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**Development of Bioelastic Material
for Aspects of
Wound Repair**

Contract No. N00014-90-C-0265

TRIANNUAL PROGRESS REPORT FOR THIRD TRIMESTER OF YEAR 3
(May through August, 1993)

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TRIANNUAL PROGRESS REPORT FOR SECOND TRIMESTER OF YEAR 3
(May through August, 1993)

A. PURPOSE OF THE CONTRACT:

Broad Goal: The synthesis and characterization of bioelastic materials designed for specific applications of wound healing.

Specific Goals: For materials with possible use as wound coverings, control of the temperature of transition by changes in hydrophobicity will be examined. In addition, the rate of water passage through the matrix will be determined. The research approach is the stepwise coordination of the synthesis and characterization of the materials. The first application phase was concerned primarily with synthesis of the basic polypentapeptide poly(VPGVG) and the analog with L-alanine substituted in position 3, poly(VPAVG) and mole fraction combinations thereof to achieve elastomeric matrices of varying elastic moduli. *And in the third year polypentapeptides containing chemical clocks are to be characterized for their rate of breakdown and their effects on drug release profiles.*

Peptide Syntheses: The three sets of syntheses are:

- 1) Synthesis of the basic polypentapeptides, poly(VPGVG) and poly(VPAVG) and mole fraction combinations thereof, $\text{poly}[f_{A3}(\text{VPAVG}), f_{G3}(\text{VPGVG})]$, and compounding of these polymers to fabrics such as gauze.
- 2) Synthesis of analogs of poly(VPAVG) with different hydrophobicities.
- 3) Synthesis of analogs with functional side chains to achieve chemical control of contraction under isothermal conditions.
- 4) *Synthesis of polymers containing chemical clocks to gain control of the rate of degradation.*

Physical Studies: The physical studies include:

- 1) Determination of the temperatures of transition.
- 2) Evaluation of the properties of aerosol sprays and foams on 37 °C surfaces and cavities.
- 3) Determination of the rates of water loss through the synthetic elastic matrices or membranes (for those materials with potential for use as wound coverings or as vascular sleeves).
- 4) Mechanical studies including: stress strain, temperature dependence of length at constant force, temperature dependence of force at constant length and rates of contraction.
- 5) *Determination of the relative rates of degradation in relevant media.*
- 6) *The release of model drugs from cross-linked matrices and coacervates with and without chemical clocks*

B. PROGRESS

1. Syntheses Completed or Begun in the Third Trimester of YEAR 03:

The synthesis of 35 grams of poly(AVGVP) has been completed. Another 30 grams of poly(GVGIP) are in final purification in addition to the first 35 grams. These syntheses are to be used to determine the biocompatibility of these polymers. Thirty-three patches of poly(AVGVP) are ready to be sent to NAmSA for biocompatibility testing.

Different ratios of poly[f_V (GVGVP), f_F (GFGVP)] have been synthesized to determine the matrices of increased strength, namely

1. poly[0.92(GVGVP),0.08(GFGVP)]
2. poly[0.84(GVGVP),0.16(GFGVP)]
3. poly[0.76(GVGVP),0.24(GFGVP)]
4. poly[0.67(GVGVP),0.33(GFGVP)]

These four polymers are in the final stages of purification.

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2. Physical Characterizations

a. Determination of Transition temperatures T_t

The transition temperatures for CG149WR, CG150WR, CG156WR and CG158WR are 26.6°C, 23.0°C, 32.2°C and 9.9°C, respectively.

b. Polymers CG149WR and CG150WR have been cross-linked and are awaiting stress/strain characterization.

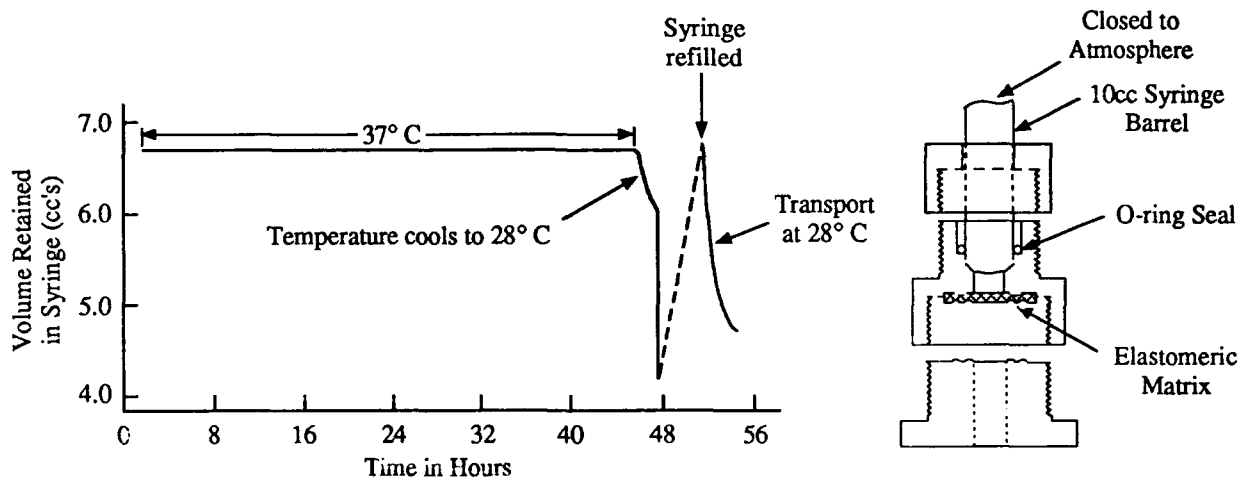
c. Water transport studies have been carried out and the results reported below.

H₂O Transport poly(GVGVP) at atmospheric pressure at 37°C.

<u>Elapsed Time</u>	<u>Total Volume Transported</u>		
0 days	0.0 cc's	05/07/93	(9.0 cc)
10 days	0.4 cc's	05/17/93	(8.6 cc)
19 days	0.4 cc's	05/26/93	(8.6 cc)
32 days	0.6 cc's	06/08/93	(8.4 cc)
48 days	1.0 cc's	06/24/93	(8.0 cc)

H₂O Transport poly(GVGVP) with Applied Pressure

Water Transport Across a Disk of Dimensions 1.314 cm² and Thickness of 0.038 cm at 100 mm Hg



C. FUTURE STUDIES

1. Biocompatibility of poly(AVGVP) and poly(GVGIP).
2. Cross-linking and dependence of elastic modulus (strength) for X^{20} -poly[f_V (GVGVP), f_F (GFGVP)]
3. Further H₂O transport studies:
 X^{20} -poly(GVGIP) and X^{20} -poly(AVGVP)
4. Cross-linking and dependence of elastic modulus (strength) of
poly[0.9(AVGVP),0.1(AFGVP)] and
poly[0.8(AVGVP),0.2(AFGVP)] and
5. Synthesis of poly[0.9(GVGIP),0.1(GNGIP)], poly[0.8(GVGIP),0.1(GNGIP)], and
poly(GVGIP) for determining rate of degradation in TP τ studies and in intraperitoneal (IP)
implants.

D. MANUSCRIPT PREPARED SINCE LAST REPORT (Copy Attached):

Luke A. Strzegowski, Manuel Bueno Martinez, D. Channe Gowda, Dan W. Urry and David A. Tirrell, "Photomodulation of the Inverse Temperature Transition of a Modified Elastin Poly(pentapeptide)," *J. Am. Chem. Soc.* 1993 (submitted).

PHOTOMODULATION OF THE INVERSE TEMPERATURE
TRANSITION OF A MODIFIED ELASTIN
POLY(PENTAPEPTIDE)

by

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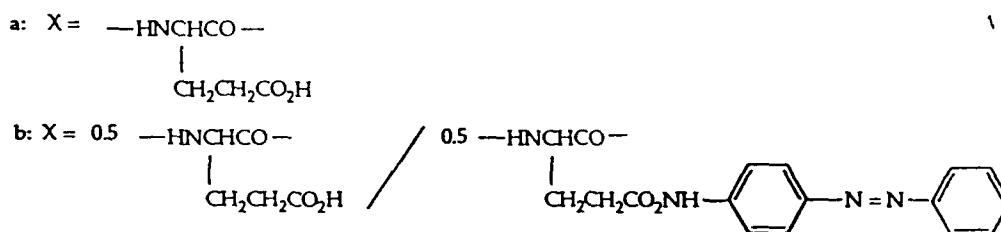
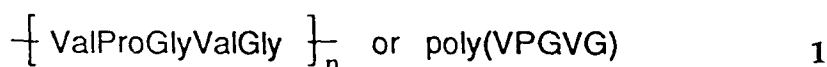
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The inverse temperature transition exhibited by elastin-like polypeptides has been shown to provide a basis for an intriguing array of energy transduction processes.¹ For example, the elastin poly(pentapeptide) **1** can be fabricated into cross-linked gels that remain swollen in water at temperatures below 25°C, but then deswell, or contract, upon a rise in temperature. Modification of sequence **1** has produced a series of related polypeptides that undergo phase mixing or swelling transitions in response to changes in pH,² ionic strength,³ or pressure,⁴ oxidation/reduction,⁵ or upon enzymatic phosphorylation.⁶ We report herein the photomodulation of the inverse temperature transition of the modified elastin polypeptide **2b**. This development provides a route to protein-based polymeric materials and gels capable of photomechanical transduction.⁷



where $n \geq 120$ and f_v and f_x are mole fractions with $f_v + f_x = 1$.

Structure **2a** was synthesized as previously described⁸ and verified by nuclear magnetic resonance. The mole fractions of pentamers were determined by amino acid analyses to be $f_v = 0.68$ and $f_x = 0.32$, i.e., **2a** may be represented as poly[0.68(VPGVG), 0.32(VPGEG)].

The photosensitive copolypeptide **2b** was prepared in the following manner. Copolypeptide **2a** (31.6 mg., 0.019 mmol of $\text{—CO}_2\text{H}$) was dissolved in 2 mL of N,N-dimethylformamide (DMF). To this solution was added 8.2 mg (0.042 mmol) of phenylazoaniline, 6.6 mg (0.049 mmol) of hydroxybenzotriazole, and 8.2 mg (0.040 mmol) of

dicyclohexylcarbodiimide. The solution was stirred for 3 days at room temperature, and 1 drop of 1M acetic acid was added to facilitate precipitation of the dicyclohexylurea by product. The precipitate was removed by centrifugation and the polymer was recovered by precipitation into excess diethyl ether, washed repeatedly with ether, and then dried overnight at 40°C. The yield was 28.2 mg (89%). Thin layer chromatography revealed no contamination by unconjugated phenylazoaniline and the ultraviolet absorption spectrum indicated amidation of 55% of the glutamic acid side chains of **2a**.^{9,10}

Figure 1 shows the changes in the electronic absorption spectrum of **2b** that occur upon irradiation of a 0.5 wt% solution of the copolymer in phosphate-buffered saline (0.15 N NaCl/0.01 M sodium phosphate, pH 3.5). The dark-adapted copolymer exhibits the expected absorption spectrum of the *trans* azobenzene chromophore, with absorption maxima at 348 nm and 428 nm (curve a). Irradiation at 350 nm (Rayonet minireactor, four 350 nm lamps) results in reduction in the intensity of the 348 nm absorption band, with the photostationary state being reached in ca. 45 sec under the conditions of this experiment (curves b-d).¹¹ Further irradiation from a longer wavelength source (a Sunpak Thyristor Auto 522 electronic flash unit with the window removed) restores the 348 nm absorption, via partial photoreversion to the *trans* form of the chromophore (curves e and f). The state represented by curve f does not change upon further irradiation, and is estimated to consist of ca. 50% *trans* and ca. 50% *cis* chromophore. The tight isosbestic points at 280 and 425 nm indicate that the *cis-trans* interconversion occurs without significant degradation of the chromophore.

Figure 2 shows that the inverse temperature transition of **2b** is sensitive to the configuration of the azobenzene chromophore. Phase separation of the polymer, as reported by an abrupt increase in the turbidity of the sample, occurs at ca. 32°C for the *trans* form and at ca. 42°C for the *cis* form of **2b** when buffered at pH 4.1. Elevation of the transition temperature upon *trans-to-cis* photoisomerization is consistent with the increased dipole moment of the *cis*

azobenzene isomer¹² and with the established correlation between the polarity of the side chain and the temperature at which phase separation is observed.^{13,14}

The shift in phase transition temperature from 32°C to 42°C upon *trans-to-cis* isomerization opens a window, near 40°C, for photomodulation of the transition at a constant pH of 4.1. Figure 3 illustrates this phenomenon. At 40°C, the relatively hydrophobic *trans* form of the polymer affords turbid biphasic suspensions. Irradiation at 350 nm results in conversion to the 70% *cis* form, with corresponding dissolution of 2b and decreasing sample turbidity. Further irradiation from the longer wavelength source reforms ca. 50% of the hydrophobic *trans* isomer and drives a second cycle of phase separation. Thermal reversion of the *cis* isomer under these conditions is negligible, and the process appears to be fully reversible under photocontrol.

These results illustrate that attachment of one azobenzene chromophore in ca. forty amino acid residues is sufficient to render photosensitive the inverse temperature transition of elastin-like polypeptides. This phenomenon can be exploited to achieve reversible photomodulation of the transition at 40°C. Extension to crosslinked gels and photomechanical transduction processes in protein-based polymers is being pursued.

ACKNOWLEDGMENTS

This work was supported in part by the NSF Materials Research Laboratory at the University of Massachusetts and by Contract Nos. N00014-90-C-0265 and N00014-89-J-1970 from the Department of the Navy, Office of Naval Research. Luke A. Strzegowski held a fellowship provided by the Research Experiences for Undergraduates program of the MRL. Manuel Bueno Martinez was a Visiting Scientist from the University of Seville, Spain and was supported by his home government.

REFERENCES AND NOTES

1. Urry, D.W. *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 819-841.
2. Urry, D.W.; Haynes, B.; Zhang, H.; Harris, R.D.; Prasad, K.U. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, 85, 3407-3411.
3. Urry, D.W.; Harris, R.D.; Prasad, K.U. *J. Am. Chem. Soc.* **1988**, 110, 3303-3305.
4. Urry, D.W.; Hayes, L.C.; Gowda, D.C.; Parker, T.M. *Chem. Phys. Lett.* **1991**, 182, 101-106.
5. Urry, D.W.; Hayes, L.C.; Gowda, D.C.; Harris, C.M.; Harris, R.D. *Biochem. Biophys. Res. Comm.* **1992**, 188, 611-617.
6. Pattanaik, A.; Gowda, D.C.; Urry, D.W. *Biochem. Biophys. Res. Commun.* **1991**, 178, 539-545.
7. Photomechanical transduction has been reported previously in modified poly(N-isopropylacrylamide) gels (Irie, M. *Pure and Appl. Chem.* **1990**, 62, 1495-1502)
8. Urry, D.W.; Peng, S.Q.; Parker, T.M. *Biopolymers* **1992**, 32, 373-379.
9. The molar extinction coefficient reported by Fissi and Pieroni (Fissi, A.; Pieroni, O. *Macromolecules* **1989**, 22, 1115-1120) for poly(L-glutamic acid) containing 85 mol % azobenzene units in the side chains was used to estimate the degree of amidation.
10. The absence of observable phenylazoaniline from the thin layer chromatogram limits the amount of the unconjugated chromophore to less than 0.5% of the amount bound to the polypeptide.

11. The photostationary state consists of ca. 30% of the *trans* and 70% of the *cis* forms of the chromophore under these conditions of irradiation (Ferritto, M.S.; Tirrell, D.A. *Biomaterials* **1990**, 11, 645-651).
12. Brown, C. *Acta Crystallogr.* **1966**, 21, 146-152.
13. Urry, D.W.; Luan, C.H.; Parker, T.M.; Gowda, D.C.; Prasad, K.U.; Reid, M.C.; Safavy, A.; *J. Am. Chem. Soc.* **1991**, 113, 4346-4348.
14. Urry, D.W.; Gowda, D.C.; Parker, T.M.; Luan, C.H.; Reid, M.C.; Harris, C.M.; Pattanaik, A.; Harris, R.D. *Biopolymers* **1992**, 32, 1243-1250.

FIGURE 1. Electronic absorption spectra of **2b** after irradiation at 350 nm or from the electronic flash unit: (a) dark-adapted 24 hrs; (b) 5 sec at 350 nm; (c) 20 sec at 350 nm; (d) 45 sec at 350 nm; (e) 15 flashes at 1 flash/sec; (f) 45 flashes at 1 flash/sec.

FIGURE 2. Temperature-dependent turbidity of aqueous samples of **2b** ($c=5$ mg/ml in pH 4.1 phosphate buffered saline solution). Transmission values obtained on a Beckman DU-7 spectrophotometer with a Lauda K-4/PD circulating bath: ($\square$) dark-adapted 24 hrs; ($\blacklozenge$) flashed 45 times.

FIGURE 3. Photomodulation of phase separation of aqueous samples of **2b** ($c=5$ mg/ml in pH 4.1 phosphate buffered saline solution, 40°C). 70% cis samples were prepared by irradiation for 5 min at 350 nm; 50/50 sample by irradiation with 50 flashes from the electronic flash unit. Hatched intervals represent periods of irradiation.

Figure 1

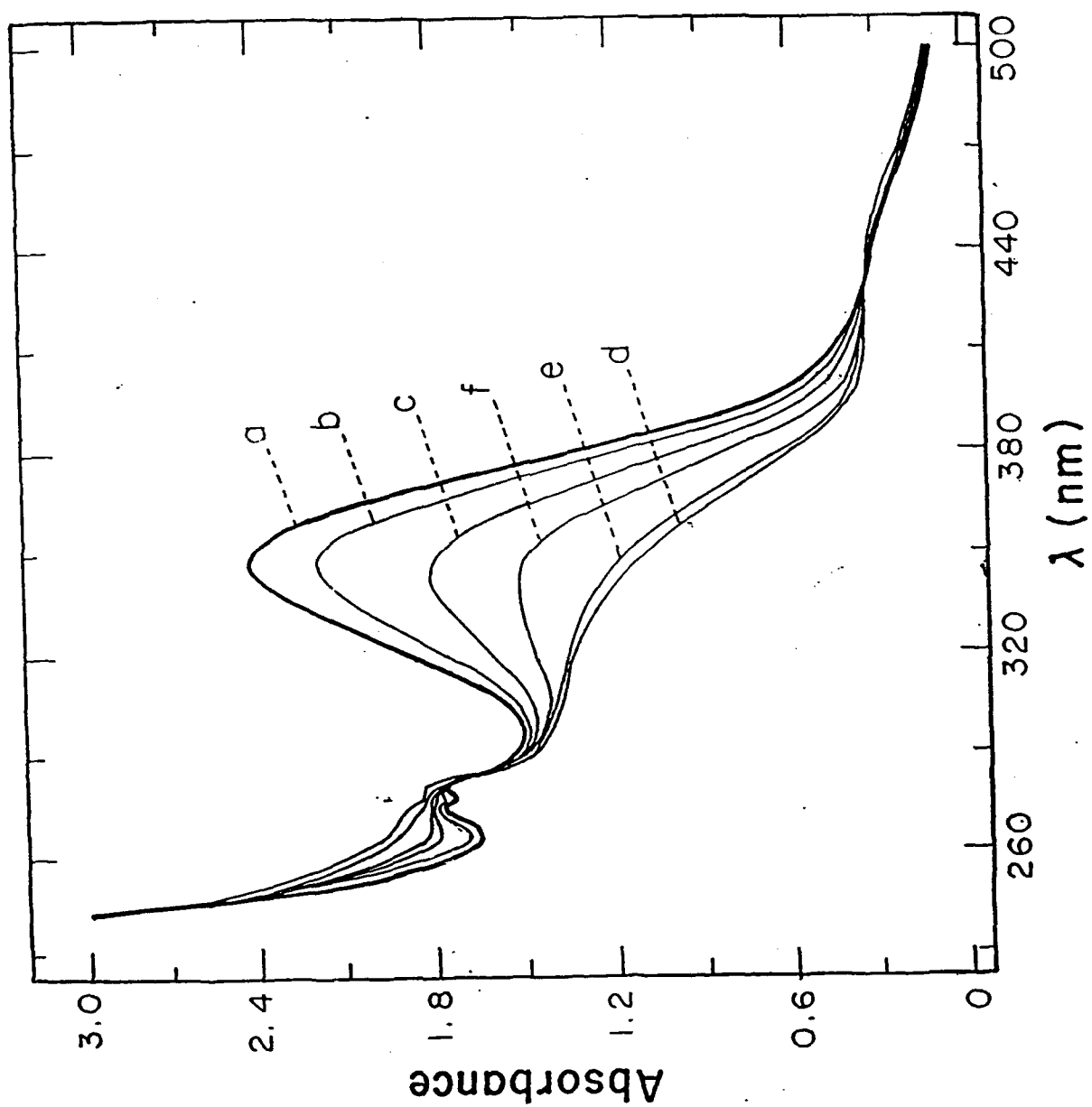


Figure 2

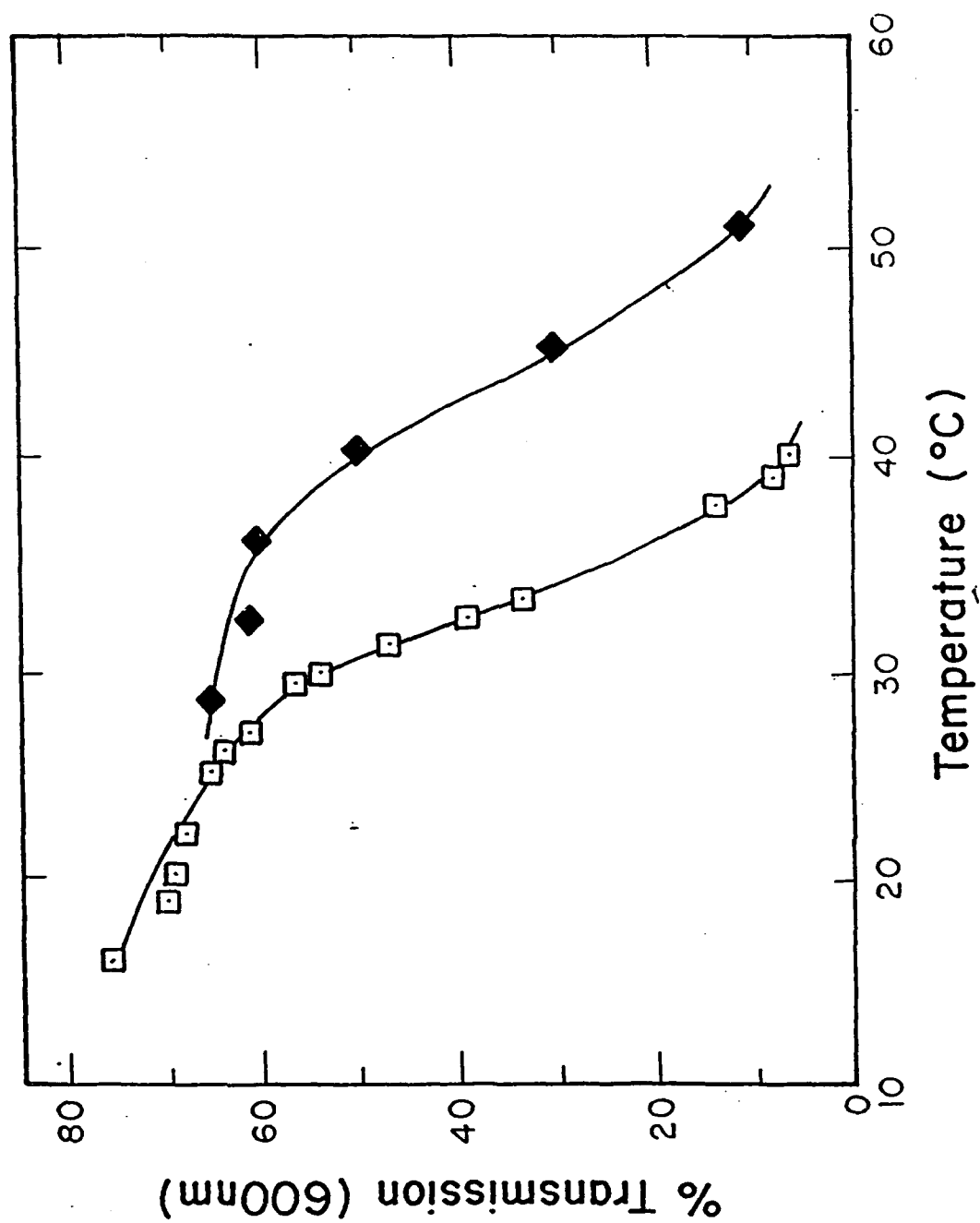


Figure 3

