CONCLUSION

The simple procedure for the isolation of l-arginine and low molecular weight oligopeptides rich in arginine is developed. The amylose, linear component of starch specifically adsorbs l-arginine. The procedure for isolation of which has been standardised. The optimum adsorption of these compounds on amylose gel is observed at acidic pH (4.6). The 70% alcohol treated amylose gel at pH 4.6 gives highest adsorption. The desorption is obtained by increasing the pH to the alkaline side. Hence adsorption and desorption of arginine is effected by changing pH and alcoholic conditions. The procedure is simple and pleasant to operate.

The alfalfa is rich in nutritive value. The chemical analysis of the low molecular weight fraction showed high content of guanidino compounds. It was first considered as an unusual, free amino acid, since it is Sakaguchi positive and is not hydrolysed by the specific enzyme arginase. However, careful analysis by gel chromatography that it is an oligopeptide having molecular weight between 1500 - 1800 dalton. It contains amino sugars confirming that it is glycosylated. It was also

observed that the oligopeptide under consideration is arginine rich and contains traces of other amino acids.

It is known that the arginine is the storage compound of the plant. Hence, arginine rich oligopeptide may be the preferred storage product to minimize excretion of nitrogen. The metabolic role of this compound including its possible role as neurotransmitter warrants detailed structural analysis.

EFFECT OF pH ON ADSORPTION OF ARGININE ON AMYLOSE GEL I

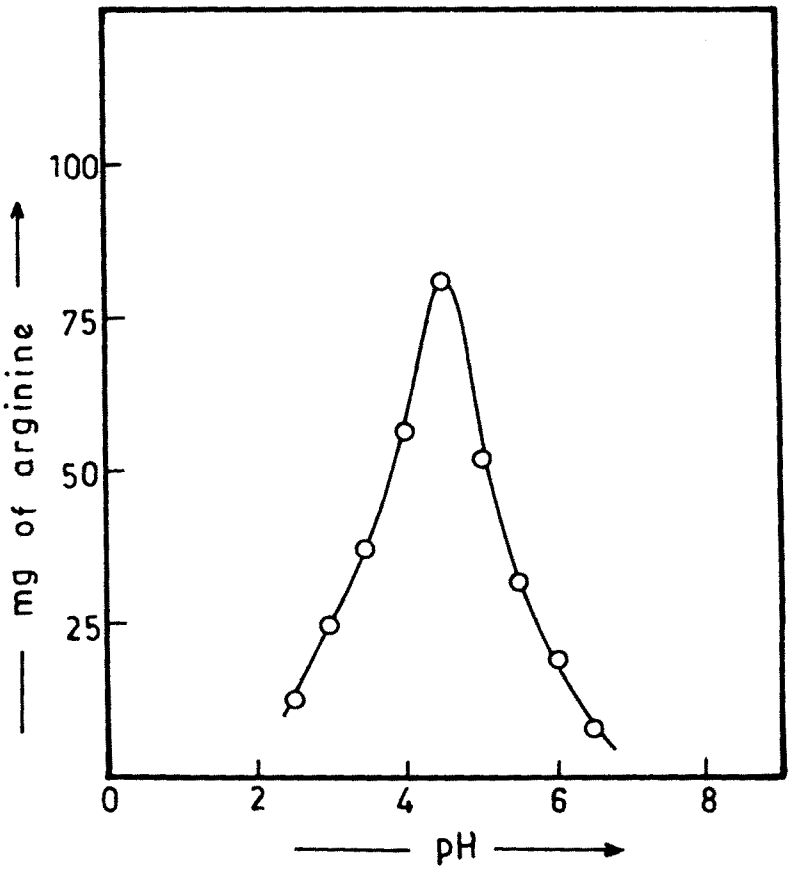


FIG. IX The arginine solution (1mg/ml) was stirred with amylose gel (1.5 ml) for 30-45 minutes. The amylose gel was washed with citrate buffer at same pH. The supernatant was assayed with ninhydrin reagent to account for unadsorbed arginine. The difference (Total - Unadsorbed) arginine is plotted against pH.

EFFECT OF pH ON ADSORPTION OF ARGININE ON AMYLOSE GEL - II

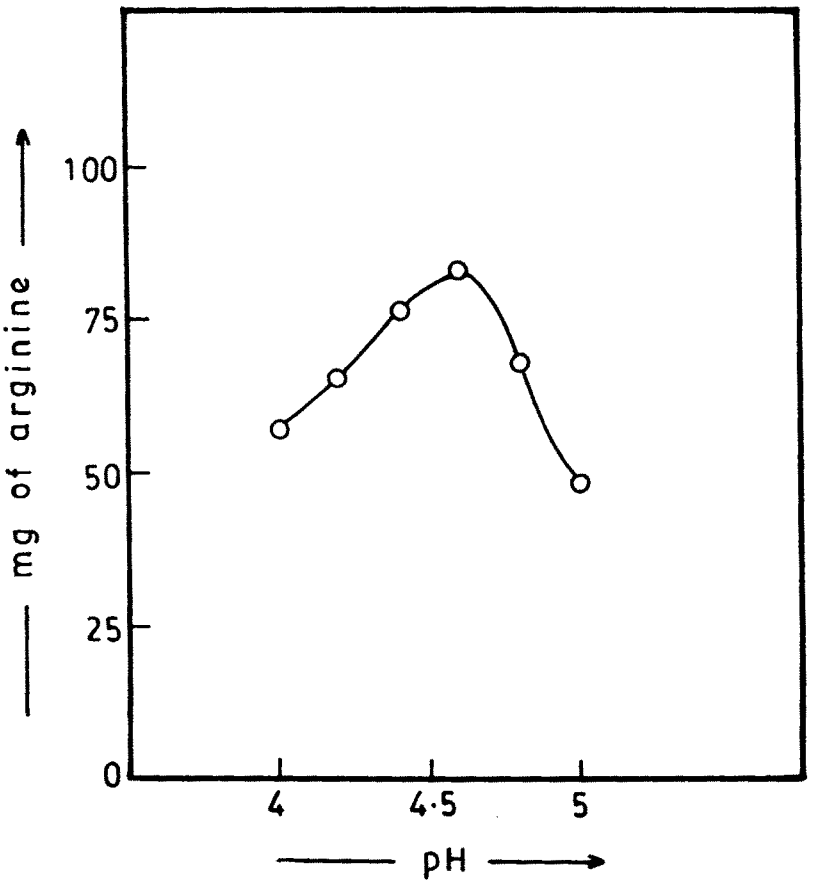


FIG. X The arginine solution (1mg/ml) was stirred with amylose gel (1.5 ml) for 30-45 min. with citrate buffer at same pH. The supernatent was assayed with ninhydrine reagent to account for unadsorbed arginine. The difference of Total - Unadsorbed arginine is plotted against pH.

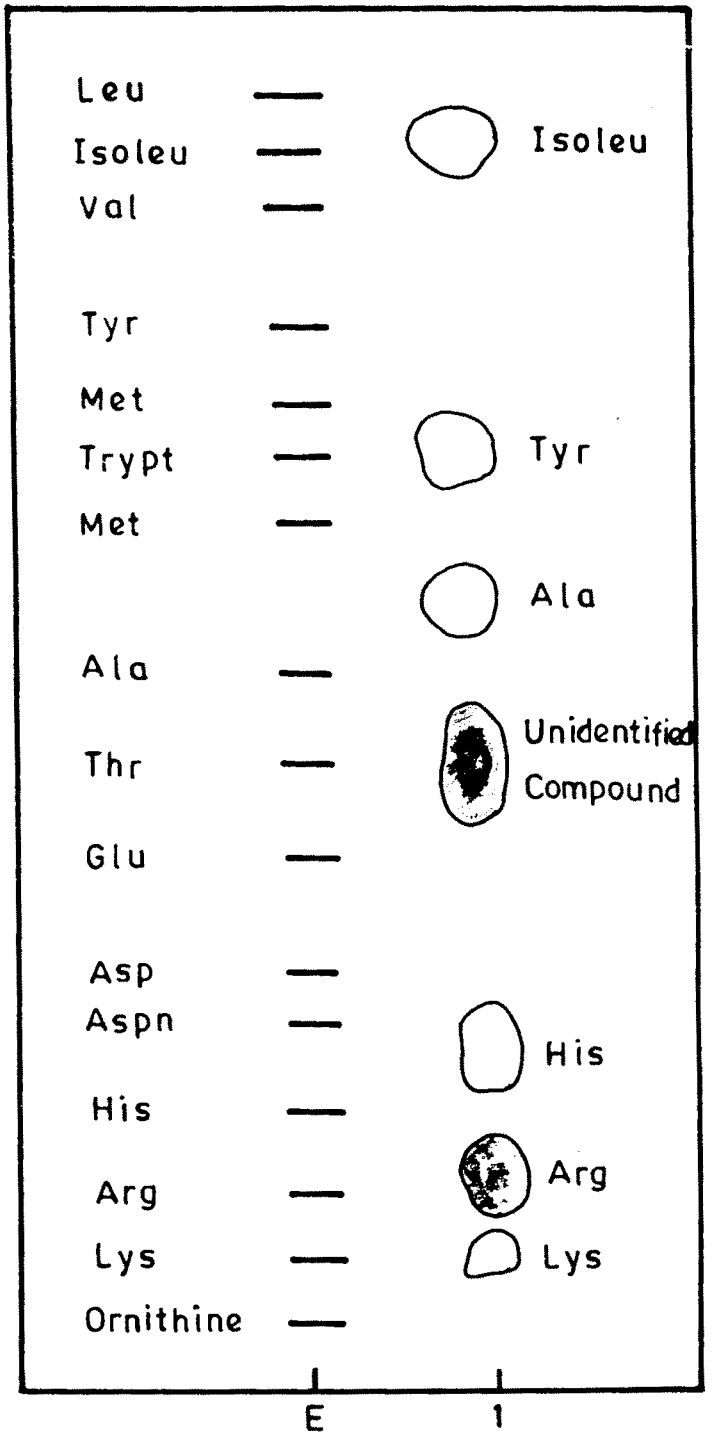


FIG. XI PAPER CROMATOGRAPHY OF AMINO ACIDS

I) An aliquot of the desorbed alfalfa filtrate from amylose gel was spotted, and run for 18 hrs using the system, butanol:acetic acid:water (4:1:5). Amino acids were detected using ninhydrin spray reagent and drying at 105° for 2min.

E) An aliquot of each of individual pure amino acid was run under similar conditions for standard Rf values.

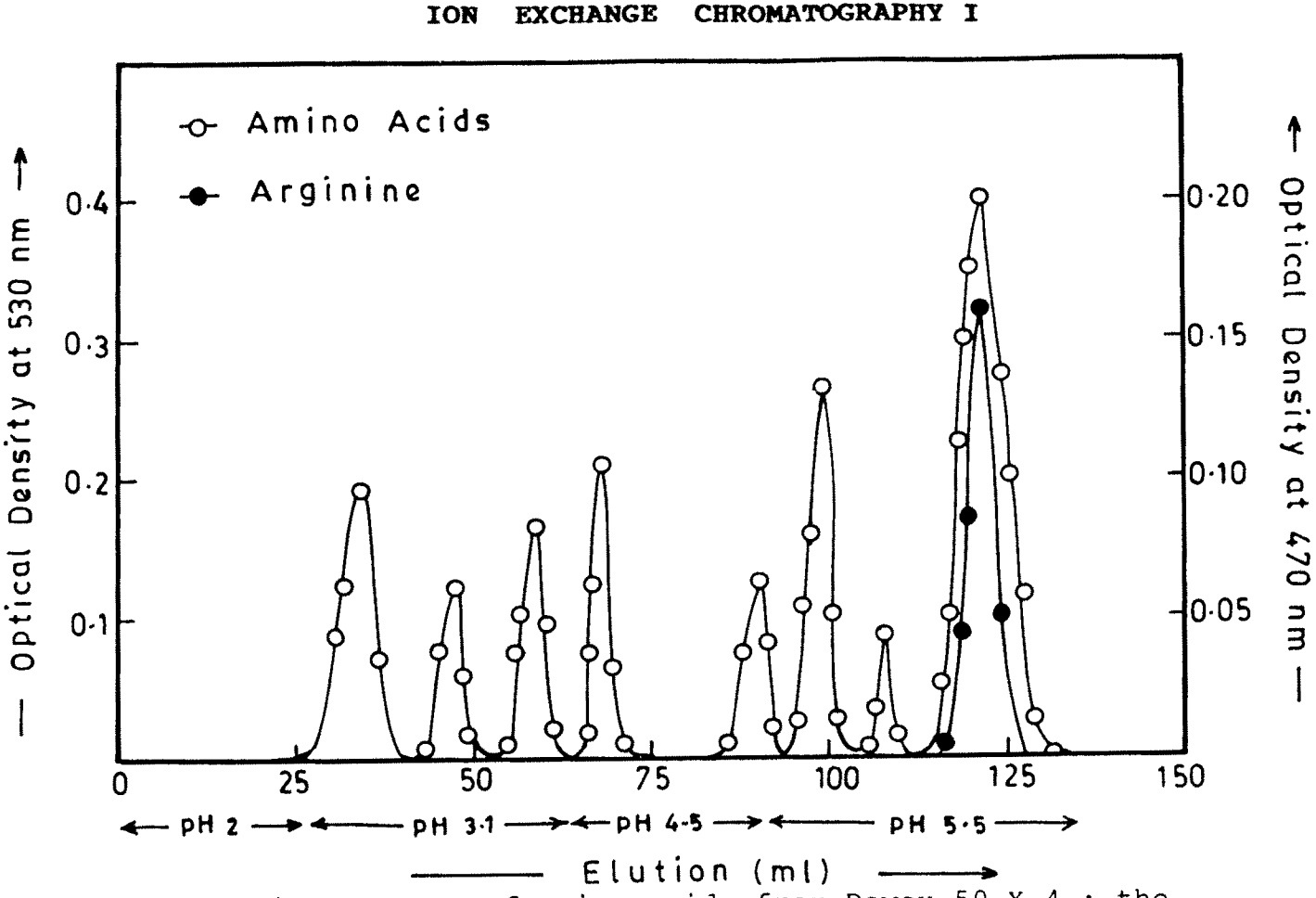


FIG.XII Elution pattern of amino acids from Dowex 50 X 4 : the column was loaded with dialysed alfalfa filtrate, previously adsorbed on amylose gel, using pyridine acetate buffer with change in strength & pH. Fractions of 2 ml were collected & aliquots (20 Ul) were analysed for amino acid with ninhydrin (-o-) & for arginine with modified Sakaguchi Reagent (-●-).

GEL FILTRATION I

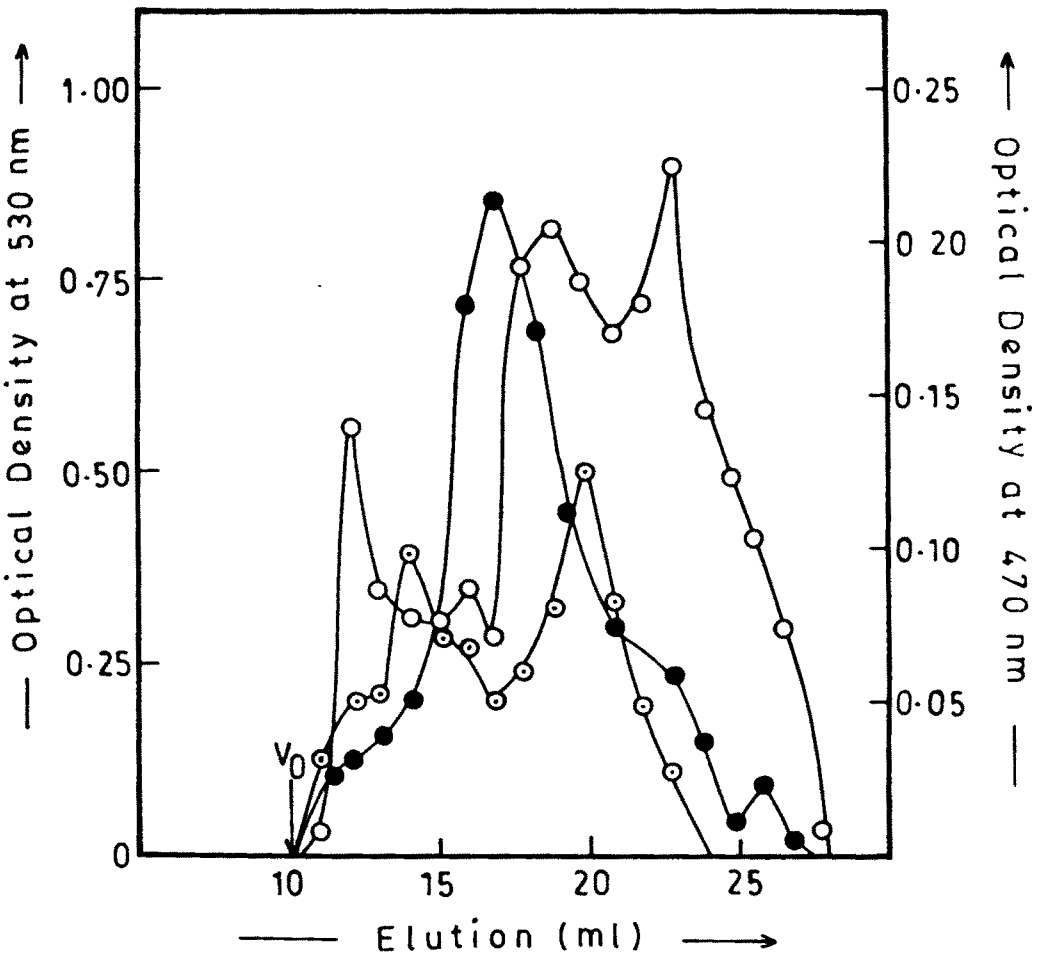


FIG.XIII Elution pattern of purified guanidine compound : the column was loaded with 2 mg of guanidine compound purified previously from Dowex 50 X 4 column. The fractions of 1 ml at the rate of 4 ml/hr were collected & aliquotes (20 Ul) were analysed for carbohydrate with phenol sulphuric acid method (-o-), amino acid with ninhydrin reagent (-●-), and arginine with modified Sakaguchi Reagent (-⊙-).

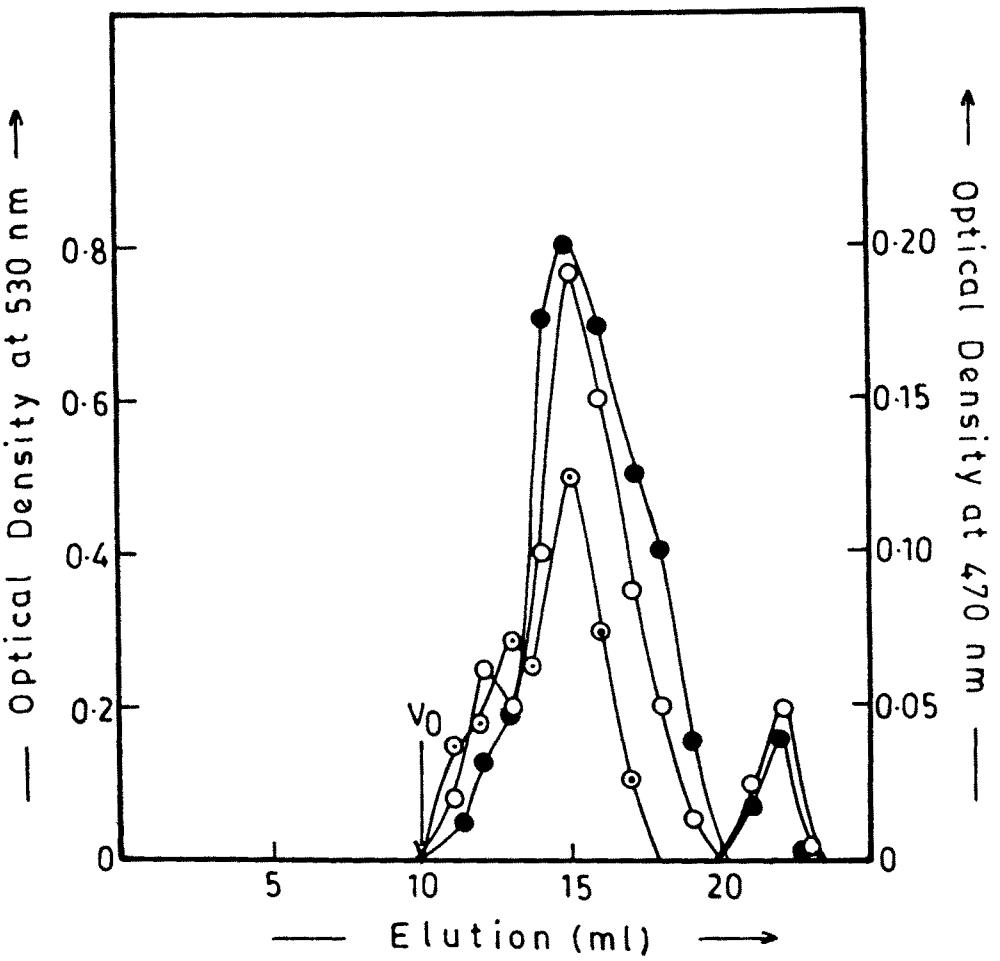


FIG.XIV Elution pattern of purified guanidine compound : the column was loaded with the guanidine compound, which was pooled (fraction No.15, 16, 17) from the previous Bio-Gel P-2 chromatography and concentrated to 0.1 ml. Fractions of 1ml at the rate of 4 ml/hr were collected & aliquots were analysed for carbohydrate with phenyl sulphuric acid method (-o-), amino acids with ninhydrin reagent (-●-) & arginine with modified Sakaguchi Reagent (-⊙-).

GEL CHROMATOGRAPHY III

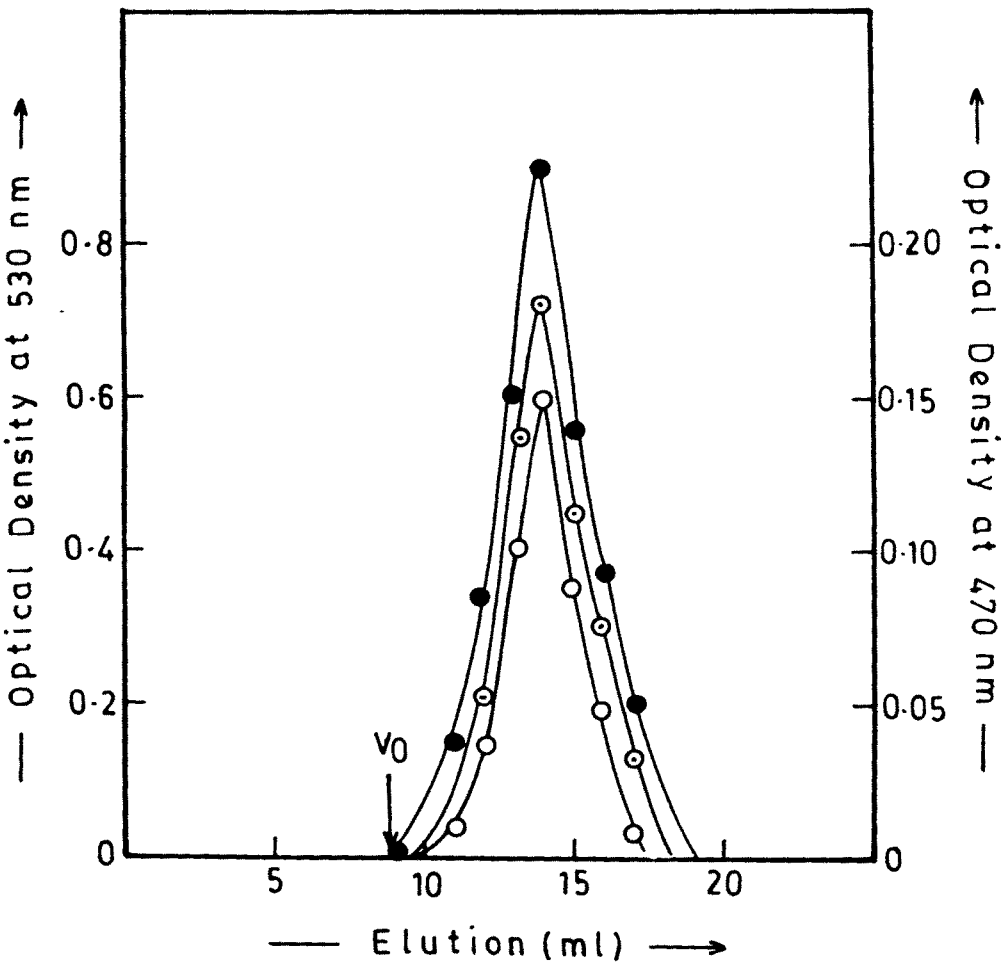


FIG. XV Elution pattern of purified guanidine compound : the column was loaded with guanidine compound, which was pooled (fraction No. 15) from the previous Bio-Gel P-2 column & concentrated to 0.1 ml. Fractions of 1 ml (flow rate 4 ml/hr) were collected & analysed for carbohydrate with phenol sulphuric acid method (-⊙-), amino acid with ninhydrin method (-●-) & arginine with modified Sakaguchi Reagent (-o-).

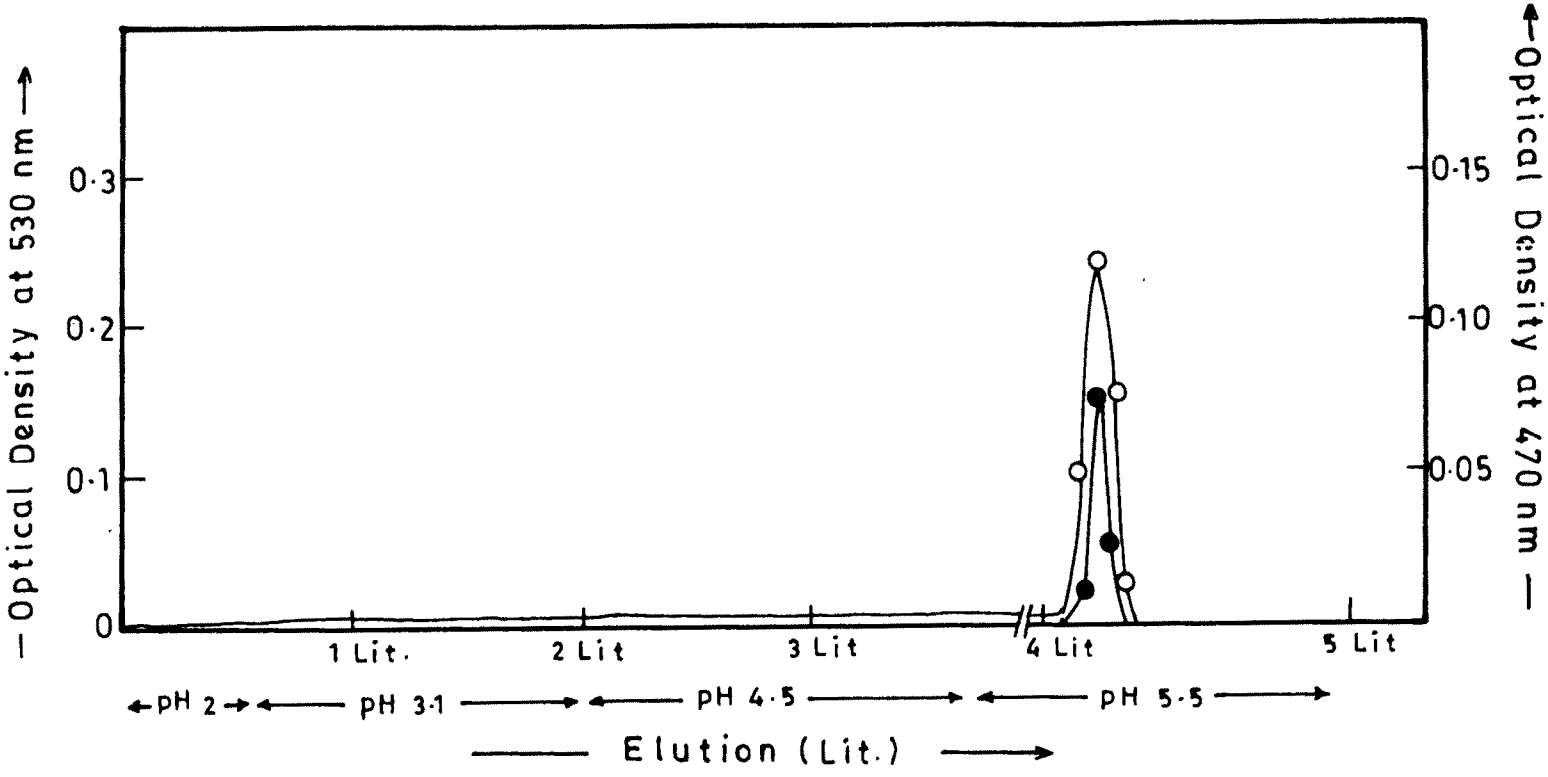
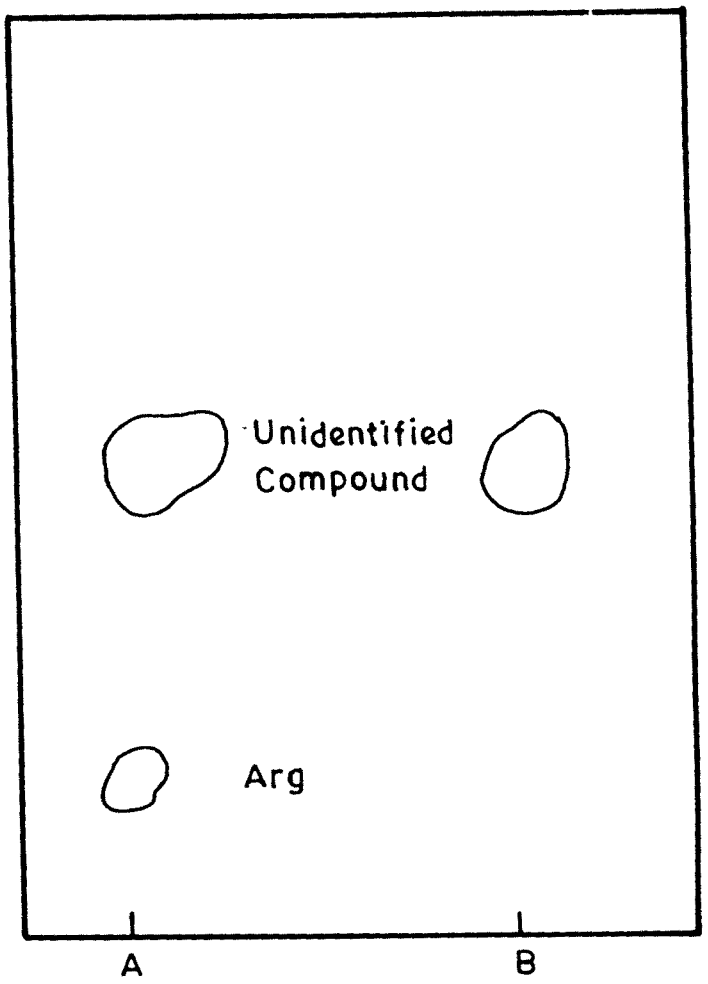


FIG. XVI Elution pattern of amino acids from Dowex 50 X 4 : the column was loaded with Arginase treated Guanidine Compound, purified previously from the same column, using pyridine acetate buffer. Two litres of buffer of pH 3.1 & 4.5 were passed. At pH 5.5, fractions of 5 ml were collected & aliquots (50 Ul) were analysed for amino acid with ninhydrin reagent (-o-) for arginine with modified Sakaguchi Reagent (-●-).



PAPER CHROMATOGRAPHY OF AMINO ACIDS

FIG.XVII A) An aliquot of arginine positive peak from ion exchange chromatography was spotted and run for 18 hrs using solvent system, butanol:acetic acid :water (4:1:5). The amino acids were detected by using ninhydrin spray reagent and drying the chromatogram at 105° for 2 min.

B) An aliquot of arginase treated guanidine positive fraction from ion-exchange chromatography was spotted, run and developed in a similar manner.

CALIBRATION CURVE FOR AMINO ACID

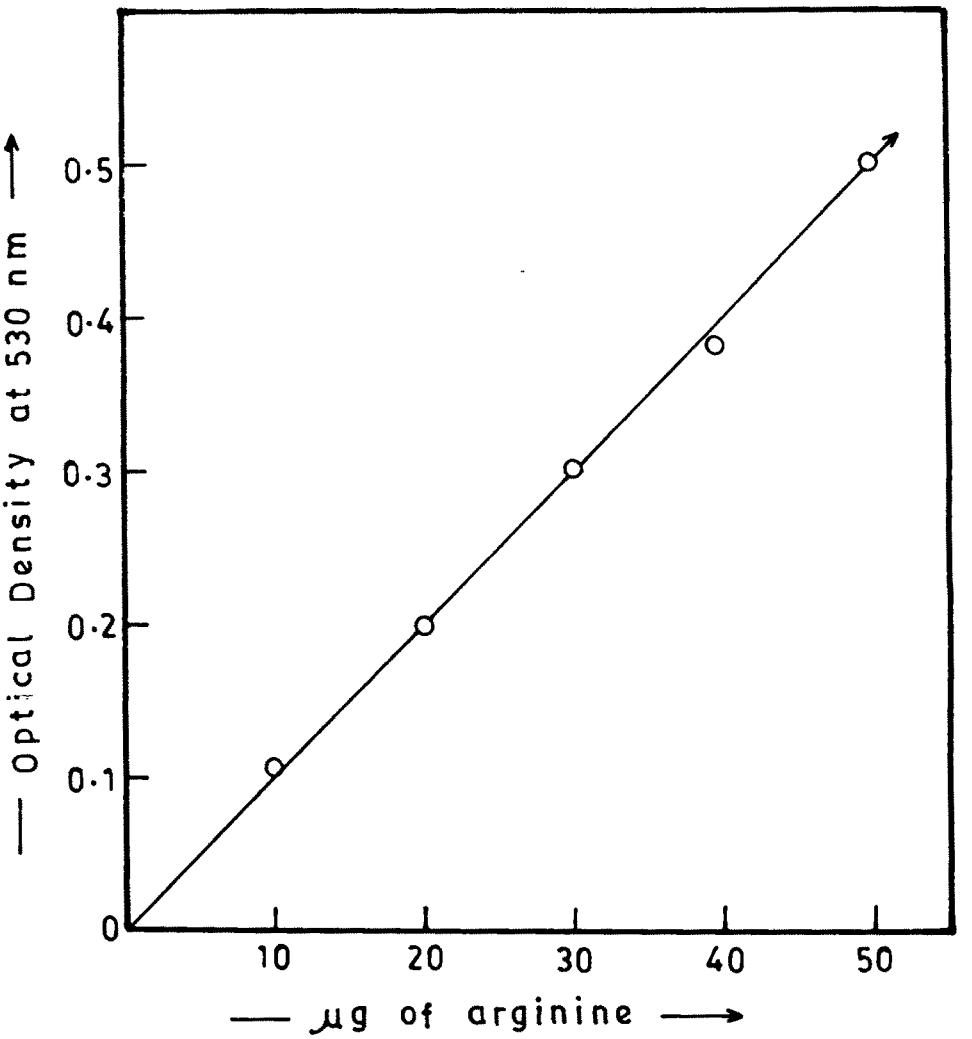


FIG.XVIII The ninhyrin reagent method of Moore & Stein was used. The reaction mixture consisted of arginine (10-15 Ul/ml) + 0.5 ml ninhydrin (3%), boiled in water bath for 20 minutes, the purple colour was read at 530 nm.

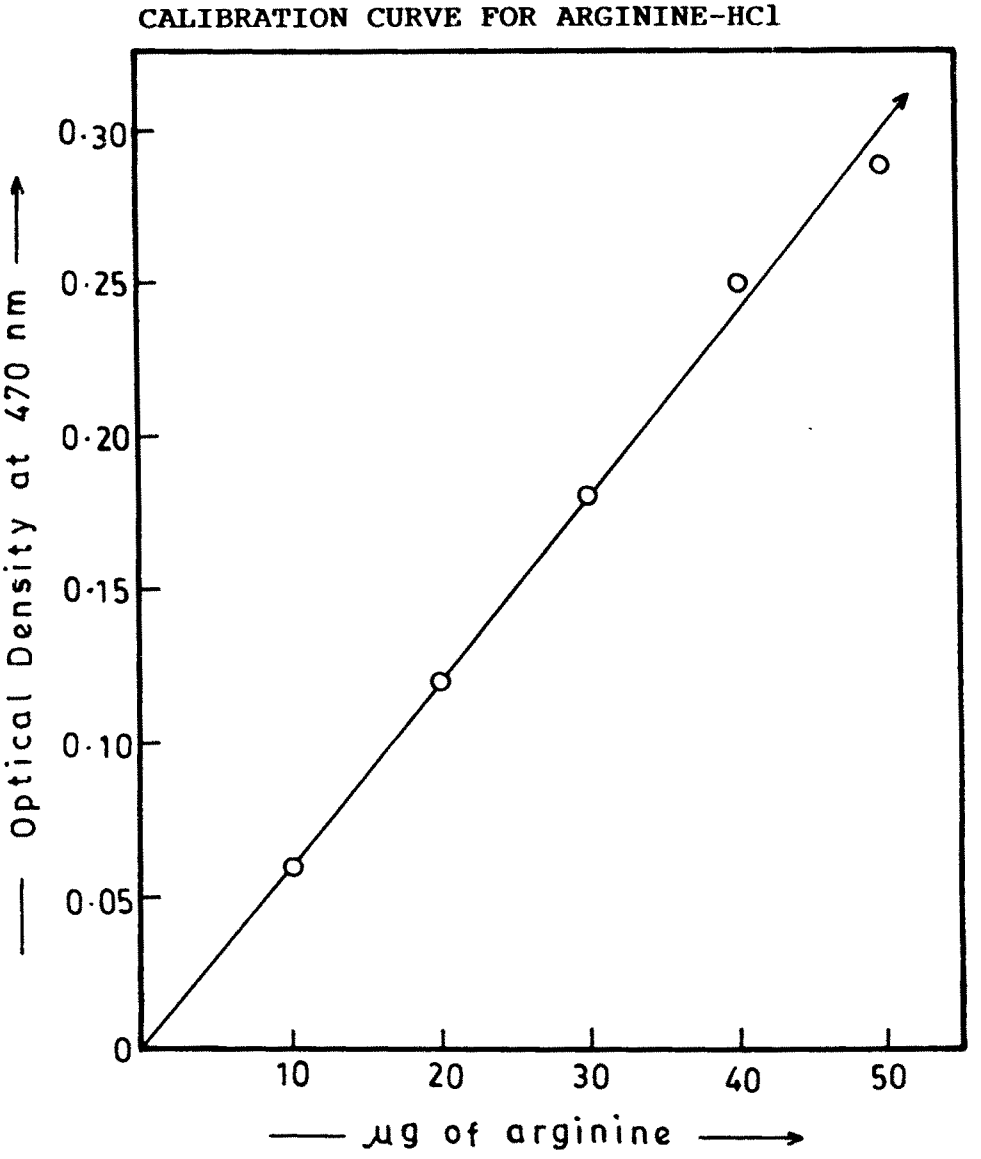


FIG.XIX The modified method of Sakaguchi Reaction was used. The reaction mixture consisted of arginine (10-50 Ug/ml) + 1 ml NaOH (40%) + 0.2 ml ∝ - naphthol (0.1%) and 1 ml N-Bromosuccinimide (0.1%) The orange red colour was read at 470nm.

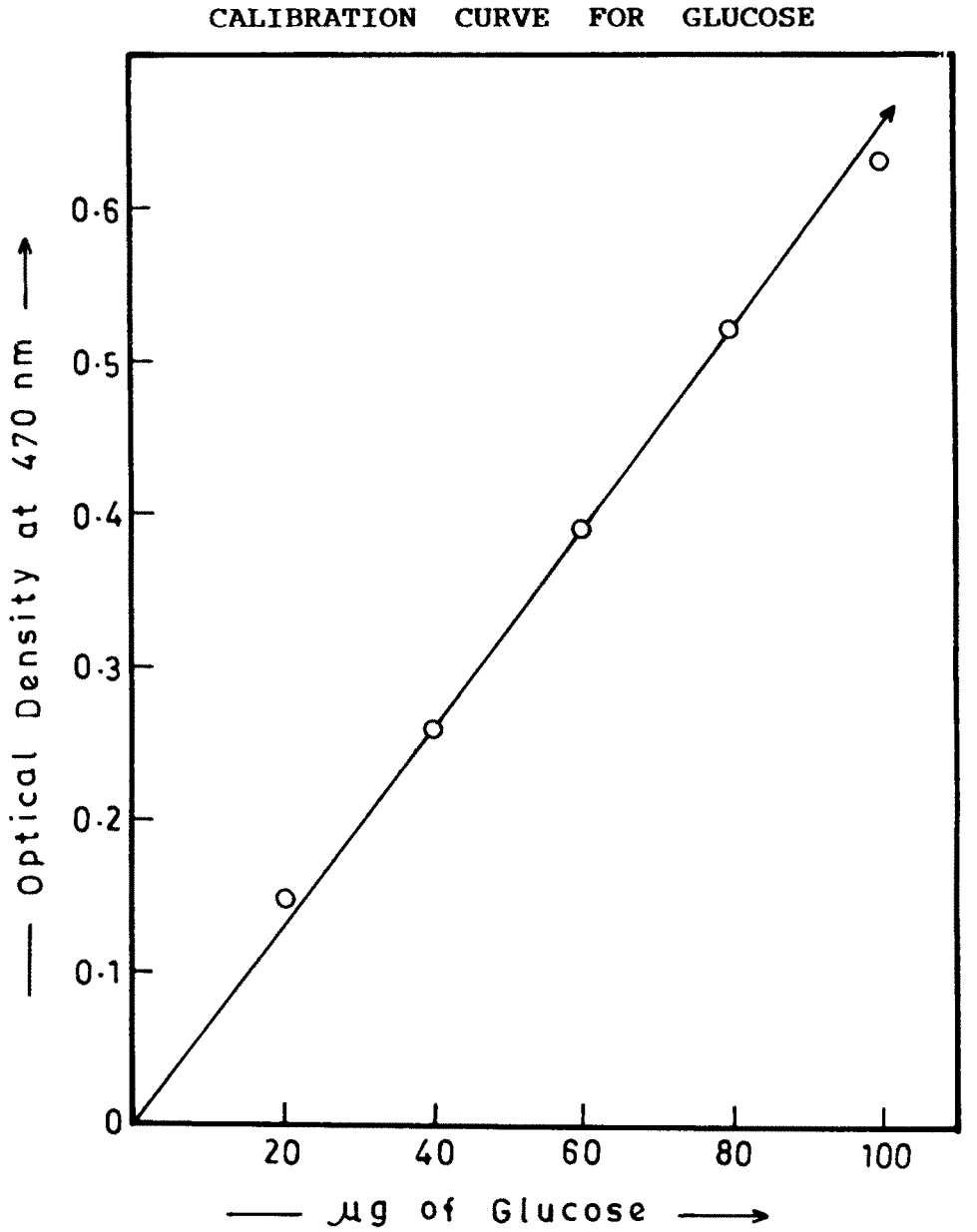


FIG. XX The phenol sulphuric acid method is used for the calibration curve. The reaction mixture consisted of glucose (20-100 Ug/ml) + phenol (0.5 ml, 7%) + Conc. H_2SO_4 (2 ml). The red colour was read at 470 nm.

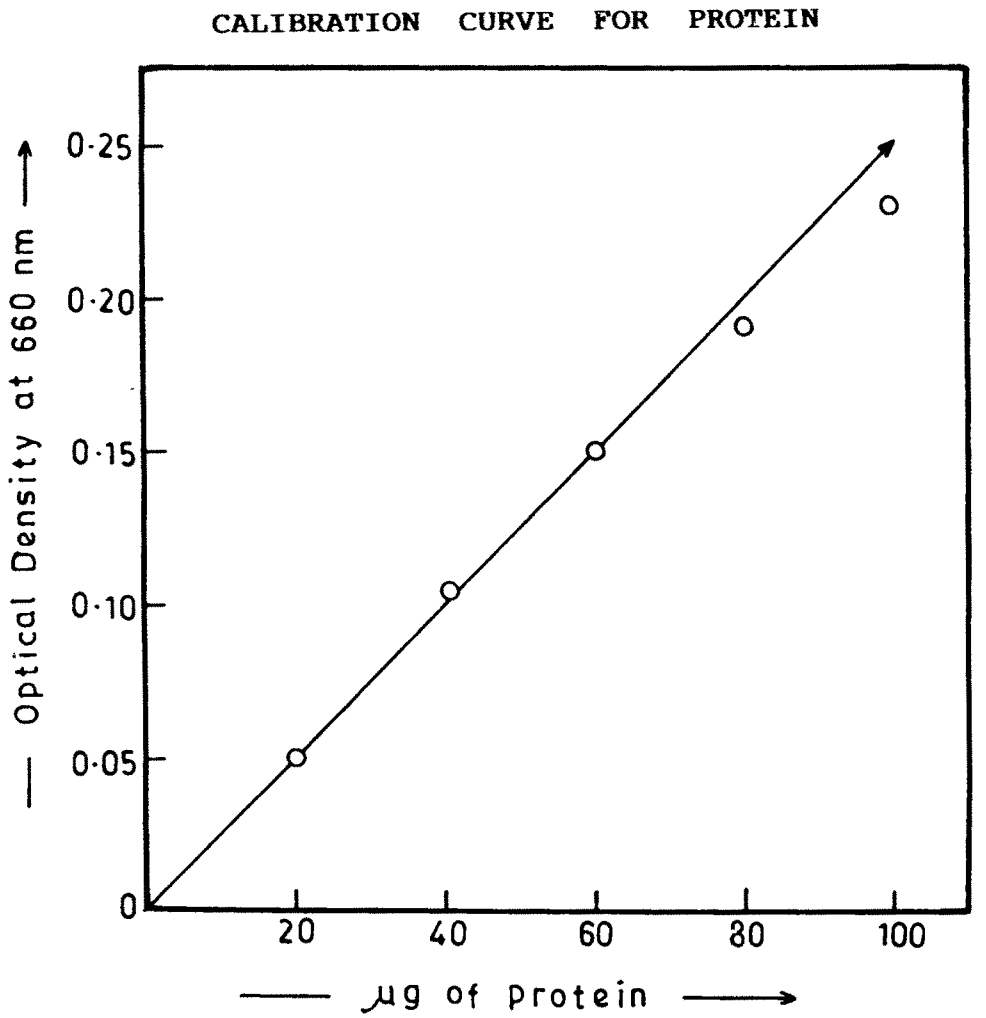


FIG. XXI The method of Lowry et al was used. The reaction mixture consisted of protein (BSA - 20-100Ug/ml) + 5 ml Lowry C and 0.5 ml of Folin Phenol Reagent. The blue colour is read at 660 nm.