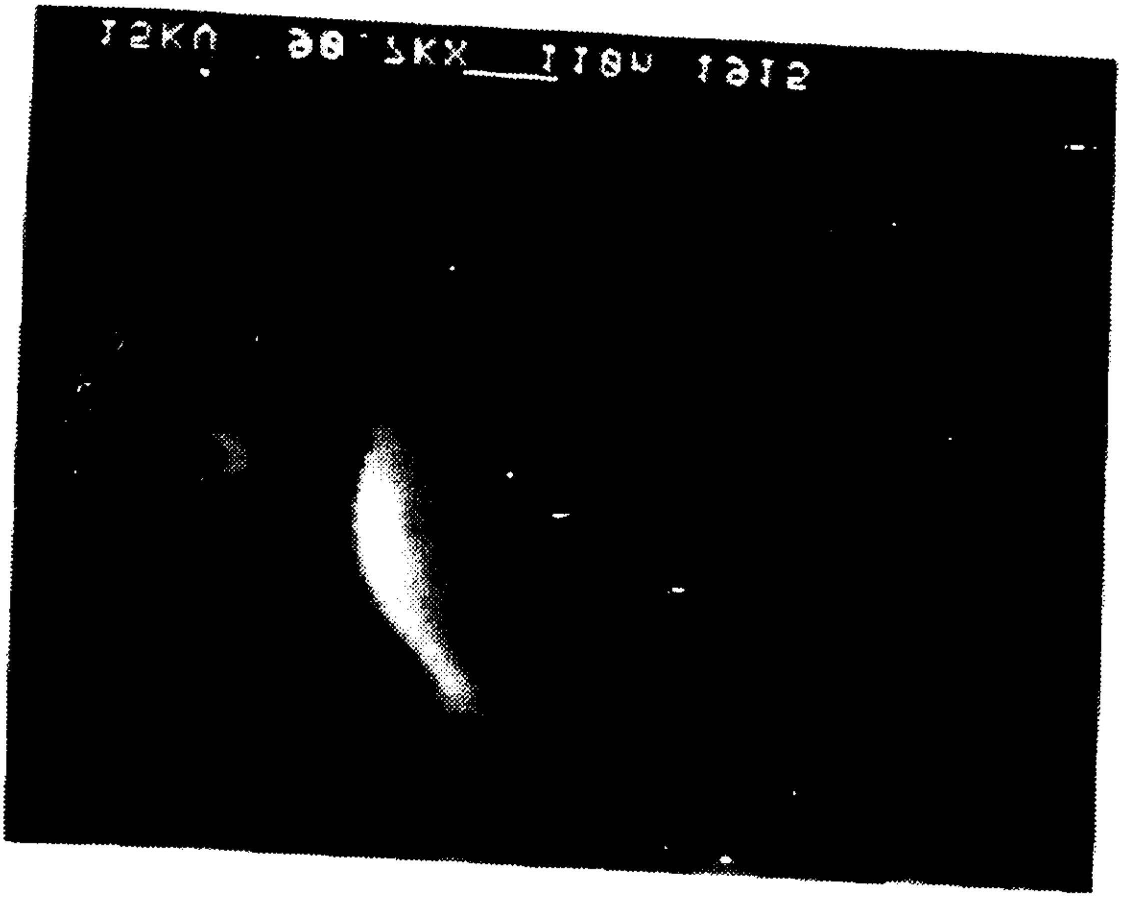
LEADS TO:

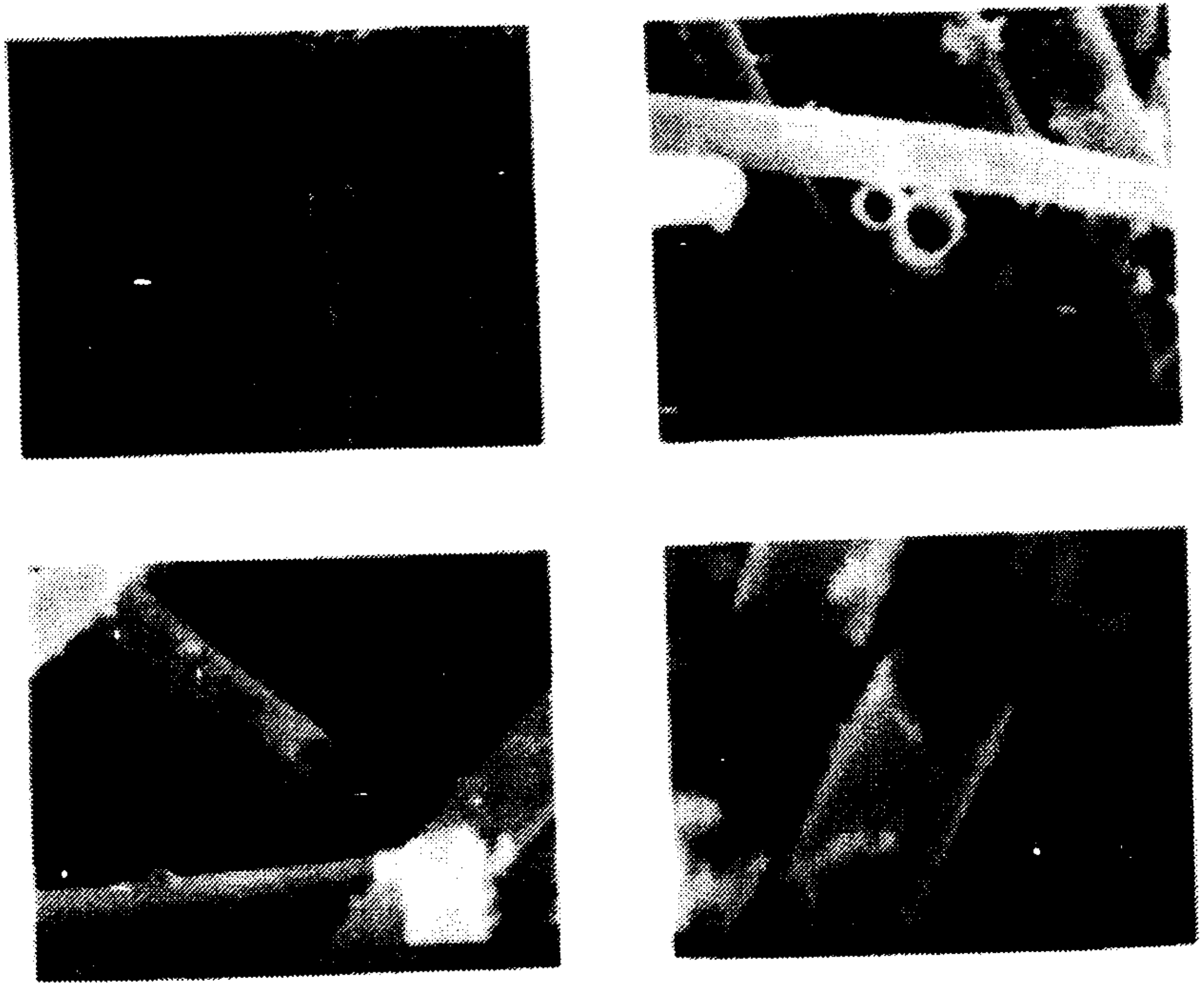
A. RUGGEDNESS
B. CONDUCTING TUBULES
C. ORIENTATION BY MAGNETIZATION
D. SUITABILITY FOR HIGH FREQUENCY DIELECTRIC SYSTEMS
E. ENCAPSULATION

2. HIGH ASPECT RATIO

3. SCALE-UP

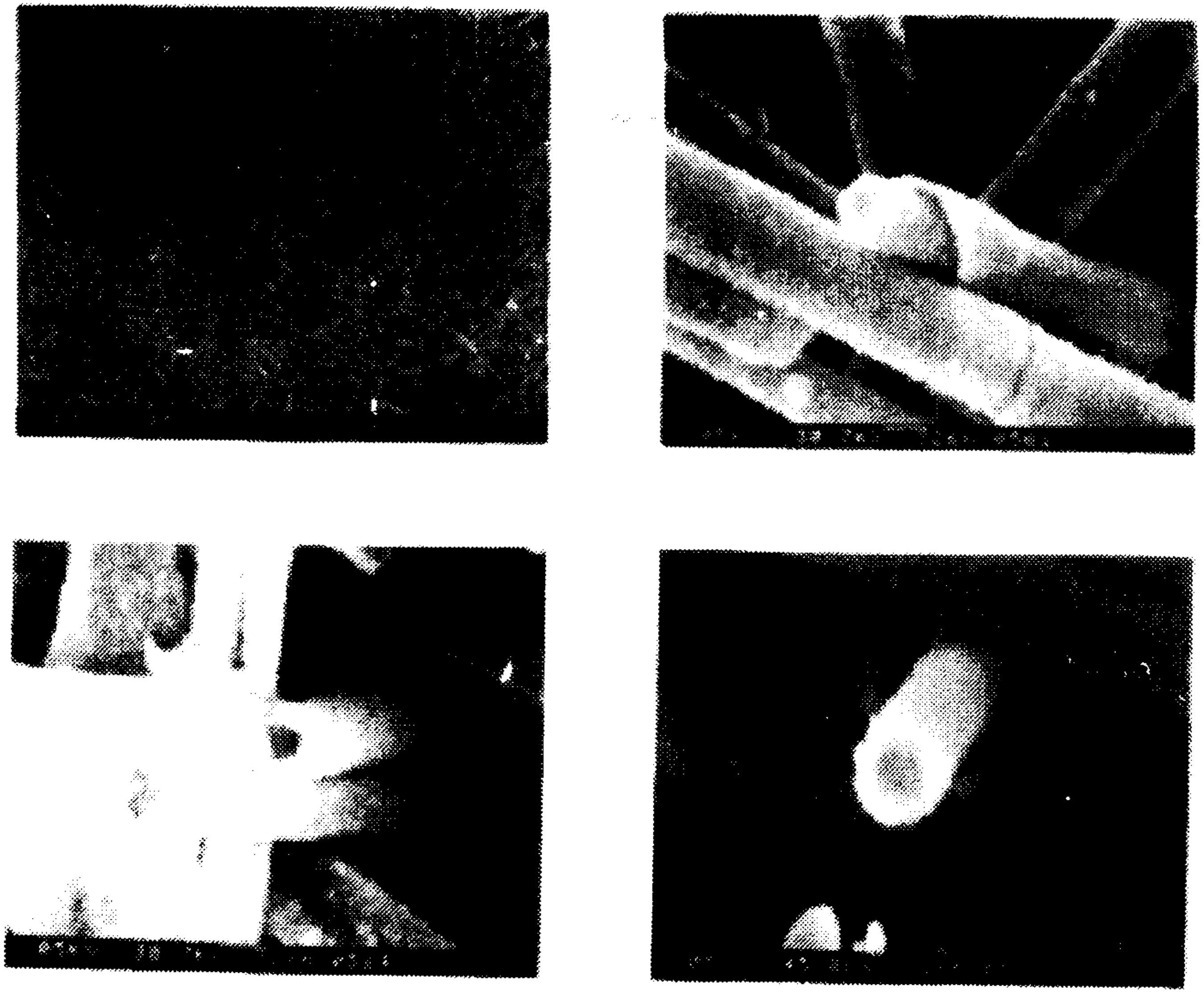






Cobalt Plated Tubes of $DC_{8,9}PC$

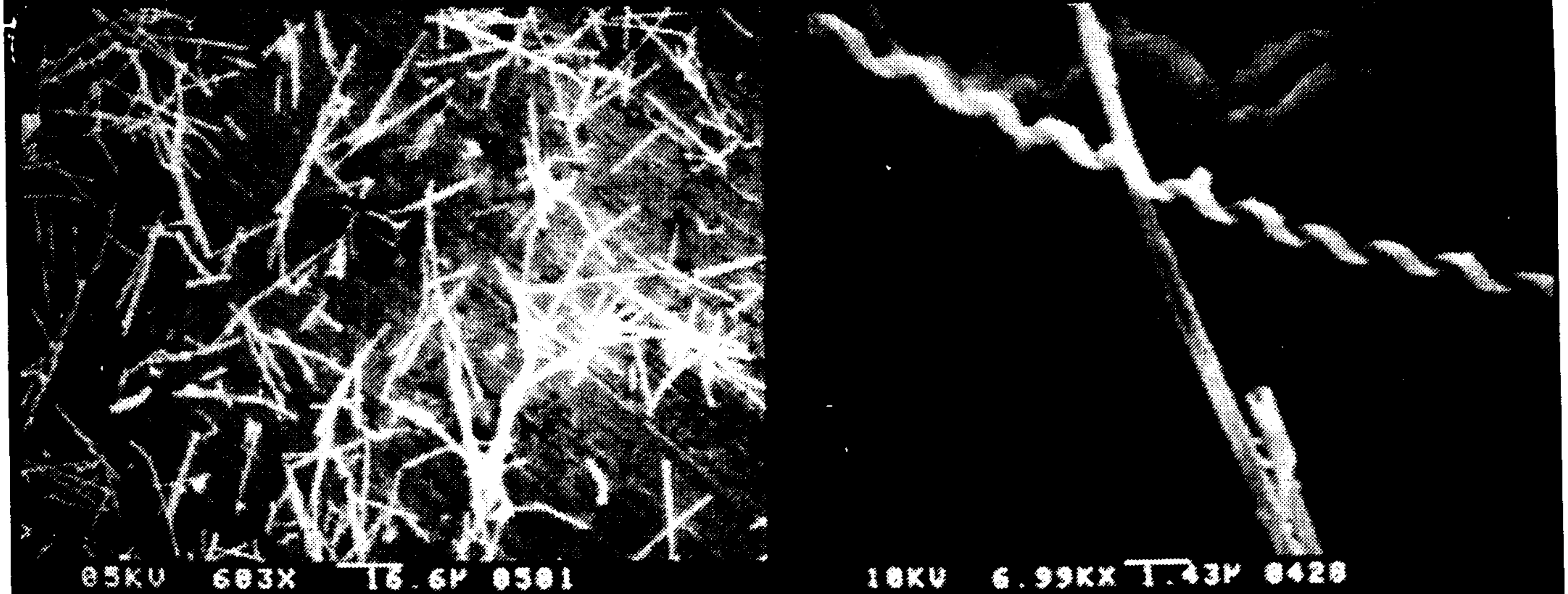
TUBULES



Gold Plated Tubes of $DC_{8,9}PC$

TUBULES

Cu-PLATED TUBULES



BACKGROUND

WHY SHOULD WE USE TUBULE-BASED COMPOSITES?

* Large, controllable shape-anisotropy

* Both dielectric permeability and magnetic permeability can be manipulated

* Large anisotropy in permeability

* Permeability and anisotropy can be "tailored" to achieve a variety of specifications applications

* Possibility of both soft-magnetic and hard-magnetic properties using the same temperature

COMPOSITE APPLICATIONS

1) High Dielectrics
   a) Capacitors
   b) Microstrip substrates

2) Lossy Dielectrics
   a) RFI
   b) Refractory composites

3) Magnetic Materials - non-reciprocal rf transmission

4) Electron Emissive Surfaces

TUBULE-BASED MAGNETIC COMPOSITES

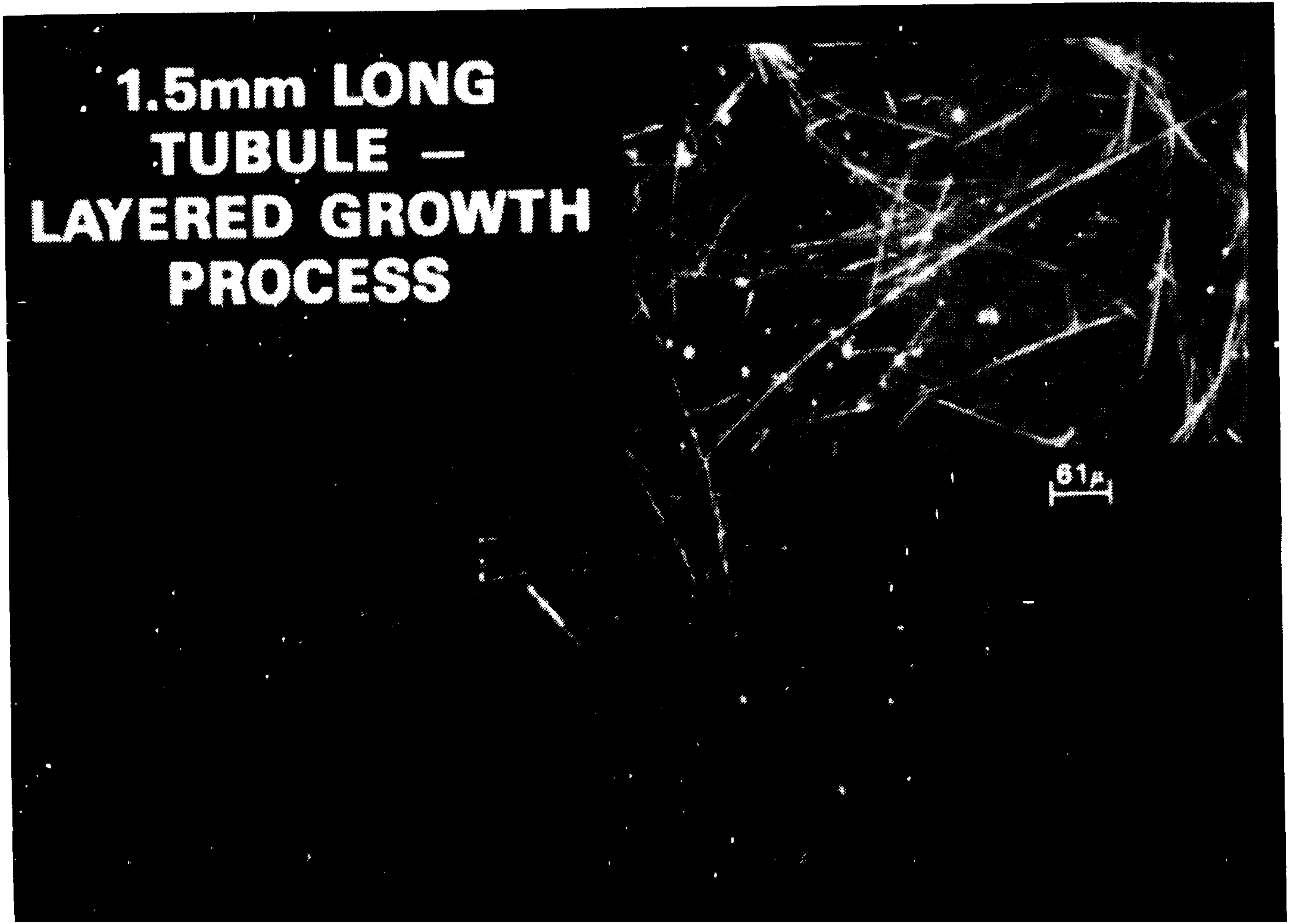
SIGNIFICANT RESULTS:

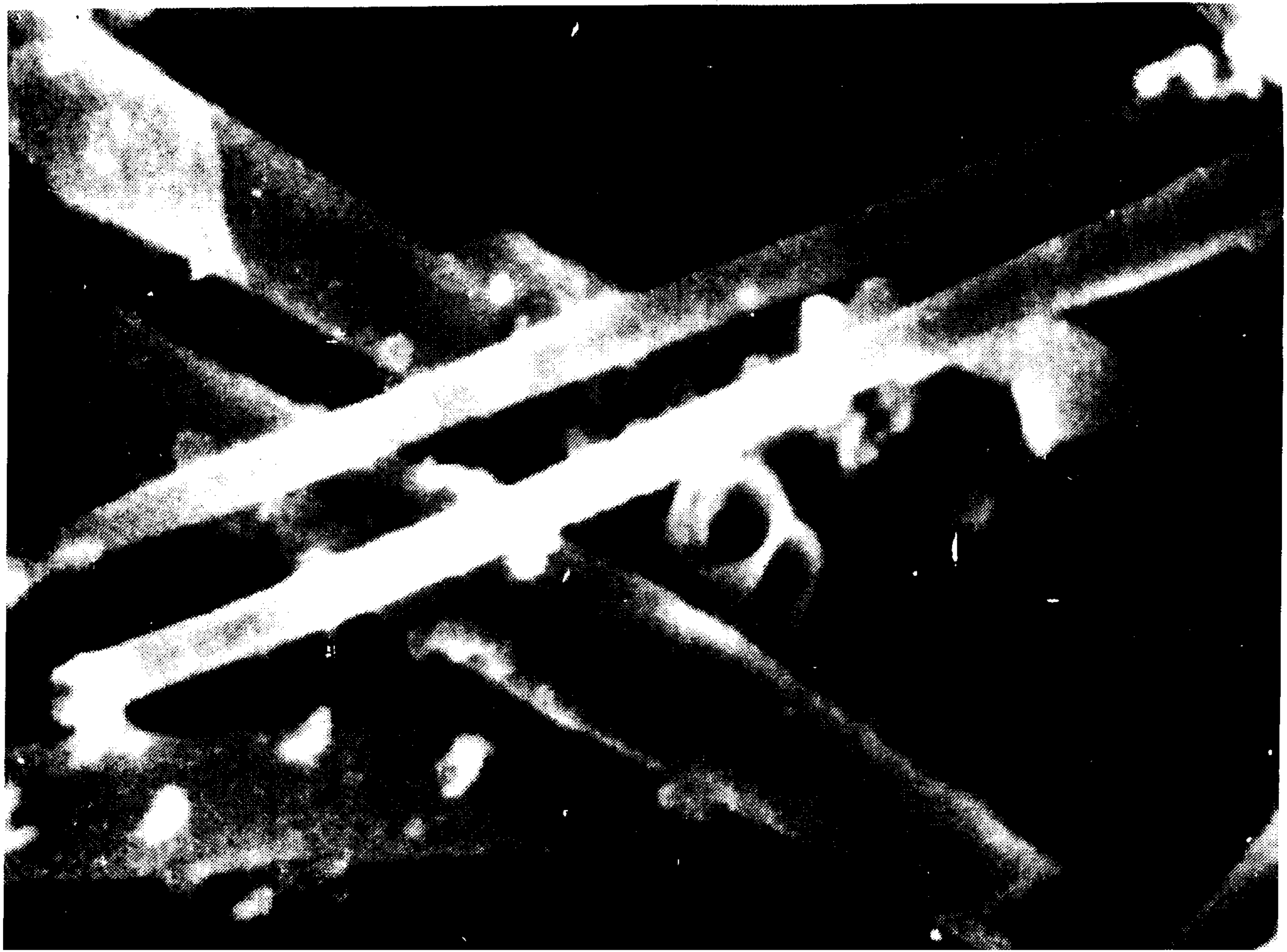
* PERMALLOY COATED TUBULES EXHIBIT MAGNETIZATION WHICH IS CLOSE TO THE BULK PERMALLOY VALUE

* A WIDE RANGE (4 - 40) OF PERMEABILITY HAS BEEN OBTAINED

* LARGE ANISOTROPY (FACTOR OF 8) OBSERVED FOR THE COMPOSITE, THE DEGREE OF ANISOTROPY CAN BE CONTROLLED

* SATURATION MAGNETIZATION SHOWS A LINEAR DEPENDENCE ON THE IRON/NICKEL RATIO OF PERMALLOY

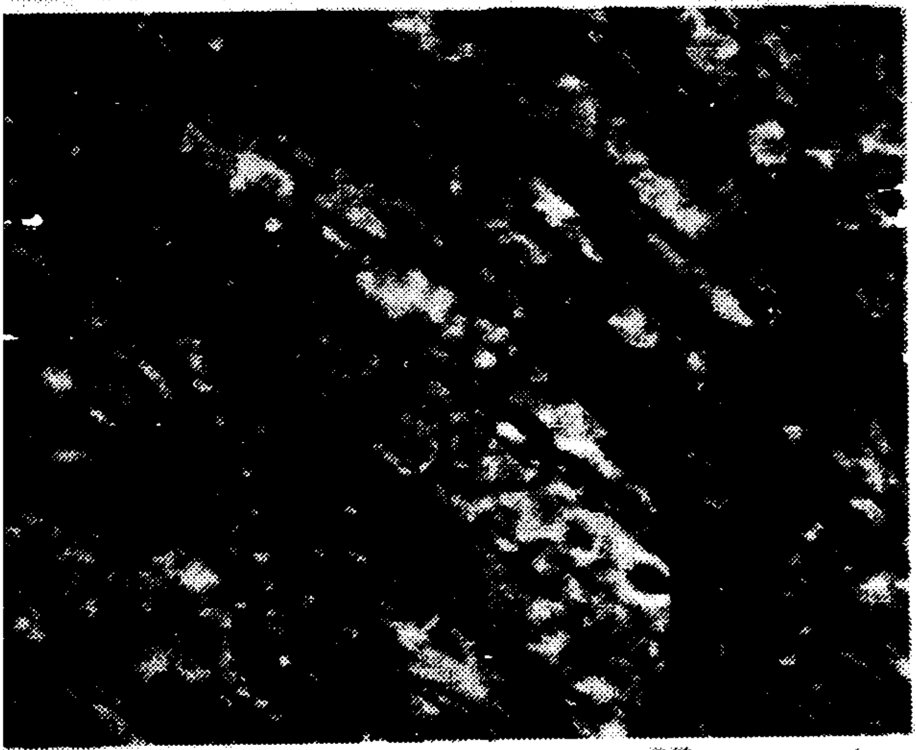

1.5mm LONG TUBULE — LAYERED GROWTH PROCESS
61μ



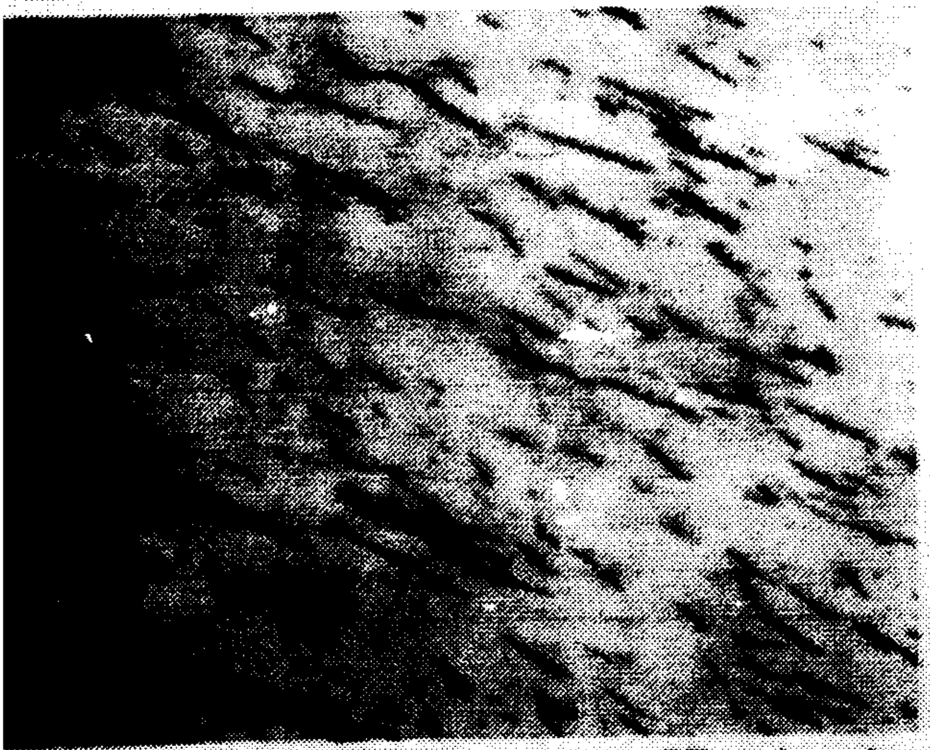
68

% BY WT. PERMALLOY TUBULES IN EPOX

AVERAGE TUBULE LENGTH ≈ 30µm



TEST SAMPLE SLICE



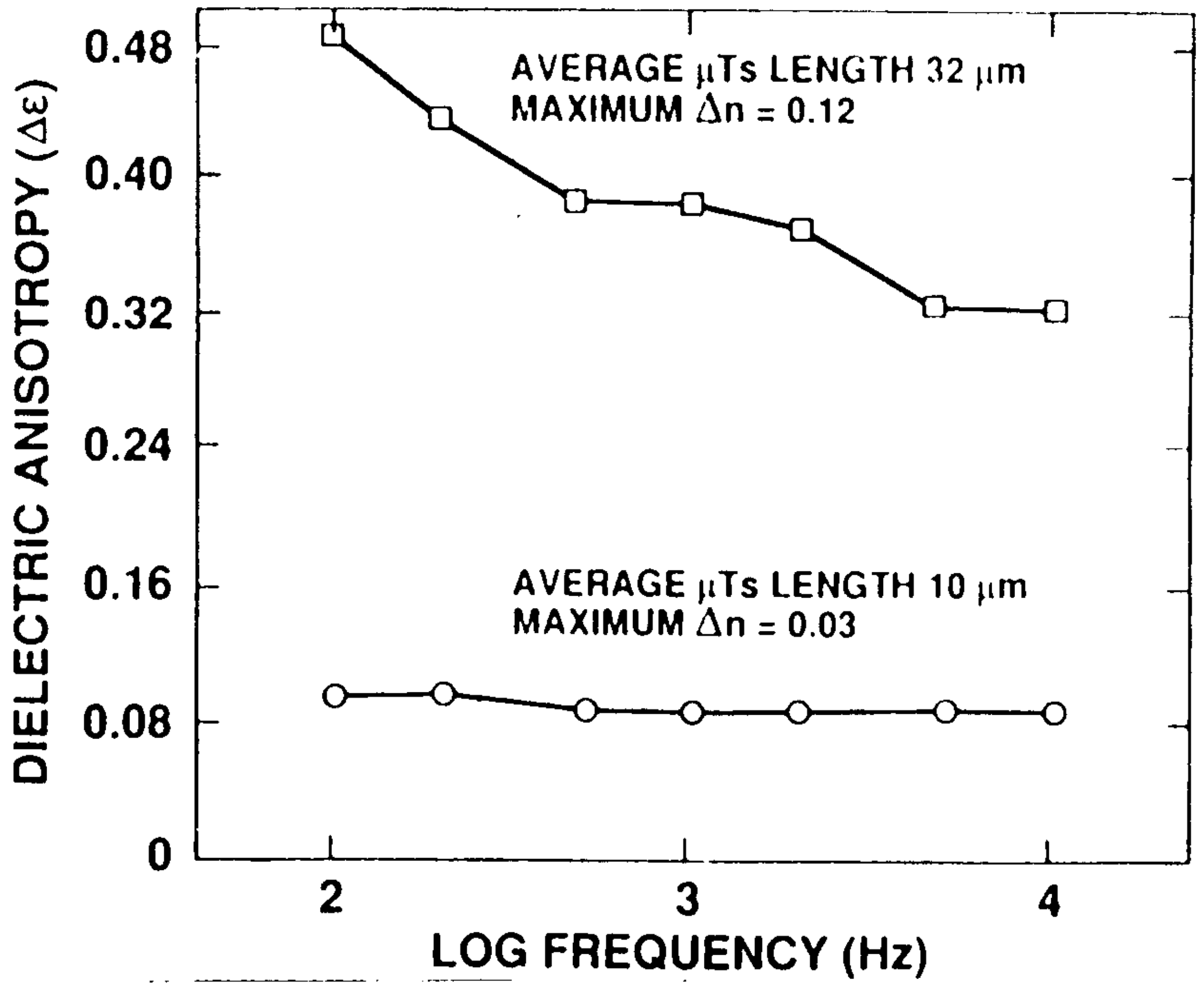
THIN FILM ON GLASS SU

FIGURE 1

DIELECTRIC ANISOTROPY OF PERMALLOY-COATED MICROTUBULES

HUGHES

0.25 wt % μTs IN PARAFFIN WAX OIL (19.6 mil) 2.0 kG MAGNETIC FIELD



TUBULES IN MAGNETIC MATERIALS

ONT

OBJECTIVE

Fabricate materials with controlled

- Magnetic Anisotropy
- Saturation magnetization
- Remanence, Coercivity
- Permeability
- Hysteresis behaviour

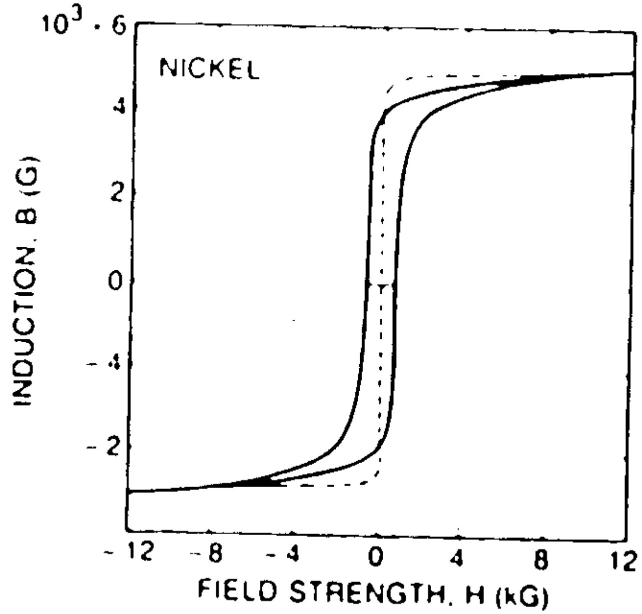
ACHIEVEMENTS

- Interesting shape anisotropy
- Low coercivity, high remanence
- Single domain tubule
- Thin film properties in bulk material

APPROACH

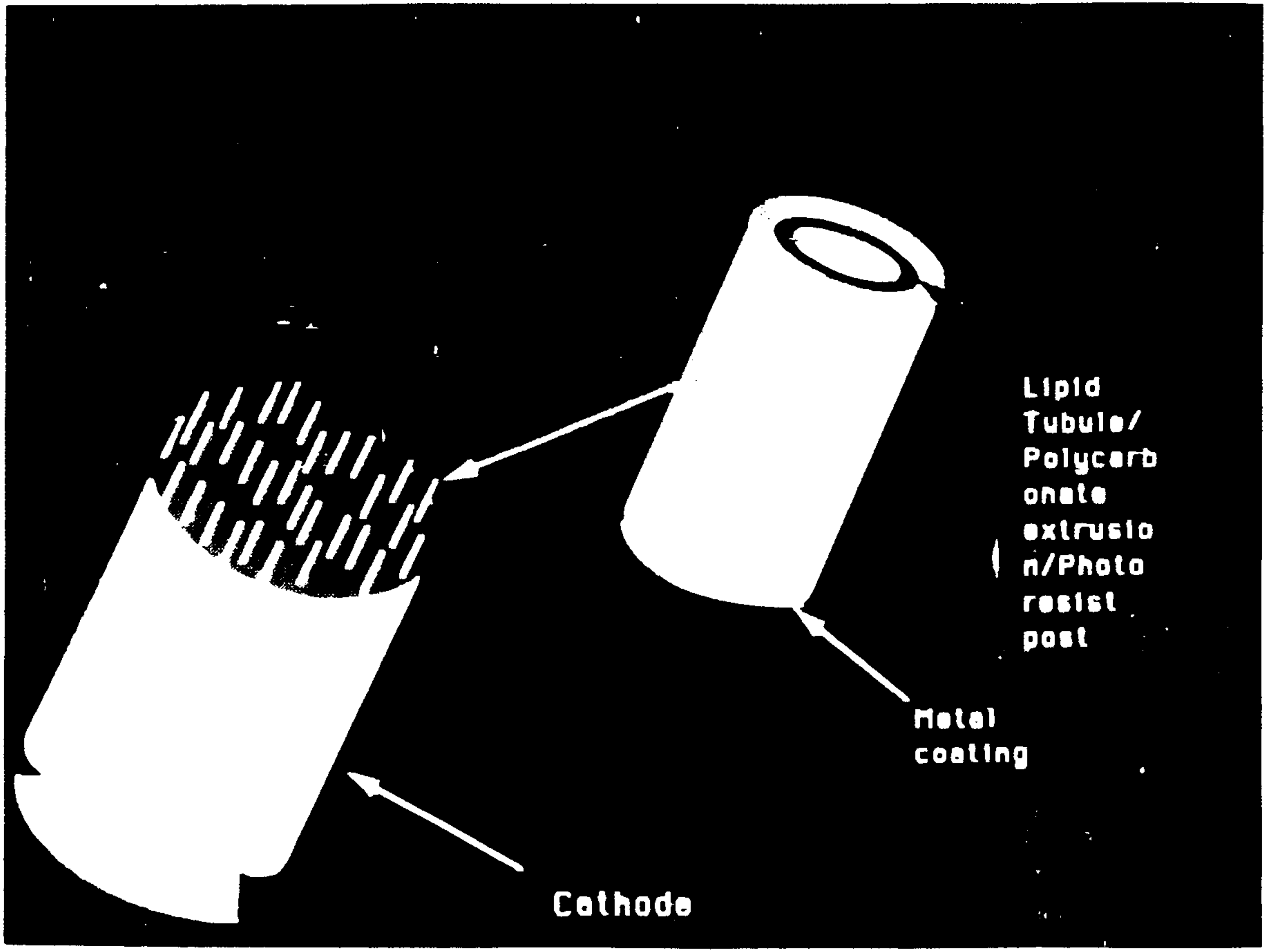
Oriented tubule composites with varying

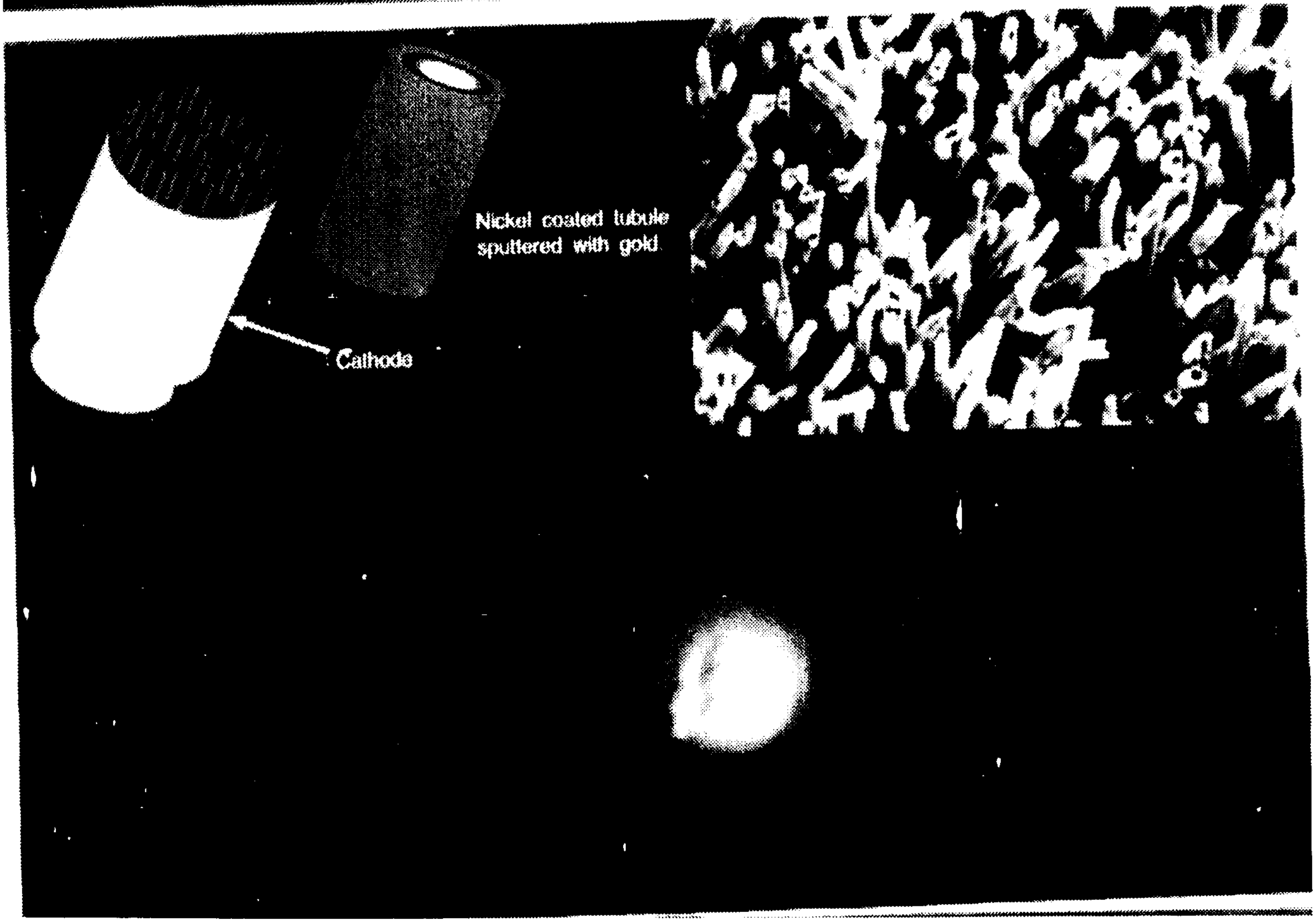
- Loading density and orientation
- Metal film thickness, grain size
- Tubule aspect ratio
- Plating in magnetic field
- Different films



HYSTERESIS LOOPS OF NICKEL AT ROOM TEMPERATURE

Tubule Based Field Emitting Cathode


Lipid
Tubule/
Polycarb
onate
extrusio
n/Photo
resist
post
Metal
coating
Cathode


Nickel coated tubule
sputtered with gold.
Cathode

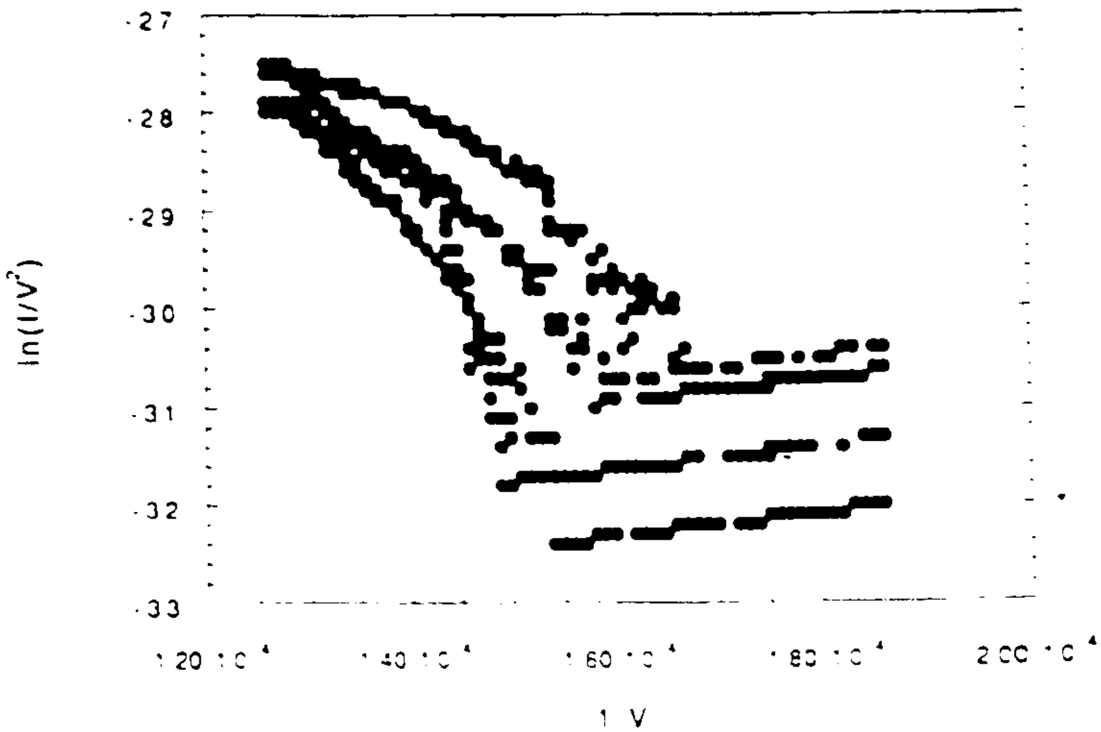
1 cm = 10 μm

(B) – 9 HRS – 5% Ni TUBULES @ 1000X

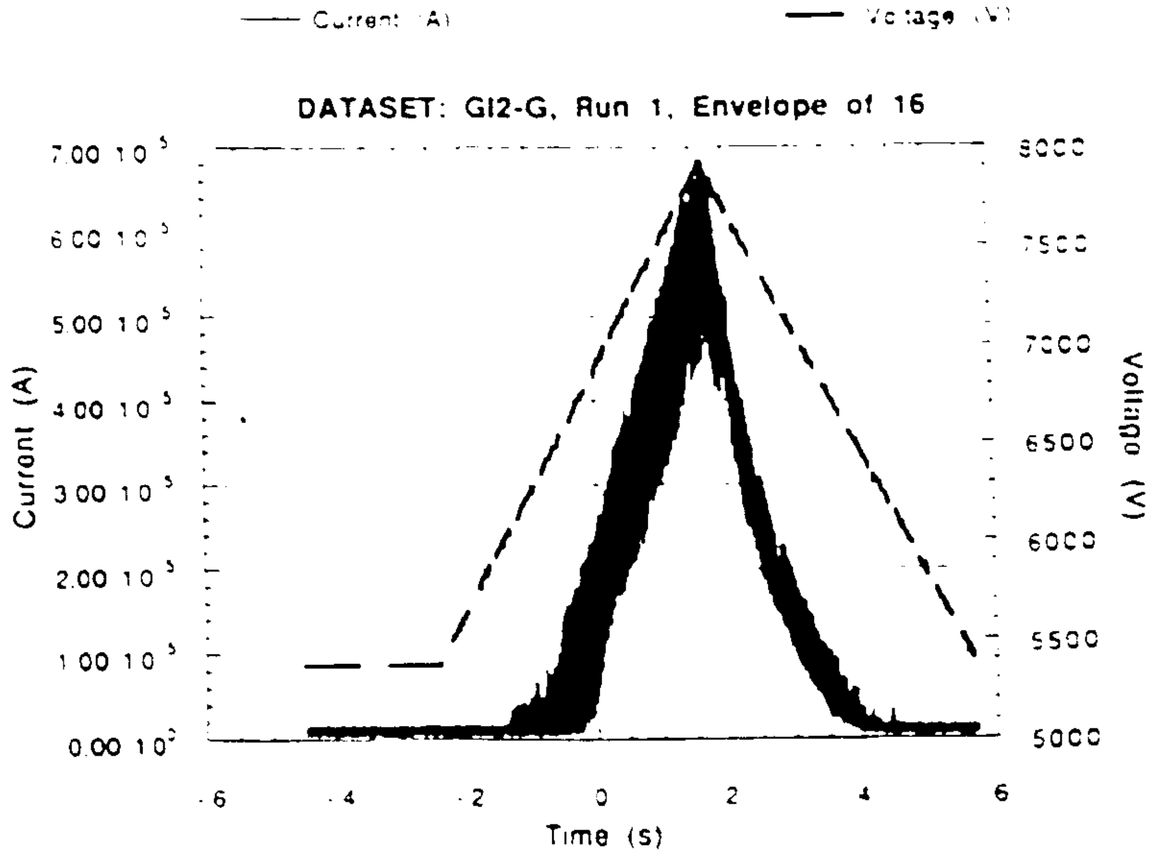
1 cm = 2 μm

(B) – 9 HRS – 5% Ni TUBULES @ 5000X

DATASET: GI2-G, Run 1, Envelope of 16



DATASET: GI2-G, Run 1, Envelope of 16



Tubule Based:

Long Term Controlled Release

APPLICATION AREAS

MARINE COATINGS

COOLING TOWERS

POWER PLANT COOLING WATER INTAKES AND
HEAT EXCHANGER

AGRICULTURAL CHEMICALS

BUILDING COATINGS

MACHINE COOLANTS AND LUBRICANTS

PLYWOOD ADHESIVES

PLASTICS AND VINYLS

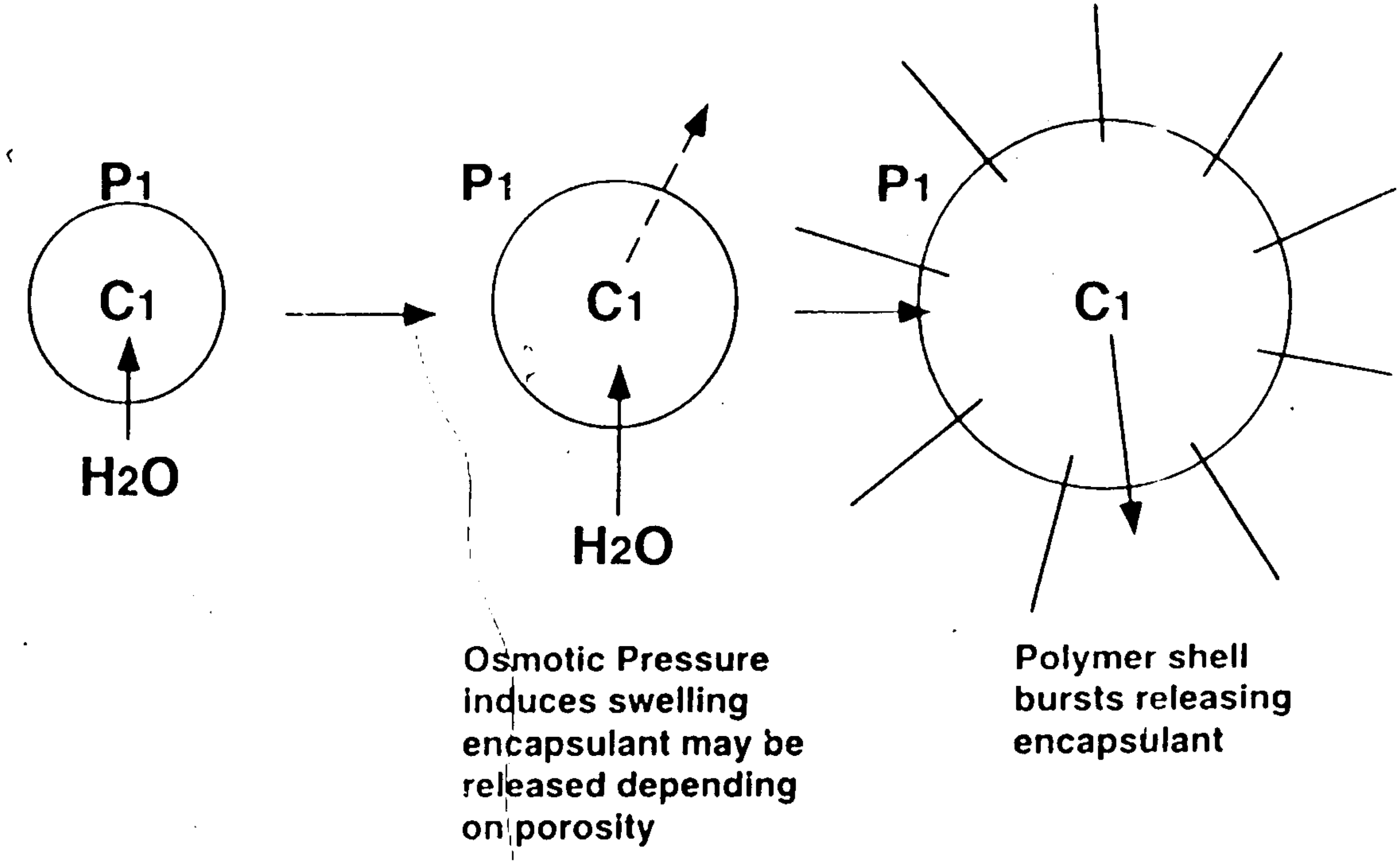
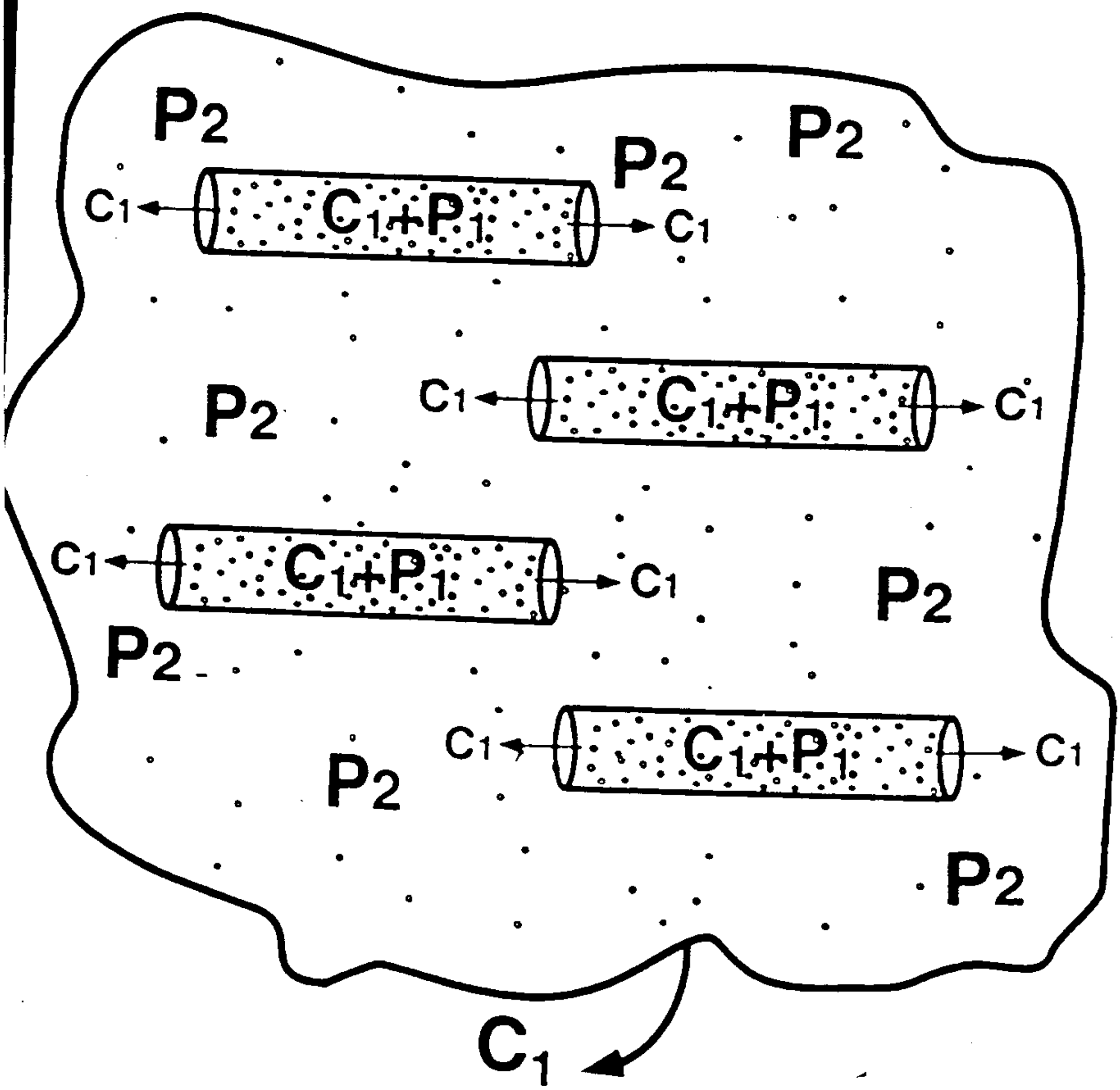


Figure 4 Cartoon showing release from spheres. Cartoon was prepared by



External Media

THEORY OF RELEASE FROM TUBULES

Monodisperse Length:

- **Parameters:**
  L = tubule length
  D = effective diffusion constant
  (determined by epoxy matrix)
  c_0 = initial concentration of solute in tubule

- **Diffusion equation:** $\dfrac{\partial c(x,t)}{\partial t} = D\dfrac{\partial^2 c(x,t)}{\partial x^2}$
- **Boundary conditions:** $c(0,t) = c(L,t) = 0$
- **Initial condition:** $c(x,0) = c_0$

- **Solution:** $$c(x,t) = \sum_{\substack{n=1 \\ n\ \text{odd}}}^{\infty} \frac{4c_0}{n\pi} e^{-n^2\pi^2 Dt/L^2} \sin\left(\frac{n\pi x}{L}\right)$$

- **Total amount released:** $$f(t) = c_0 L - \sum_{\substack{n=1 \\ n\ \text{odd}}}^{\infty} \frac{8c_0 L}{n^2\pi^2} e^{-n^2\pi^2 Dt/L^2}$$

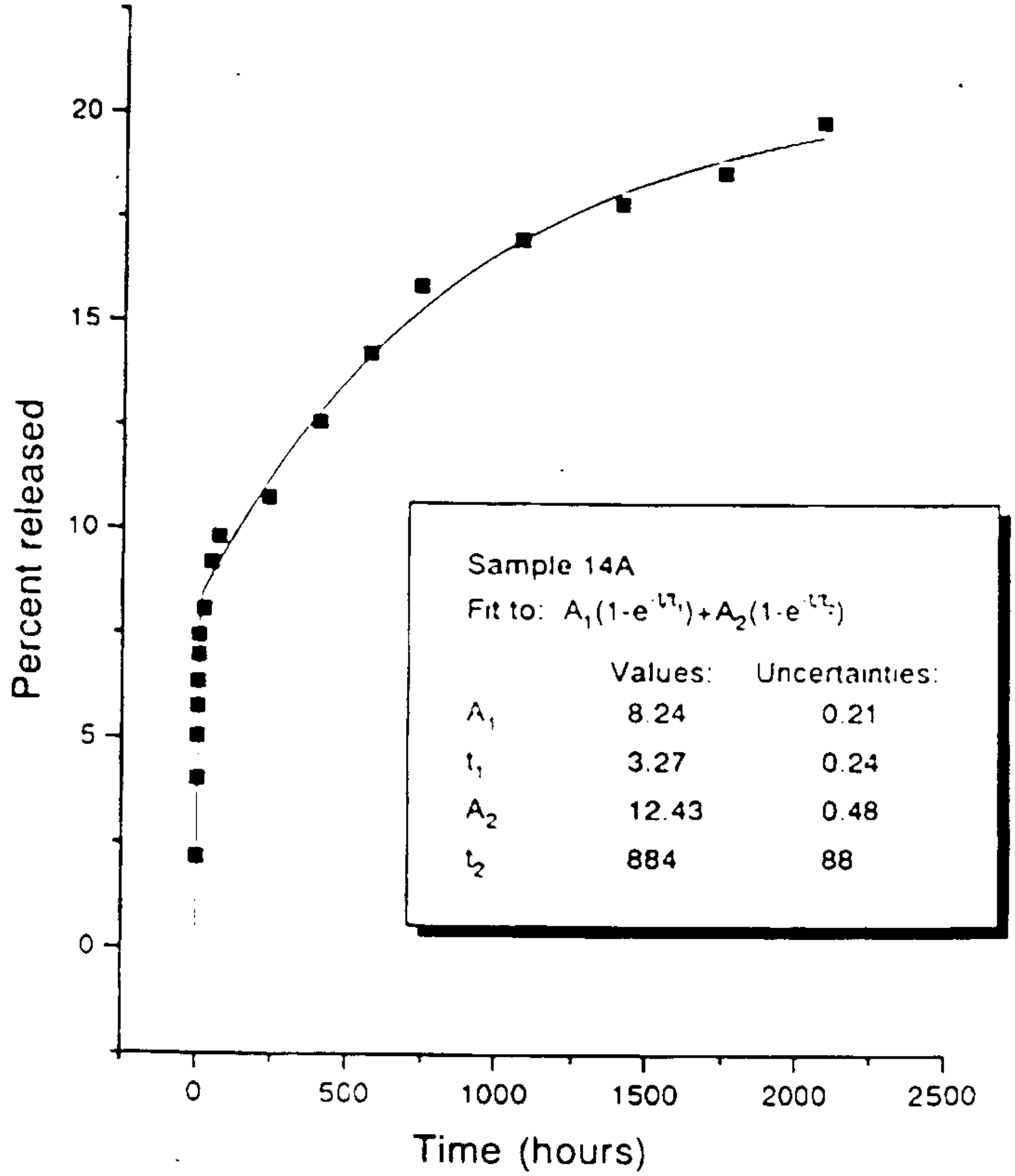
- **Long time: release looks like single exponential, release time** $\tau(L) = \dfrac{L^2}{\pi^2 D}$.

Polydisperse Mixture:

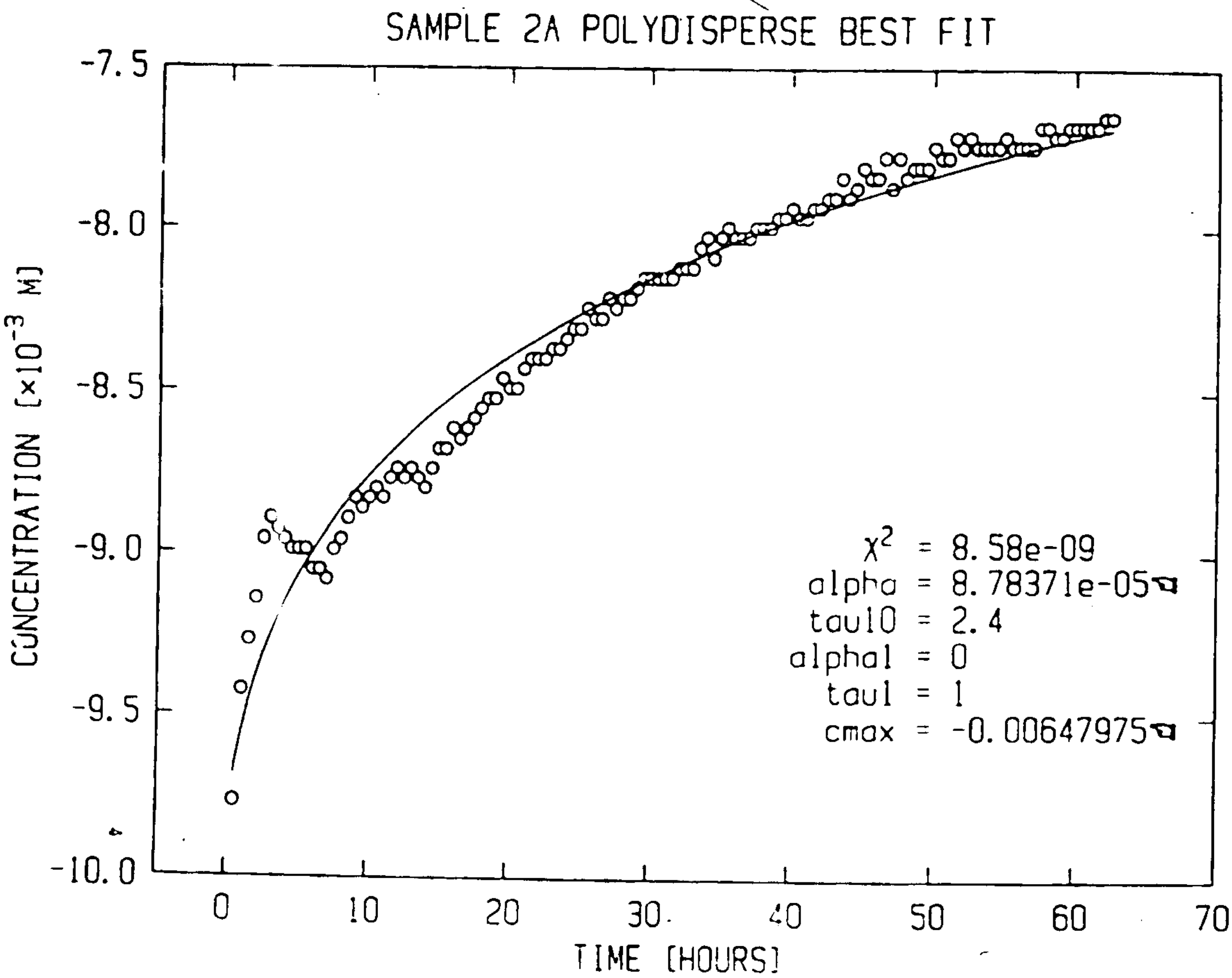
- **Distribution of lengths:** $\rho(L) = \dfrac{1}{L_0} e^{-L/L_0}$
- **Integrate over lengths to obtain amount released:**

$$f(t) = c_0 L_0 - \int_0^{\infty} dL \frac{1}{L_0} e^{-L/L_0} \sum_{\substack{n=1 \\ n\ \text{odd}}}^{\infty} \frac{8c_0 L}{n^2\pi^2} e^{-n^2\pi^2 Dt/L^2}$$

- **Long time: release looks like stretched exponential** $e^{-[(t/\tau(L_0))]^{1/3}}$.



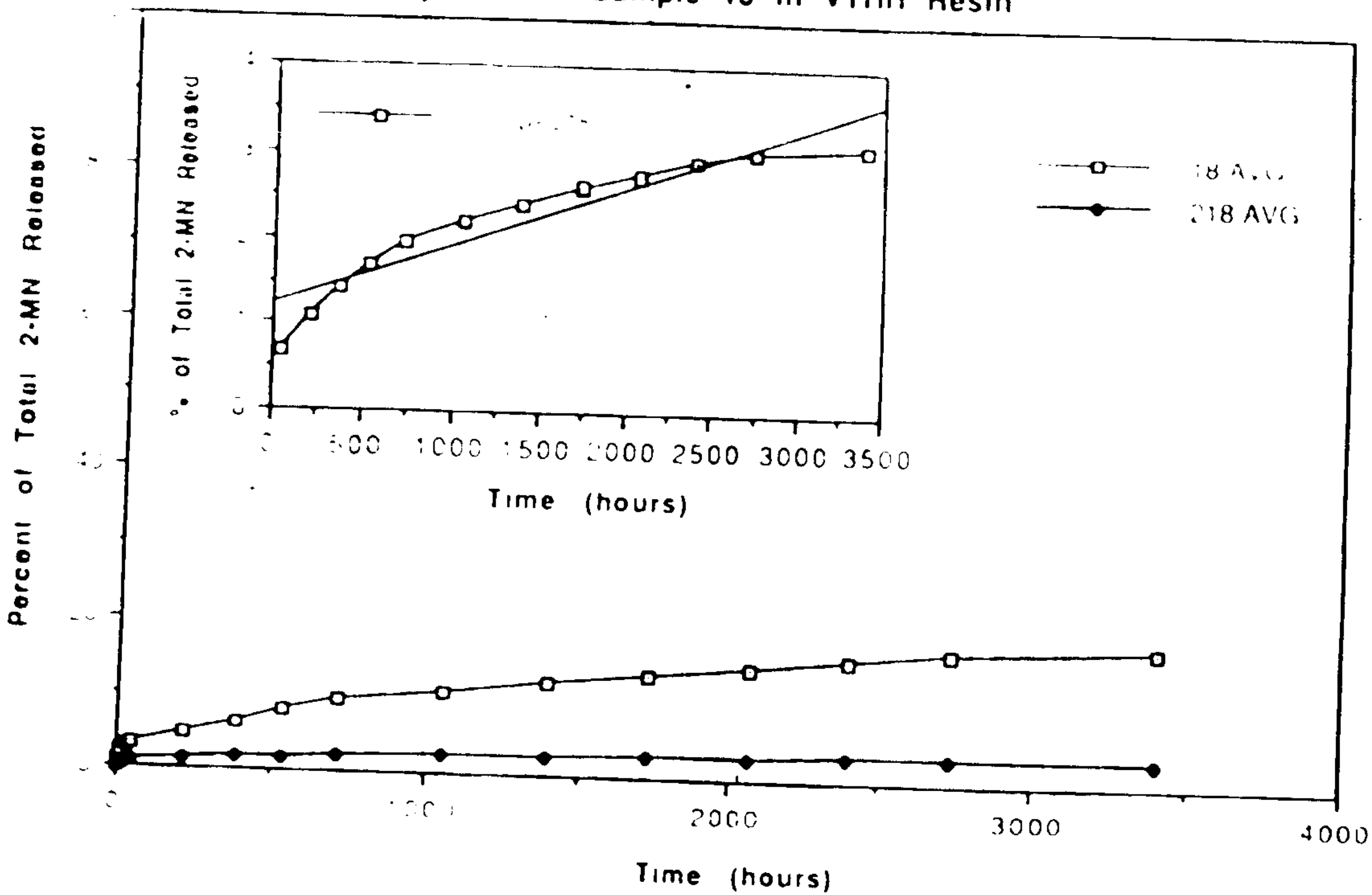
Sample 14A
Fit to: A1(1-e-t/τ1)+A2(1-e-t/τ2)
Values:
Uncertainties:
A1 8.24 0.21
t1 3.27 0.24
A2 12.43 0.48
t2 884 88
Percent released
Time (hours)
0
5
10
15
20
500
1000
1500
2000
2500



SAMPLE 2A POLYDISPERSE BEST FIT
CONCENTRATION [×10^-3 M]
TIME [HOURS]
-7.5
-8.0
-8.5
-9.0
-9.5
-10.0
0
10
20
30
40
50
60
70
χ² = 8.58e-09
alpha = 8.78371e-05
tau10 = 2.4
alpha1 = 0
tau1 = 1
cmax = -0.00647975

Release of 2-MN

Sample 18 - Polymeric cap (Epon SurwelR)
Sample 218 - Sample 18 in VYHH Resin



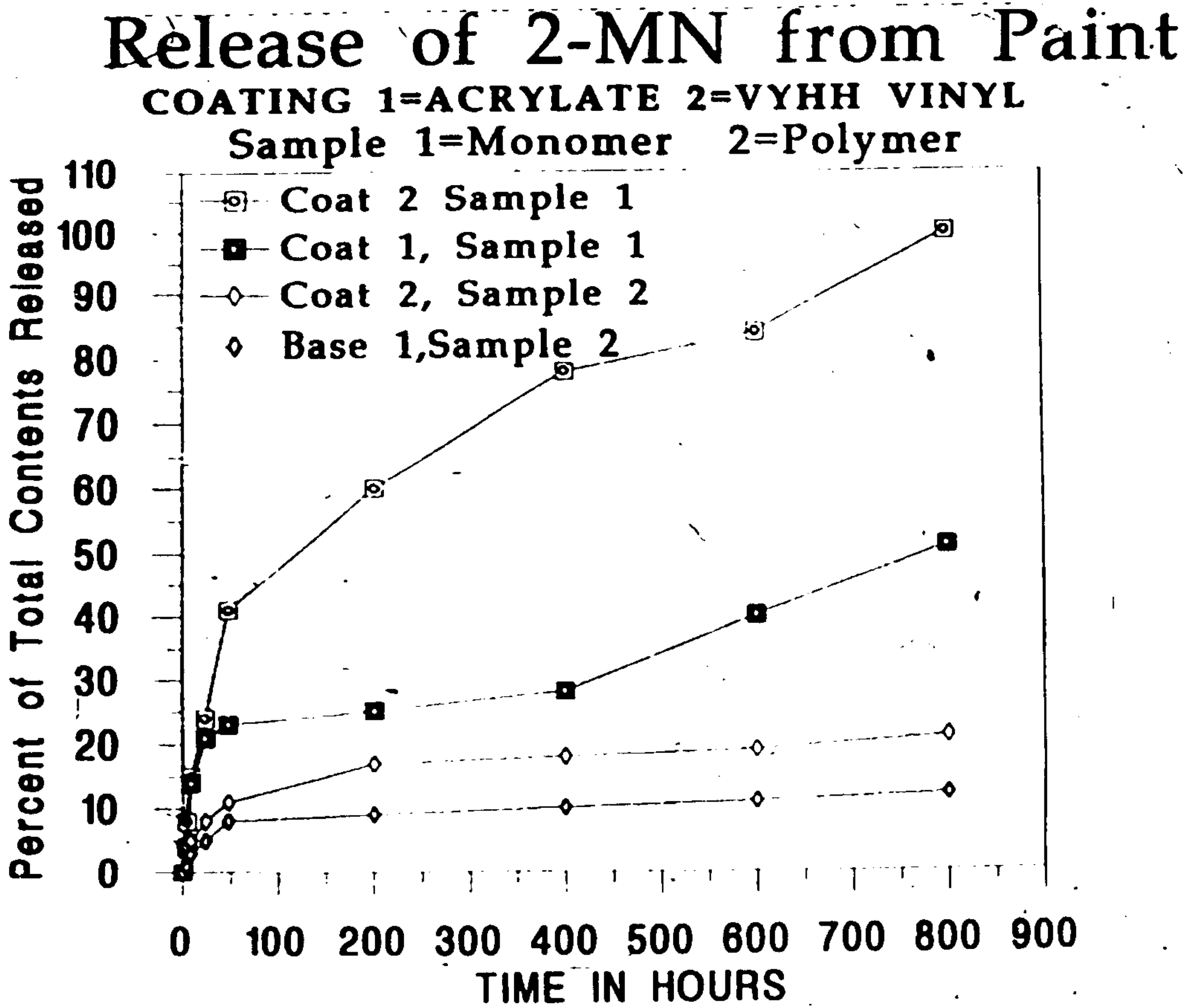


Figure 6 Graphic demonstration of an experiment studying the effect of varying the polymer (P1) on release rates

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