slurry was washed with 20% hydrochloric acid. The washings were repeated until the filtrate was free from titanium. The residue was then treated with excess of THF and the filtrate collected and evaporated to dryness Bright yellow product with brilliant fluorescence was obtained. The mechanism of polymerisation is probably as follows. Ti(II) species is first produced by the action of LiAlH₄ on Ti(III). This is evidenced by the formation of black colour, characteristic of Ti(II)[17]. Ti(II) species reacting with the carbonyl group forms a cyclic Ti(II) complex followed by the concerted loss of TiO₂ or stepwise loss of oxygen, forming the olefinic linkage. The polymer chain grows by this stepwise mechanism
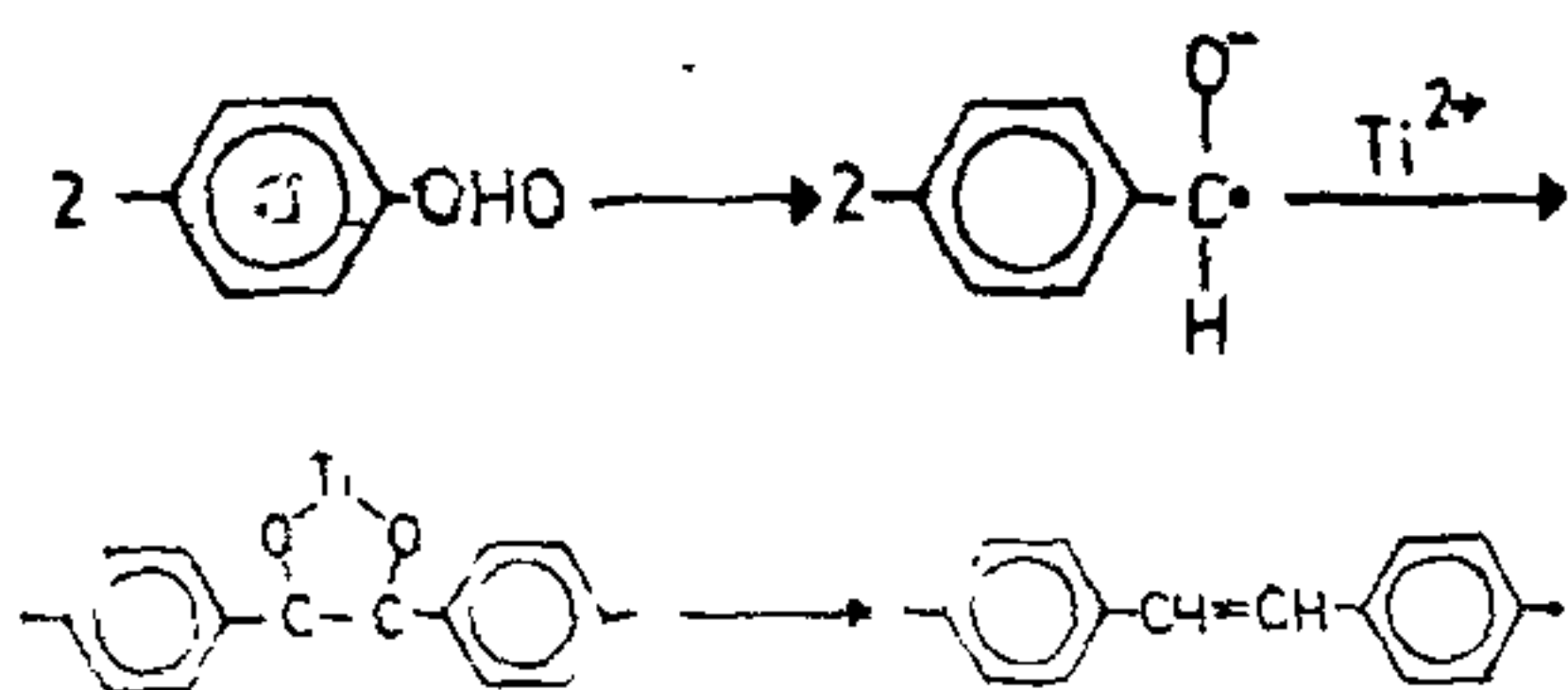
3. *Polymer Characterisation*

The solubility of the polymer was tested in most of the organic solvents. It was sparingly soluble in THF and CHCl₃. This solubility was not sufficient to carry out viscosity measurements. The UV spectrum of the polymer in CHCl₃ showed absorption maxima at 385 nm, 364 nm, 323 nm, 305 nm and 292 nm. This spectrum with many bands stretching into the visible region, shows the presence of long conjugated aromatic chromophore[18]. The IR spectrum of the polymer in KBr pellet exhibited absorption maxima at 835 cm⁻¹ (para-disubstituted benzene ring), 967 cm⁻¹ (trans-ethylenic unsaturation) and 1620 cm⁻¹ (ethylenic bond conjugated with aromatic rings) thus confirming that the coupling has taken place with the formation of olefinic double bonds. This IR spectrum of the polymer is identical with the spectrum of the polymer synthesised by other procedures by earlier workers[10]. The polymer solution in CHCl₃, fluoresced with maximum emission intensity at 455 nm, normalised at 400 nm. DSC measurements showed no apparent endothermic or exothermic transitions upto 550° K at a heating rate of 8°/min. When the heating rate was 32°/min sudden changes in heat capacity of the sample were observed at 510° and 580° K. Visual observation of the sample showed that the sample tarnished at about 420° K and gas evolution occurred at 580° K. Conductivity measurements of the polymer pellet sandwiched between platinum foils, showed a conductivity $\sigma = 1 \cdot 28 \times 10^{-8}$ ohm⁻¹ cm⁻¹. Further work with the polymer, like X-ray analysis, etc., is in progress,

Department of Physical   LALITHA RAJARAMAN.
  Chemistry,   M. BALASUBRAMANIAN.
University of Madras,   M. J. NANJAN.
Madras 600 025,
July 19, 1979.

1. Goodings, E. P., *Chemical Society Reviews*, 1976, 5 (1), 96.
2. Eley, D. D., *J. Polym. Sci.*, Part C, 1967, 17, 78.
3. Rembaum, A. and Landel, R., Eds. "Electrical conduction of polymers," *J. Polym. Sci.*, 1967, Part C, 17.
4. Berlin, A. A., *Ibid*, 1961, 55, 621.
5. Becker, M. and Mark, H. F., *Angew Chem.*, 1961, 73, 641.
6. Kallmann, H. and Silver, M. Eds., *Symposium on Eectrical Conductivity in Organic Solids*, Interscience, New York, 1961.
7. Rembaum, A., *J. Polym. Sci.*, 1970, Part C, 29, 158.
8. Hartel, M., Kossmehl, G., Menecke, G., Willie, W., Wohrle, D. and Zerpner, D., *Angew. Makromol. Chem.*, 1973, 29/30, 307.
9. McDonald and Campbell, W., *J. Amer. Chem. Soc.*, 1960, 82, 4669.
10. Hoeg, D. F., Lusk, D. I. and Goldberg, E. P., *J. Polym. Sci.*, 1964, Part B, 2, 697.
11. McMurry, J. E., and Fleming, M. P., *J. Amer. Chem. Soc.*, 1974, 96, 4708.
12. — and —, *J. Org. Chem.*, 1975, 40 (17), 2555.
13. —, *Accounts of Chemical Research*, 1974, 7 (9), 281.
14. Blaszezak, L. C. and McMurry, J. E., *J. Org. Chem.*, 1974, 39, 258.
15. McMurry, J. E. and Meltin, J., *J. Amer. Chem. Soc.*, 1971, 93, 5309.
16. —, *J. Org. Chem.*, 1973, 38, 4367.
17. Van Tameleen, E. E. and Schwartz, M. A., *J. Amer. Chem. Soc.*, 1965, 87, 3277.
18. Williams, D. H. and Fleming, I., *Spectroscopic Methods in Organic Chemistry*, McGraw-Hill, 1973, p. 8.

PRESENCE OF TWO FORMS OF β-GLUCOSIDASE IN THE CULTURE FILTRATE OF *MACROPHOMINA PHASEOLINA*

A CONSIDERABLE progress has been achieved in the last few years in characterizing enzymes of cellulose catabolic pathways. Three types of enzymes, *viz.*, endo-β-1,4-glucanase, exo-β-1,4-glucanase and β-glucosidase are involved in the conversion of native cellulosic substrate to soluble sugars by synergistic action[1,2]. Again, cellulase activity has been corre-
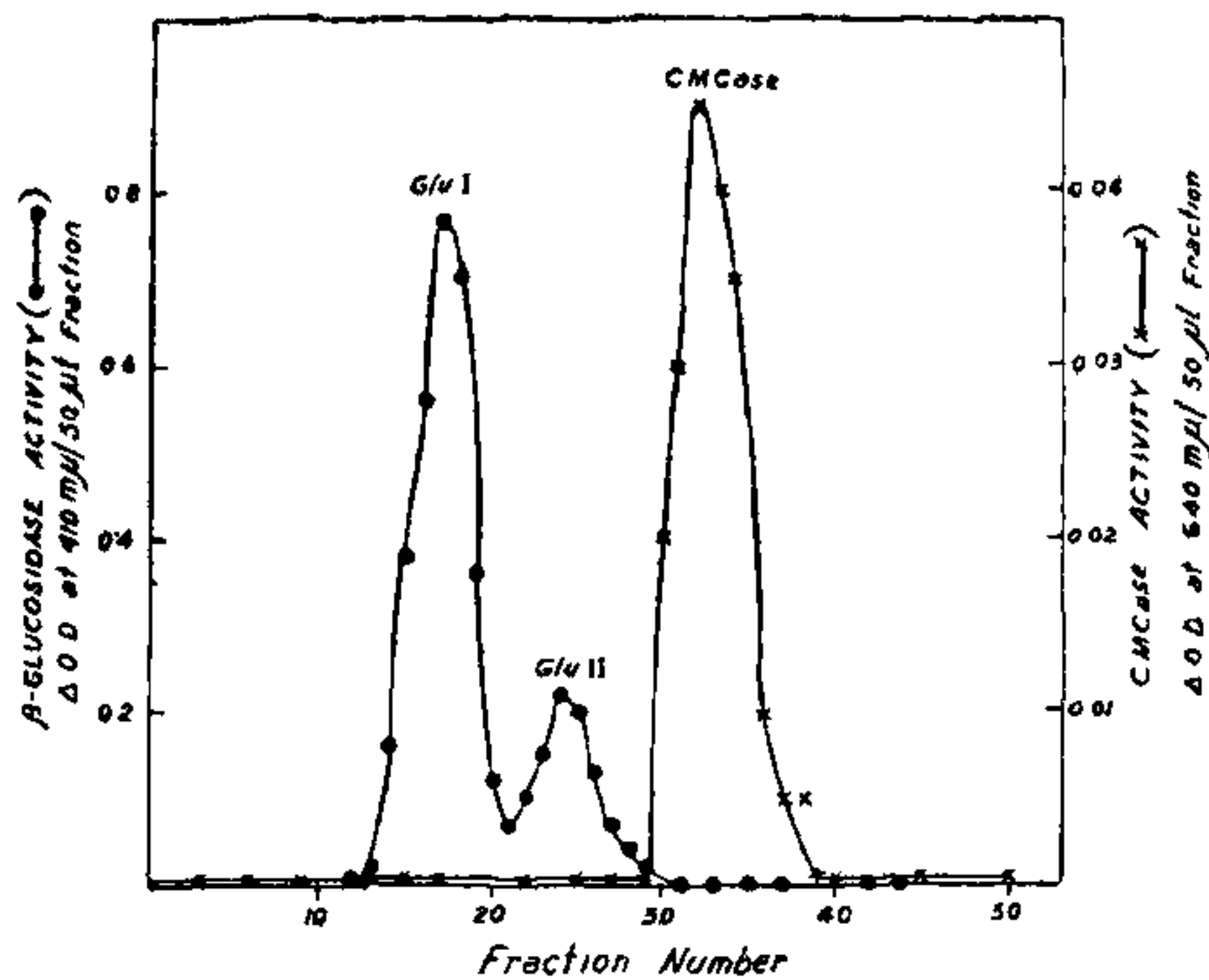
FIG. 1. *Gel-filtration of ammonium sulphate fraction of the culture filtrate.* Sephadex G-100 (10° xl cm) was previously equilibrated with 50 mM Tris-HCL buffer (pH 7·0). The charging material was 5 ml of ammonium sulphate fraction containing 5 mg

lated with the pathogenicity of phytopathogenic fungus. Hence β-glucosidase may play an important role when the phytopathogenic fungus degrades cellul se of the host cell[3]. We have already reported the phenomenon of induction of extra-cellular cellulolytic enzymes in the jute pathogenic fungus *M. phaseolina* by the soluble carboxymethyl cellulose[4]. In the present communication we report the existence of two forms of β-glucosidase enzyme in the culture filtrate of *M. phaseolina* when the jute pathogen is grown in the medium supplemented with CMC as the sole carbon source.

protein and 140 Units of β-glucosidase (1 unit is defined as liberation of 1 μmole of p-nitrophenol per 30 minutes at 55° C). The column was eluted with Tris-HCl buffer (pH 7·0). Fifty fractions each containing 2 ml were collected in an automatic fraction collector (Fractometer 200, Buchler). The activities of β-glucosidase as well as CMCase were estimated with 50 μl aliquot from each fraction according to the method of Michell *et al.*[5] and Mandel *et al.*[6] respectively.
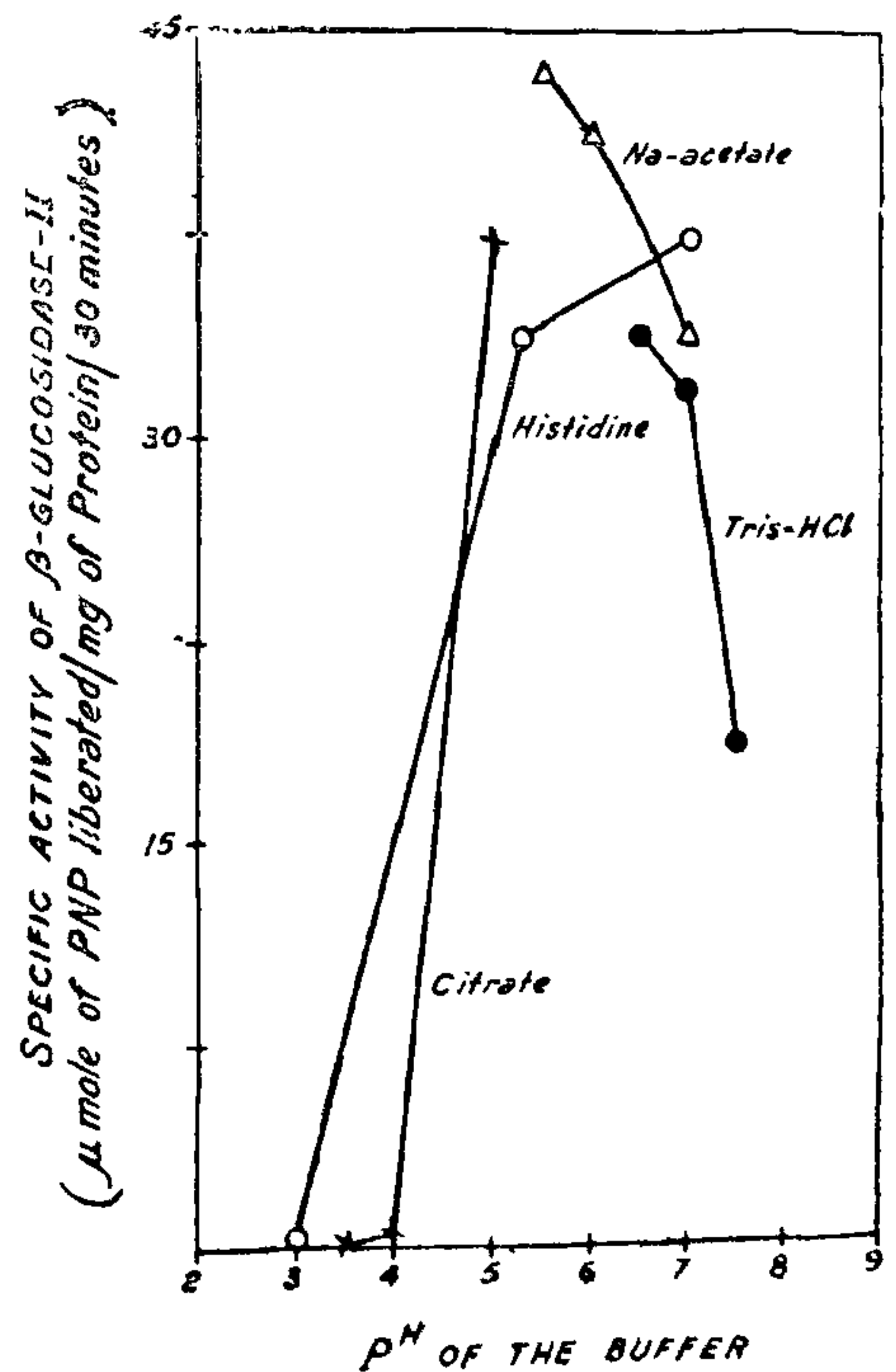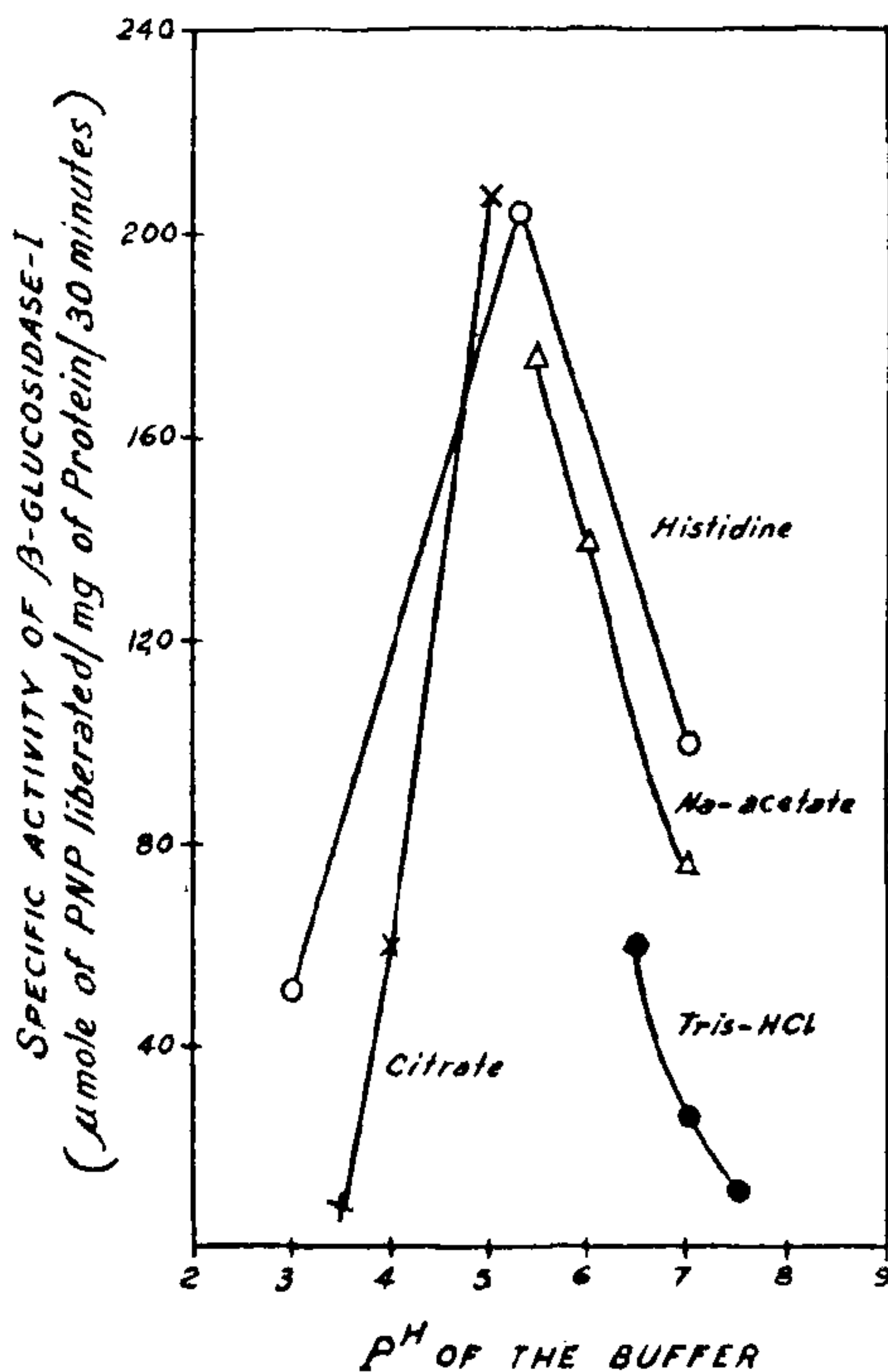

FIG. 2. *pH optima of β-Glucosidase I and II* isolated from the culture filtrate of M. phaseolina β-glucosidase activity was measured using different buffers at various pH as indicated in the figure at optimum temperature. ×—× : Citrate buffer. ●—● : Tris-HCl buffer; O--O : Histidine buffer △--△ : Sodium-acetate buffer.
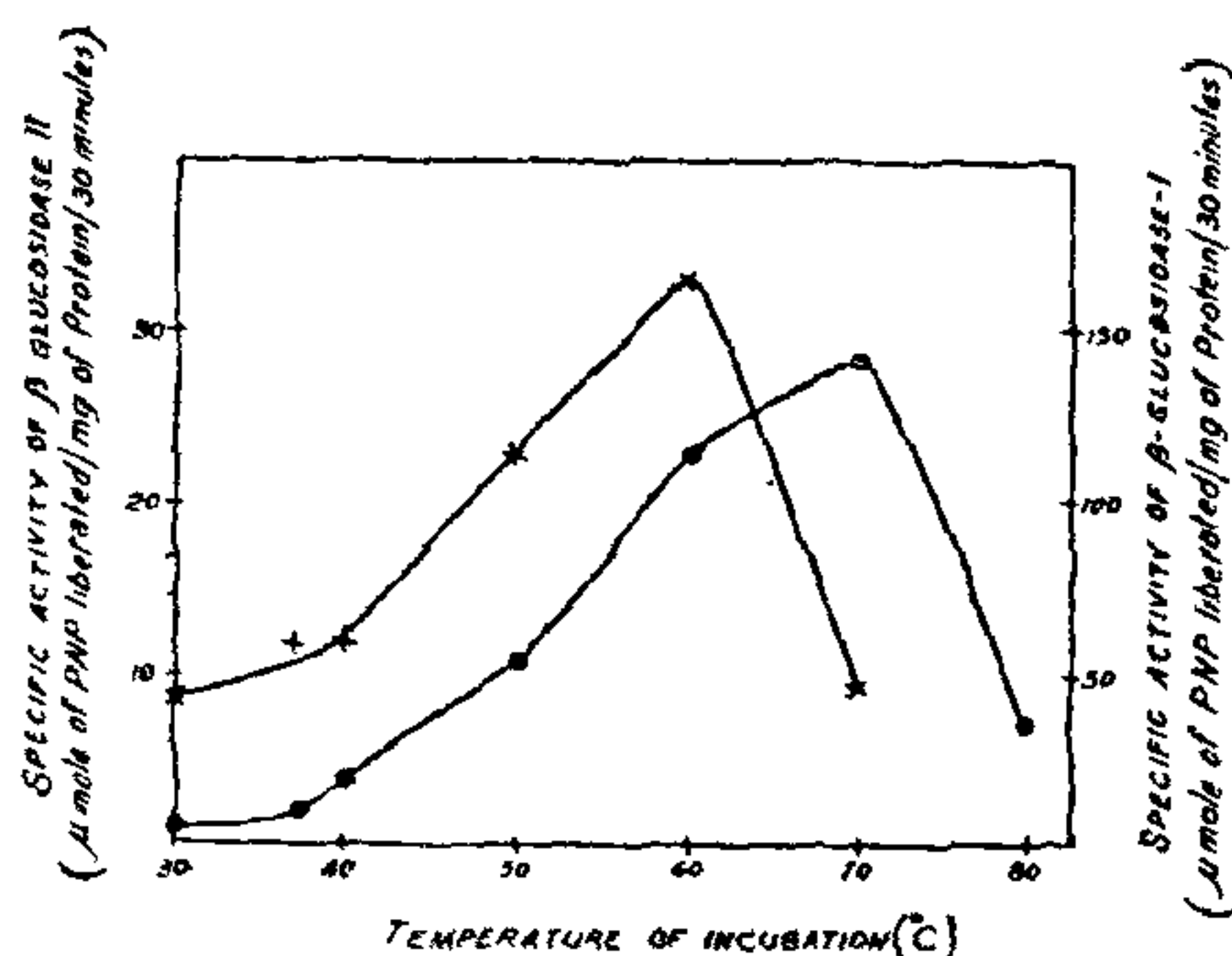
FIG. 3. *Optimum temperature for the activity of Glucosidase I and Glucosidase II isolated from the culture filtrate of* M. phaseolina.
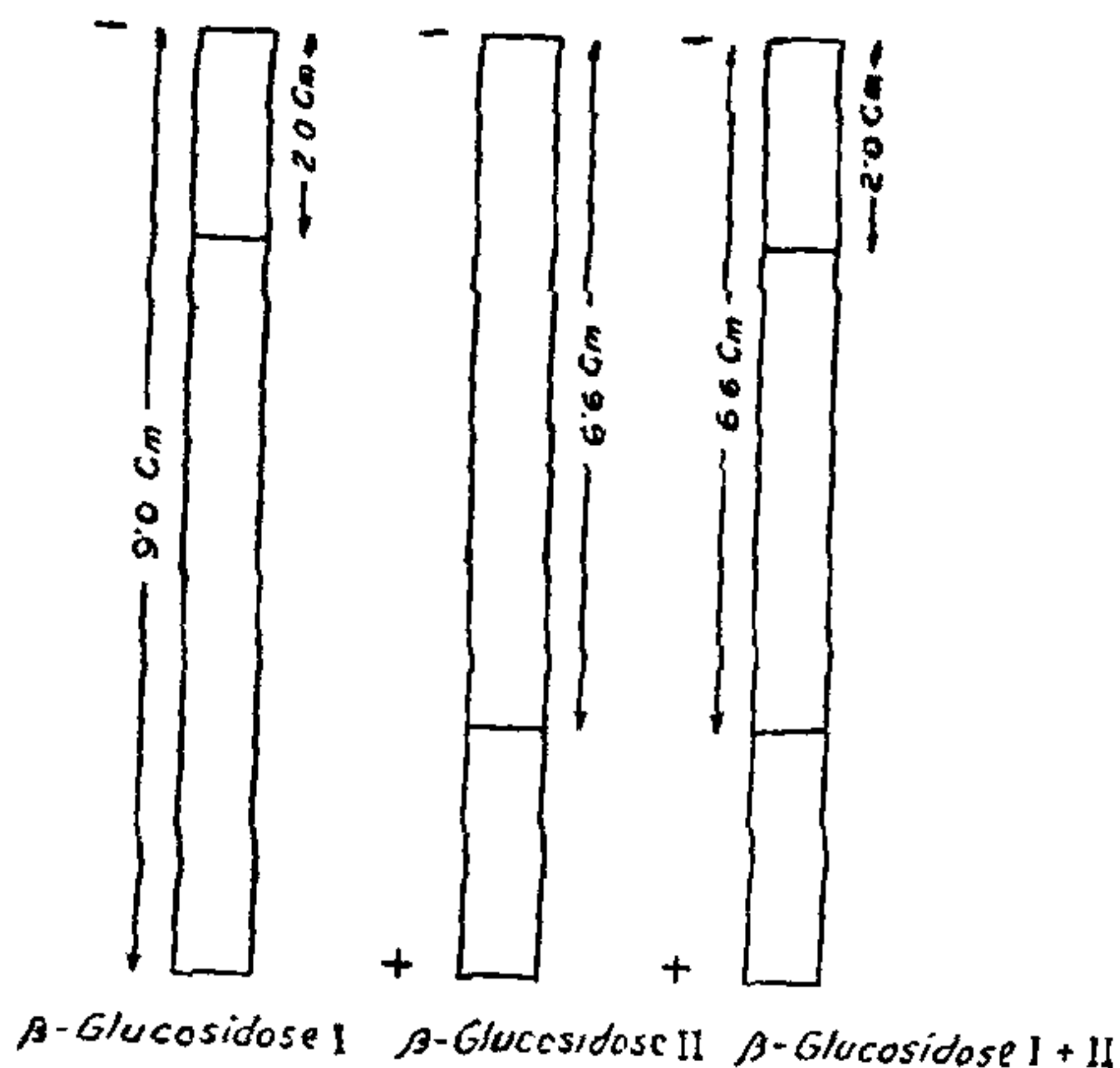

β-*Glucosidase* I   β-*Glucosidase* II   β-*Glucosidase* I + II

FIG. 4. *Zymorgram pattern of Glucosidase I and Glucosidase II.* Purified enzymes were electrophoresced in 7% polyacrylamide gel according to the method of Davis[7]. When the electrophoresis was over the gels were incubated at 50° C in citrate buffer (pH 4·8) containing o-nitro-phenyl β-D-glucopyranoside (1 mM) for 30 minutes. The citrate buffer was then drained out and 0·01 M Na_2CO_3 was added to each tube. A faint yellow band appeared at the position as indicated in the figure. However the colour is too faint and transparent to be photographed in ordinary black and white film. Hence the sketchy diagram of the electrophoretic pattern is furnished.

In our earlier experiment it was shown that the crude culture filtrate of *M. phaseolina* grown in presence of CMC exhibited higher activity towards the hydrolysis of o-nitrophenyl β-D glucopyranoside. So to the

crude culture filtrate, solid ammonium sulfate was added slowly with constant shaking upto 80% saturation. The whole mixture was then kept at 4° C for two hours. Insoluble material was removed by centrifugation at 10,000 × g for 30 minutes in cold. The sediment was dissolved in 10 mM Tris-HCl buffer (pH 7·0) and was designated as 'Ammonium sulfate fraction'. An aliquot of ammonium sulfate fraction was charged onto a Sephadex G-100 column previously equilibrated with 50 mM Tris-HCl buffer. The results given in Fig. 1 depict the separation of activities of two β-glucosidases and also CMCase. The recovery of the total β-glucosidase activity from the Sephadex column was about 70-75%.

In order to characterize the two different β-glucosidase activities, viz., Glucosidase I and Glucosidase II, some physical and biochemical properties of the two enzymes were also being investigated. The results given in Fig. 2 show that the pH optima of two enzymes are different. Glucosidase II is more active than the other above pH 5·5. They have different optimum temperatures for their activities. Optimum temperature for the activity of Glucosidase I is 55° while that for Gluc_sidase II is 65° (Fig. 3). Moreover the two forms differ in the electrophoretic mobility in the polyacrylamide gel and it has been found that Glucosidase II is the faster moving one. The present results along with some unpublished observations (chromatographic behaviour on DEAE cellulose, thermal stability and kinetic properties) strongly suggest that the two β-glucosidases are different in their activities.

Department of Biochemistry,   SAGAR C. SAHA.
University College of Science,   ARUNIK SANYAL.
35, Ballygunge Circular Road,   SYAMALIMA DUBE.
Calcutta University,   DIPAK K. DUBE.
Calcutta 700 019,
September 11, 1979.

1. Eriksson, K. E., *Biotechnology and Bioengineering,* 1978, **20**, 317.

2. Wood, T. M., *Biotechnol and Bioeng. Symp.,* 1975, No. 5, 111.

3. Sudo, T., Nagayama, H. and Tamari, K., *Agric. Biol. Chem.,* 1973, **37**, 2535.

4. Saha, S. C., Dube, S., Sanyal, A. and Dube, D. K., *Curr. Sci.,* 1979, **48**, 484.

5. Michell, K. H., Karonovsky, N. J. and Kornovsky, M. L., *Biochem. J.,* 1970, **116**, 207.

6. Mandels, M., Andreotti, R. and Roche, C., *Biotech. Bioeng. Symp.,* 1976, **6**, 21.

7. Davis, B. J., *Ann. N.Y. Acad. Sci.,* 1964, **121**, 404.