0273–2289/84/9000–0073$02.00

Radiopolymerized Mixture of Acrylic Acid, Methyl Methacrylate, and Polyethylene Glycol as an Enzyme Support System

K. PISKIN,[1] E. ARCA,[2] AND E. PISKIN[2,*]

[1]*Department of Biology and* [2]*Department of Chemical Engineering, Hacettepe University, Ankara, Turkey*

Received November, 1983; Accepted December, 1983

ABSTRACT

An enzyme support system is prepared by dropping a mixture of acrylic acid, methyl methacrylate, polyethylene glycol (mw, 1000) and enzyme into supercooled petroleum ether followed by γ-radiation. The particles obtained by this procedure are spherical (1.5 mm in diameter) in shape and are in the form of a white gel. After radiation, the spherical polymer granules are placed in a vessel and the noncrosslinked polyethylene glycol is dissolved in water in order to achieve a porous gel structure. To test the feasibility of using this system as an enzyme support, urease is immobilized by optimizing the radiation dose and ratio of components. Activity yield of enzyme is found to be 30–80% of the native enzyme. The highly porous structure of this system offers the advantage of enabling a large enzyme loading per gram polymer. Besides this, the gel form leads to the achievement of high rates of diffusion into the particles. It is thus reasonable to conclude that these hard, spherical gel particles might be a suitable support system for enzyme immobilization.

INTRODUCTION

Recently, the mobilization of enzymes and of microbial cells has become the subject of increased interest (*1*). There are four fundamental methods of immobilization: (i) chemical binding, with a covalent bond

*Author to whom all correspondence and reprints request should be addressed.

between enzyme and carrier; (ii) ionic or physical adsorption; (iii) microencapsulation; and (iv) a physical trapping or composing method for a biocomponent with the matrix. The radiation crosslinking and the low temperature-supercooled phase polymerization are very convenient means for physical composing immobilization (2,3). We have prepared the immobilized enzyme in a bead-shaped form by dropping the enzyme–acrylic acid, methyl methacrylate, and polyethylene glycol solution into supercooled petroleum ether, followed by polymerization with radiation. Urease is used as a model enzyme to test the immobilization capacity of this system. This paper reports the preparation and properties of this polymer–enzyme conjugate.

MATERIALS AND METHODS

The procedure for preparation of bead-type gel particles is given in Fig. 1 schematically. In this procedure acrylic acid, methyl methacrylate, and polyethylene glycol (mw, 1000) are mixed in different ratios. This mixture is then heated to increase the viscosity of the mixture. Urease, dissolved in phosphate buffer (pH 7) is added to this mixture and the mixture is agitated well in order to have a well-distributed heterogeneous system. The mixture of enzyme and polymerizing substances are then injected into a precooled organic solvent. Petroleum ether that is put in a cryostat then cooled by liquid nitrogen is used as organic solvent. The cryostat that contains the glassy particles is placed in a cobalt (^{60}Co) source and irradiated at a dose of 0.15 Mrad. Liquid nitrogen is added to the cryostat at 30 min intervals during irradiation to keep the spheres frozen. After irradiation the spheres are removed from the cryostat and placed in a vessel then the noncrosslinked polyethlene glycol is dissolved in water in order to obtain a porous structure. The spheres are kept in phosphate buffer (pH 7) at 4°C.

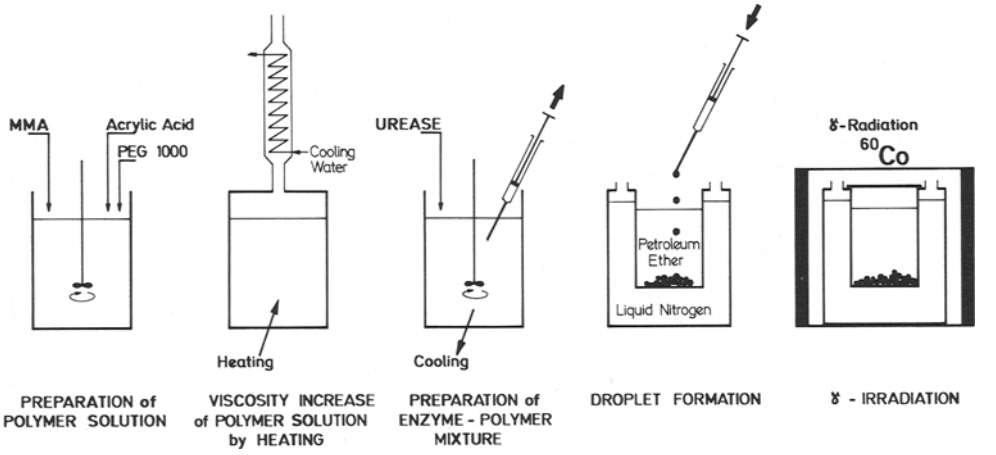

Fig. 1. Preparative procedure for bead-type gel particles.

The examination of the activity yield is carried out by means of spectrophotometrical methods. The urea nitrogen formed in 10% urea solution by hydrolysis is determined with Nessler's reagent. The activity yield of the immobilized enzyme may be represented as follows:

$$\text{Activity yield } (\%) = \frac{I_a}{N_a} \times 100 \quad (1)$$

where I_a is the quantity of hydrolysis product formed by immobilized enzyme during the reaction and N_a is the quantity of hydrolysis product formed by native enzyme. The enzyme leakage is detected by testing the phosphate buffer media after removing the gel spheres for enzyme activity.

The optimum ratio of components and the effect of radiation dose on the activity yield of immobilized urease is examined by changing the duration of irradiation and the amount of components.

RESULTS AND DISCUSSION

The polymer–enzyme conjugates obtained by radiation-induced polymerization at low temperature are spherical and are in the form of white porous hard gel. The scanning electron microscopic photographs of the gel sphere surface are given in Fig. 2. Figure 2a is the SEM photograph of the gel sphere taken right after polymerization procedure. Figure 2b is the SEM photograph of the same type of material swollen after being kept in phosphate buffer.

The polymer–monomer and enzyme mixture is prepared in different proportions keeping the total volume of mixture constant. In this group of experiments the irradiation period was 15 h. The immobilized enzyme activity yield in selected samples are given in Table 1. Acrylic acid leads to gel formation, methyl methacrylate provides a hard structure and polyethlene glycol gives the porous structure and also forms gel. As can be seen in Table 1, when the initial PEG ratio to the other components is increased soft gels with high enzyme activity are obtained. But some enzyme leakage is detected in these samples. When MMA is in high ratio, the hard spheres are obtained and the activity is comparably less. In conclusion, the optimum combination is less PEG (20%) and same amount of MMA and acrylic acid (40% each). This combination gives white sponge-like gel spheres with the highest immobilized enzyme activity and no leakage.

The relationship between the radiation dose and the activity yield of immobilized urease is given in Table 2. The gel spheres used in this set of experiments were prepared using acrylic acid, methyl methacrylate and PEG in the volume ratio of 40:40:20, respectively. The results of these experiments show that increasing the dose of radiation causes a reduction in the enzyme activity yield. This may result from the formation of a

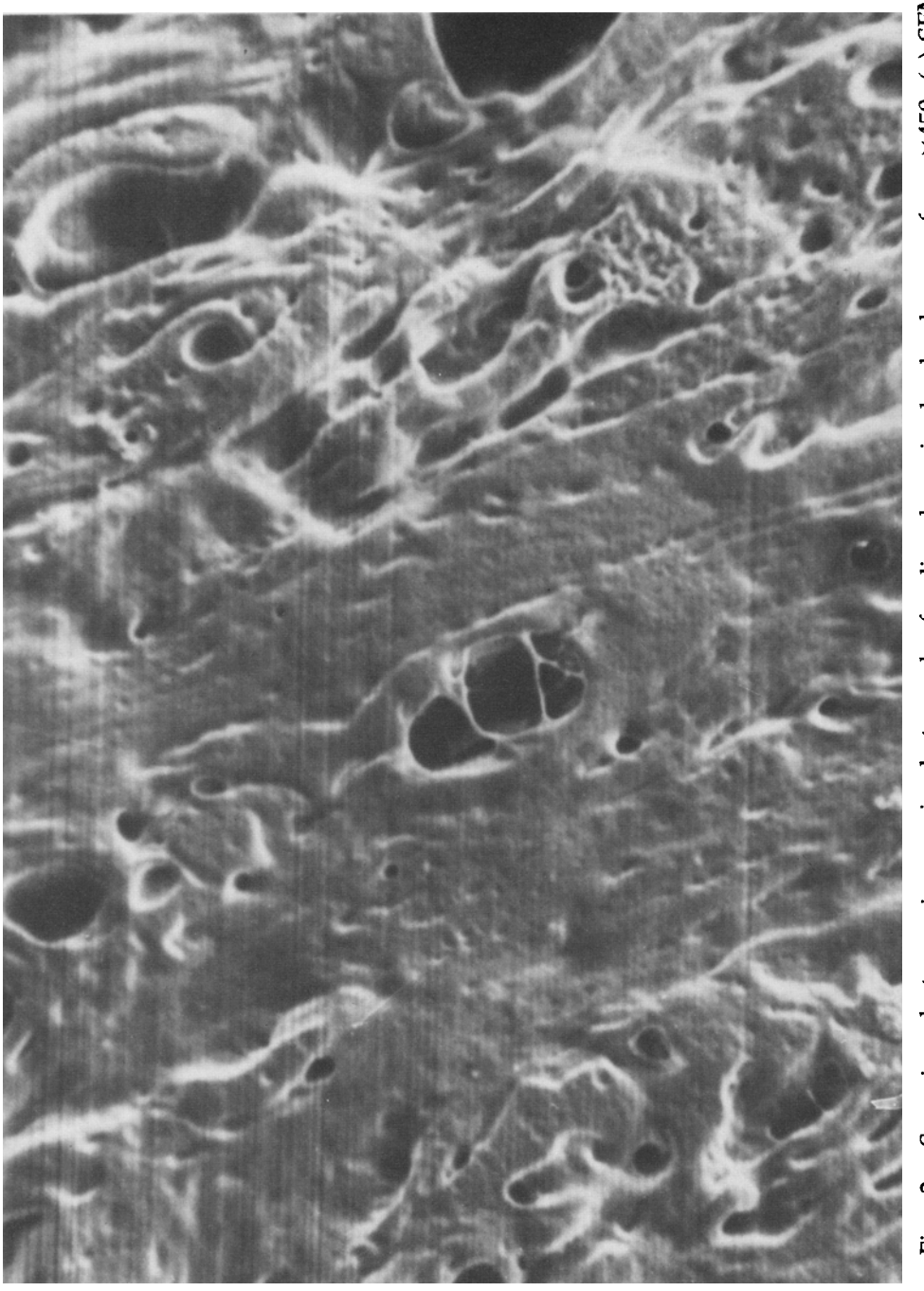

Fig. 2. Scanning electron microscopic photograph of radiopolymerized gel sphere surface, ×450. (a) SEM photograph of gel spheres after preparation.

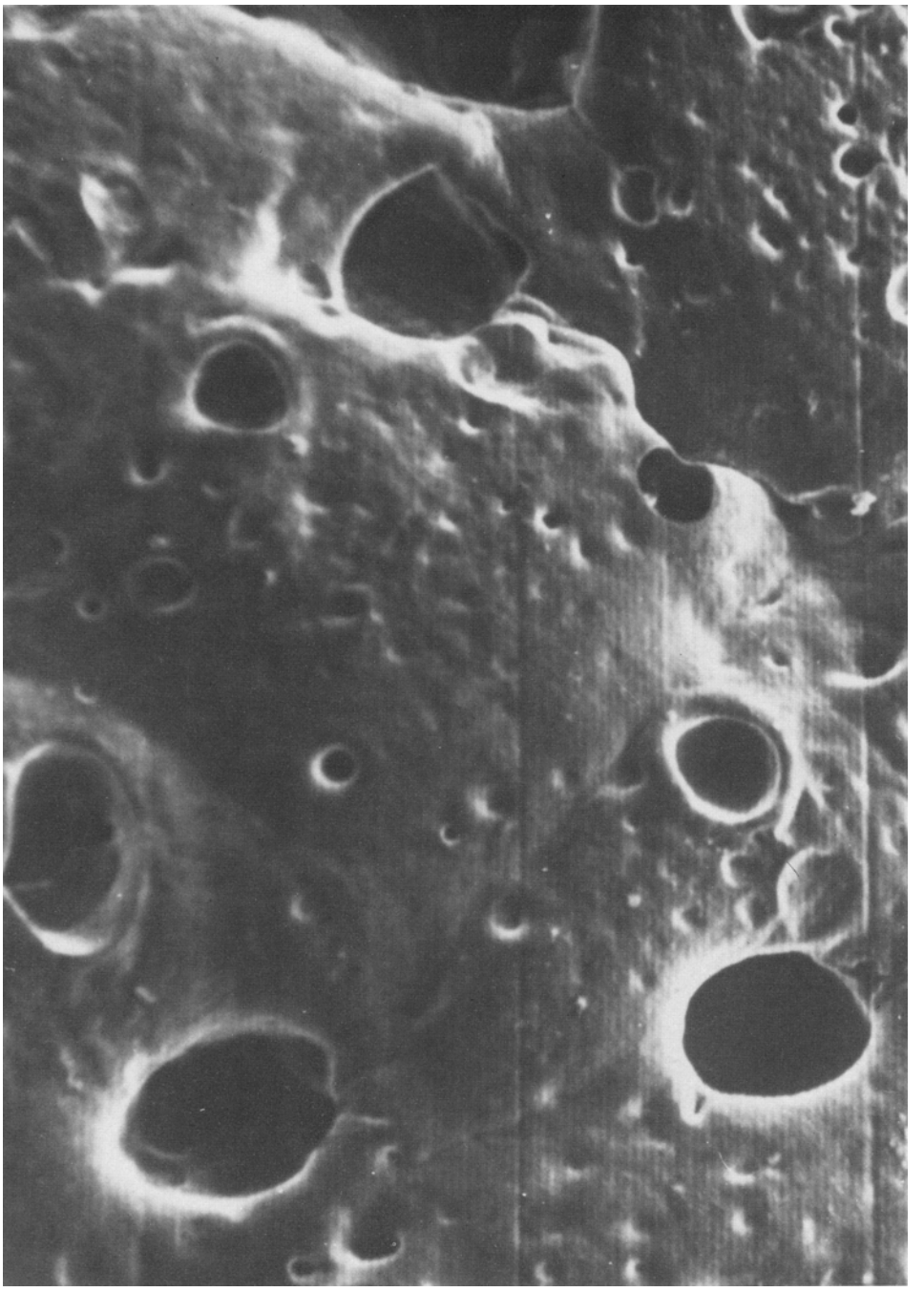

Fig. 2(b) SEM photograph of swollen gel particles.

TABLE 1
Effect of Ratio of Components on the Formation of Gel Spheres and Immobilized Enzyme Activity

Sample	Ratio of components (% v/v)			Property of polymer sphere	Immobilized enzyme activity yield (%)	Leakage
	Acrylic Acid	MMA	PEG			
1	60	20	20	white sponge like gel spheres	50±2.1	-
2	20	60	20	hard spheres	30±1.9	-
3	40	40	20	white sponge like gel spheres	65±0.8	-
4	20	40	40	curdy composite	47±4.2	+
5	20	20	60	soft gel (nonspherical)	74±3.0	+
6	40	20	40	soft gel (nonspherical)	80±2.4	+

TABLE 2
Effect of Radiation Dose on the Activity Yield of Immobilized Urease

Dose period, h	Activity yield, %
10	70 ∓ 1.0 (there is some leakage)
15	65 ∓ 0.6
30	48 ∓ 0.2
45	35 ∓ 0.2

highly crosslinked polymer network that prevents substrate diffusion. However, a low radiation dose causes some enzyme leakage. This may also be explained by the insufficient level of crosslink formed in the polymer structure. It is concluded that irradiation for 15 h (total radiation dose, 2.75 Mrad) is optimum.

In conclusion, these highly porous gel spheres may be a suitable enzyme support system that offers the advantage of high enzyme loading per gram of polymer.

ACKNOWLEDGMENT

The authors are grateful to Mr. C. Tan of Middle East Technical University for kindly taking the SEM photographs.

REFERENCES

1. Zaborsky, O. R. (1979), *Immobilized Enzymes*, CRC Press, Cleveland, Ohio.
2. Kumakura, M., Yoshida, M., and Kaetsu, I. (1979), *Appl. Environ. Microbiol.* **37,** 310.
3. Kaetsu, I., Kumakura, M., and Masaru, Y. (1979), *Biotech. Bioeng.* **XXI,** 863.