UREASE IMOBILIZATION BY ELECTRON BEAM CURING.

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Urease was immobilized onto cellulose fibril sheets by radiation curing of tetradecaoxyethylene glycol diacrylate (A-14 G), an hydrophilic monomer. the relationship between the preparation conditions and the activity of the membranes was studied,such as monomer concentration,irradiation dose and protective agents. Since urease is a very labile enzyme for irradiation, bovine serum albumin (BSA) or cysteine (cys) was added in the immobilization procedure . Various properties of the immobilized urease were evaluated. The optimum pH for the enzyme reaction of the immobilized urease was identical to the native one, while the activity of the former urease decreased less than that of the latter one at both acidic and alkaline sides of the optimum pH Up to 30ºC, a decline in the activity was observed, more deeply for the free enzyme while the immobilized enzyme was stable from 50 to 70ºC. The maintenance of activity was examined by repeated batch enzyme reactions. The speed of processing, simplicity and uniformity of the insolubilized reagent on the membrane beam irradiation is emphasized.

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INTRODUCTION

The immunobilization of biofunctional components can be carried out by means of radiation polymerization. Recent development of radiation polymerization involves radiation are processes were mixtures of oligomers or monomers are polymerized into a thin homopolymer film using ionizing radiation from electron beam (E.B.) sources.

The curing system possesses great potential for the current immobilization methods since complete polymerization is achieved in a short time. The possibility of using radiation curing processes with electron beam source for immobilization of enzyme was explored and found to be possible.

MATERIALS

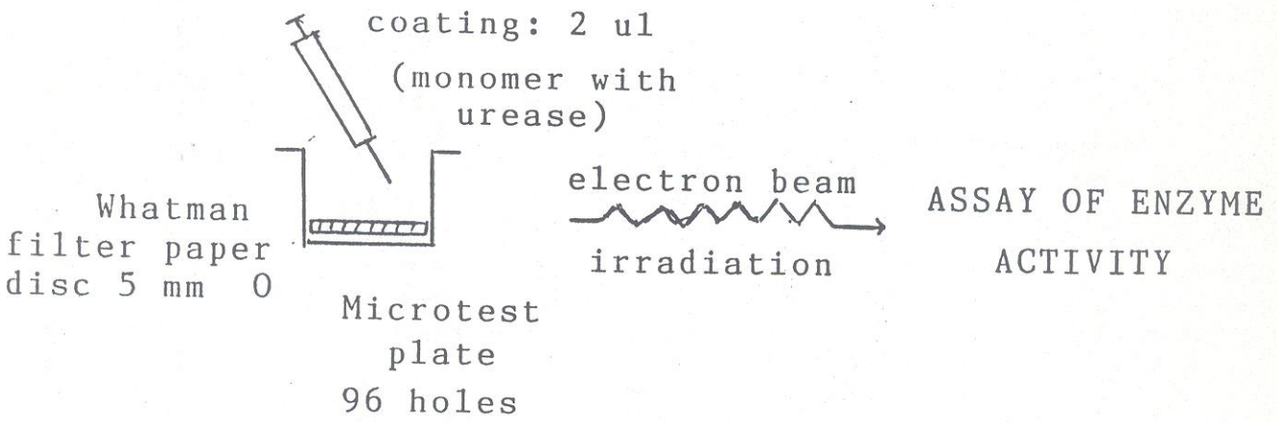
Urease was immobilized on cellulose sheets by radiation curing of tetradecaoxyethylene glycol diacrylate (A-14G), an hydrophylic monomers.

Thin filter paper was used as support for the urease solution mixed with monomer that was coated onto the sheet using micro-automatic pipette. Urease paper-discs were irradiated in a Dynamitron type electron accelerator, in which the electron beam accelerator voltage was 0.55 MeV. The irradiation at 1mA electron beam current and speed of the belt conveyor at 4m/min., gave the irradiation dose of 10 Gy. The samples were irradiated at room temperature.

METHODS:

PROCEDURE OF IMMOBILIZATION

I. IMMOBILIZATION



II. ASSAY OF ENZYME ACTIVITY

Substrate: 0.2 urea in phosphate buffer 0.04M pH 7.2, with 0.2% arabic gum and 0.6N sodium tartrate - 3 ml.

Enzyme reaction: 30°C, 30 min.

Analysis of amonia: Nessler's method
reagent volume: 3 ml
developing time: 15 min
absorbance: 420 nm

RESULTS

The irradiation effect of electron beam on the activity of native urease was studied before immobilization. A decreased activity of the enzyme was detected after being irradiated (Fig. 1).

Since urease is a very labile enzyme for irradiation, protective substance, as cysteine or BSA, were added in the immobilization procedure. In Fig. 2 and 3 are shown the effect of addition of Cys and BSA in the urease solution to prevent the reduction in the enzyme activity during irradiation.

The effect of irradiation dose of electron beam on the activities of free and immobilized urease is represented in Fig. 4. Acording to Kaetsu et al. (1987), one possible reason for the higher activity of the immobilized enzyme, is that the formed polymer also acts as a screening substance to protect the enzymee molecules from irradiation and damage.

As is shown in Fig. 5 the activity of the immobilized enzyme has a maximum at a certain monomer concentration. It can be explained by an optimum balance between the leakage of enzyme decreasing with an increase of monomer concentration, and the occlusion of enzyme increasing with the increase of monomer concentration (Yoshida et al., 1980).

In Fig. 6 the maintenance of activity was observed with the repeated uses for enzyme reactions.

The optimum pH for the enzyme reaction of the immobilyzed urease was identical to the native one. The activity of the former urease decrease less than the latter one at both acidic an alkaline sides of the optimun pH (Fig.7).

A decline in the activity was observed up to 30ºC, more deeply for the free enzyme while the immobilized enzyme was stable from 50ºC to 70ºC (Fig. 8).

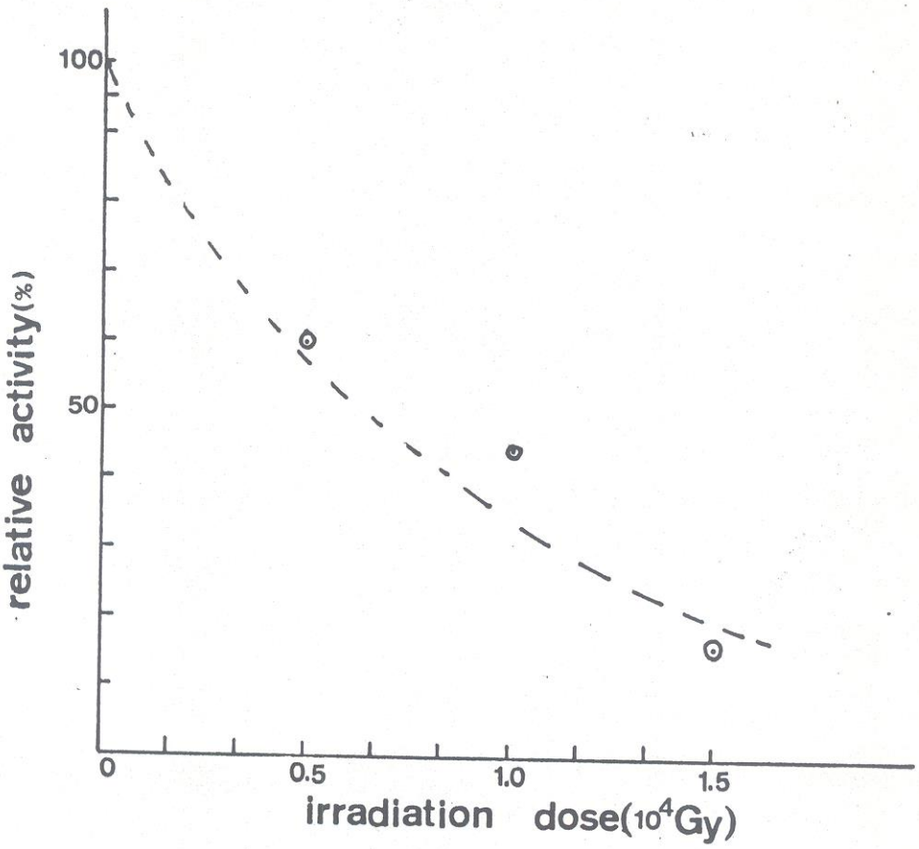


FIG.1 - EFFECT OF IRRADIATION DOSE OF ELECTRON BEAM ON THE ACTIVITY OF UREASE SOLUTION. Enzyme: 0.3% in buffer.

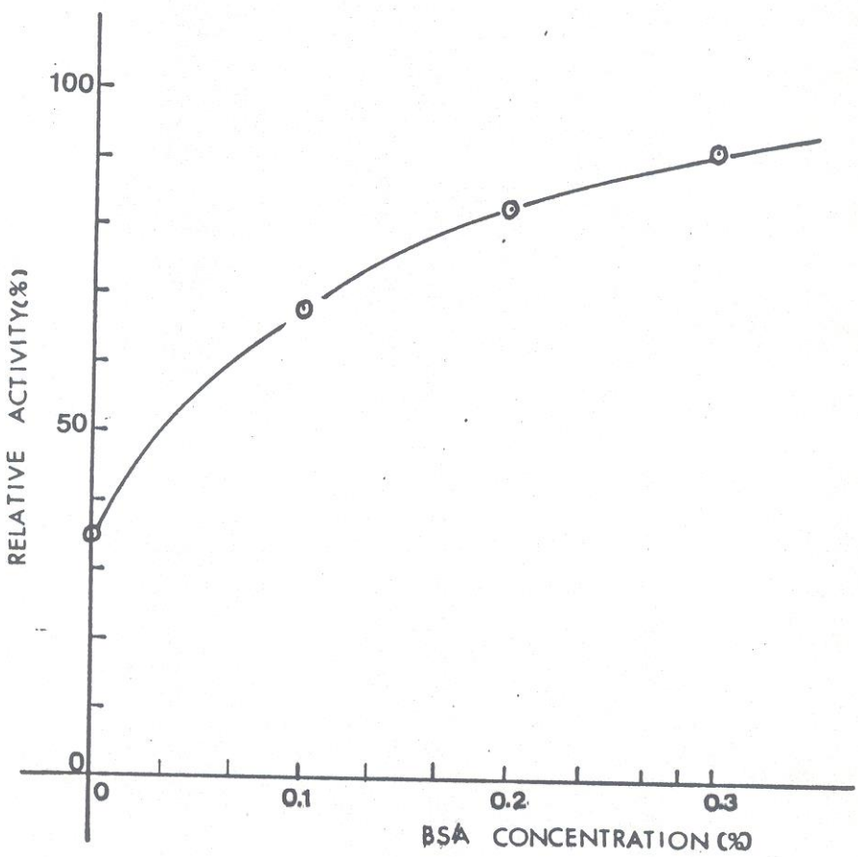


FIG.2 - EFFECT OF ADDITION OF BSA ON THE ACTIVITY OF UREASE. Irradiation: 10^4 Gy r.t.; enzyme: 0.3% in buffer.

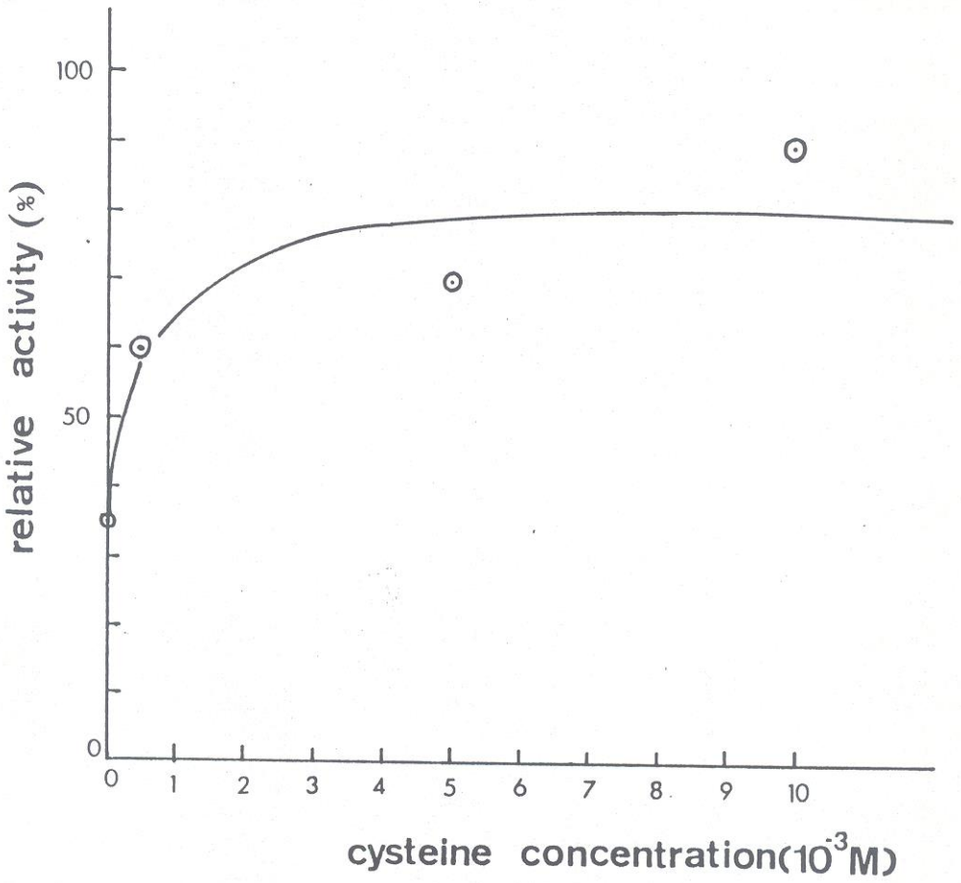


Fig.3 - EFFECT OF ADDITION OF CYSTEINE ON THE ACTIVITY OF UREASE. Irradiation: 10^4 Gy r.t.;enzyme: 0.3% in buffer.

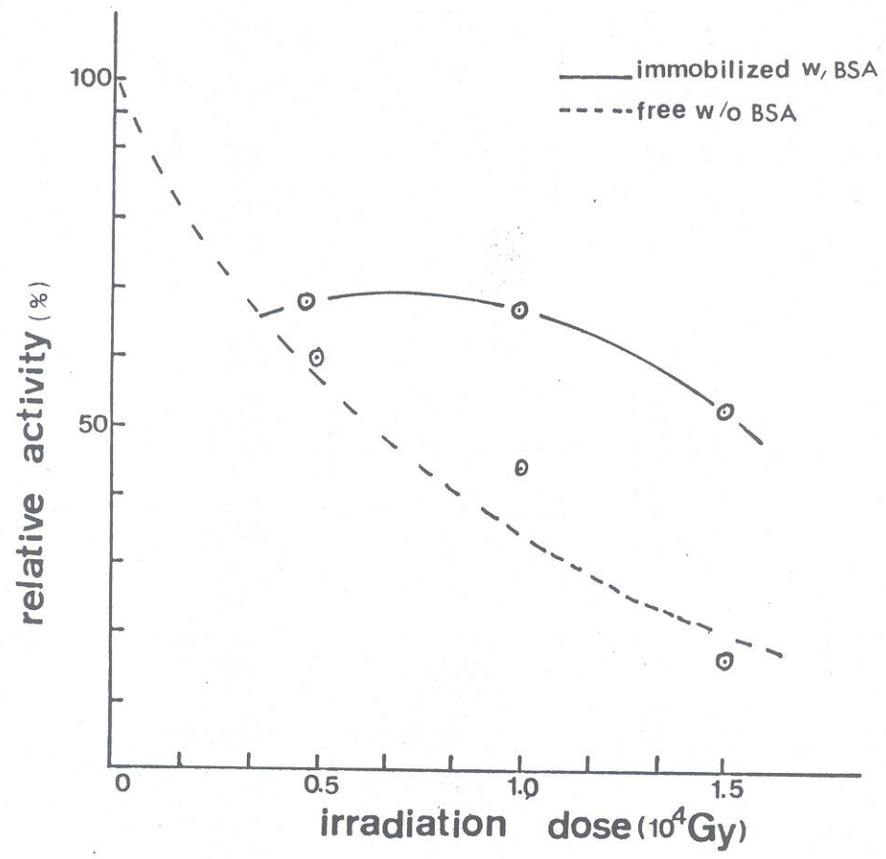


Fig.4 - EFFECT IRRADIATION DOSE OF E.B.ON THE ACTIVITIES OF FREE AND IMMOBILIZED UREASE. Irradiation: 10^4 Gy. Enzyme:0.3% BSA: 0.2% ; Monomer: 50% A-14 G.

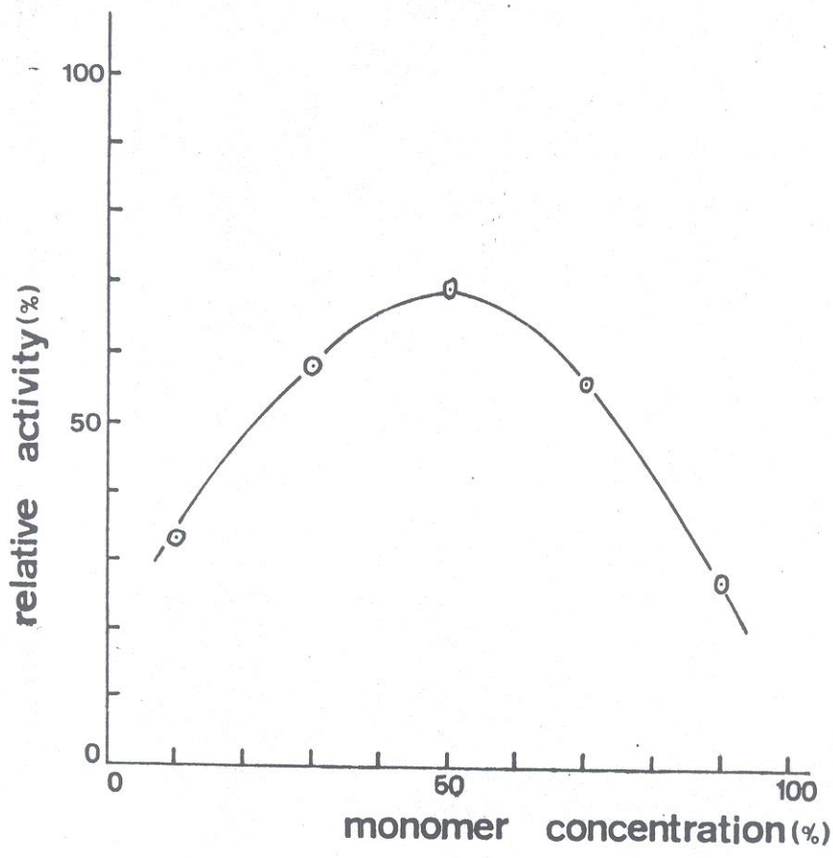


Fig. 5 - EFFECT OF MONOMER CONCENTRATION IN IMMOBILIZATION BY E.B. ON THE ACTIVITY OF IMMOBILIZED UREASE. Enzyme: 0.3%: BSA: 0.2%: Monomer: A-14G.

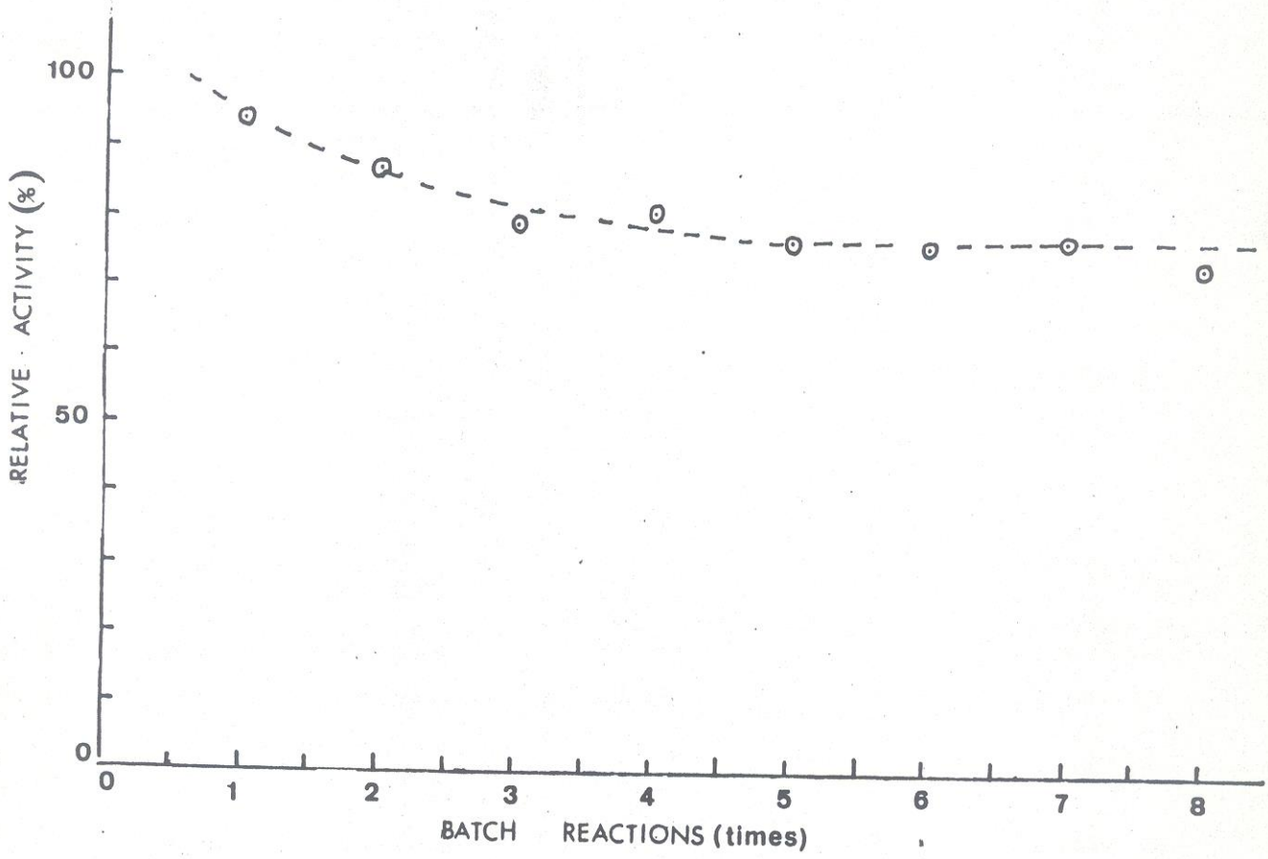


Fig.6 - RELATION BETWEEN THE ACTIVITY OF IMMOBILIZED UREASE AND THE NUMBER OF REPEATED USES FOR ENZYME REACTION.

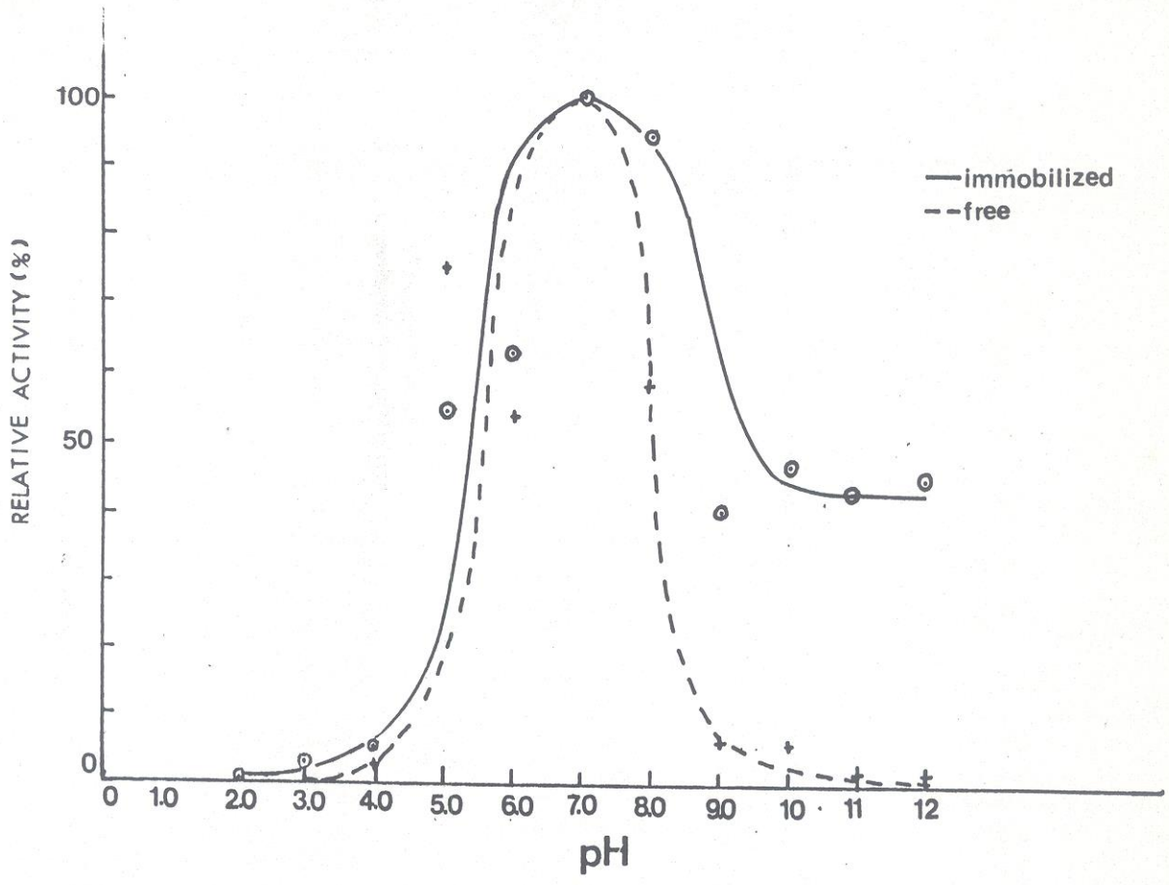


Fig. 7 - EFFECT OF pH TREATMENT IN THE ACTIVITIES OF FREE AND IMMOBILIZED UREASE. Treatment: 1 hour at r.t.

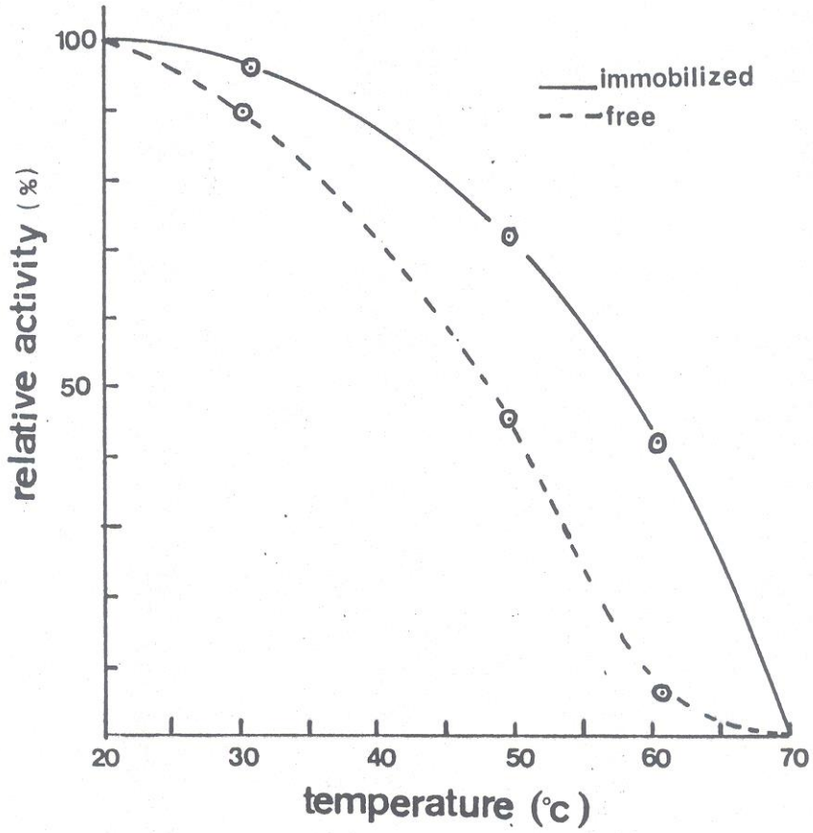


Fig. 8 - EFFECT O TEMPERATURE TREATMENT IN THE ACTIVITIES OF FREE AND IMMOBILIZED UREASE. Treatment: 1 hour.

CONCLUSIONS

1. The immobilization onto filter paper doses is a simple technique and applicable to different biocompounds. It does need large amount of bioactive substance and the preparation conditions are mild and fast by the use of E.B. irradiation.

2. The immobilized enzyme retained significant activity showed by maintenance of the products yield in the repeated batch reactions.

3. The paper disc immobilized urease can be useful for medical and pharmaceutical purposes for urea dosage.

REFERENCES

1. KAETSU, I & KUMAKURA, M. - Radiat. Phys. Chem. 30 (4), p. 263, 1987.

2. YOSHIDA, M.; KUMAKURA, M.; KAETSU, I. - J. Macromol. Sci. Chem. A-14 (4), p. 541, 1980.