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Isolation of CA 125 from Breast Tumor Tissue Using Gel Chromatography

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ABSTRACT:-

Gel filtration chromatography technique was used (Sepharose CL – 6B) of CA 125 from malignant breast tumor homogenate and the isolation of (CA 125 / ^{125}I – anti CA 125 antibody), complex was prepared from crude CA 125 , isolated CA 125 and standard human CA 125. The results revealed the presence of two forms of CA 125 (AgI & AgII) and found to be 517 Kda and 38 Kda respectively. The isolation of CA 125 showed a purification fold of 17.5 AgI and 15 for AgII.

عزل ال (CA 125) من انسجة ثدي مصاب بالسرطان باستخدام الكروماتوكرافي

نزار أحمد ناجي

المستخلص:-

تم في هذا البحث استخدام تقنية الترشيح الهلامي (Sepharose CL – 6B) لفصل مستحضر الكربوهيدرات (CA 125) المفصول من متجانس سرطان الثدي الخبيث و قد حضر معقد المضاد (CA 125/^{125}ICA 125) من متجانس الخام لل (CA 125) ومن (CA 125) المفصول و من (CA 125) البشري القياسي. أظهرت النتائج وجود شكلين لل (CA 125) هما (AgI & AgII) . ثم استخدمت تقنية الترشيح الهلامي ذاتها في قياس الوزن الجزيئي لل (CA 125) المعزول (AgI & AgII) حيث كان (38 Kda & 517 Kda) على التوالي. أظهر فصل (CA 125) عدد مرات تنقية مقدارها (15 AgI & 17.5 AgII).

INTRODUCTION:-

Chromatography encompasses a diverse and important group of separation methods that permits the scientist to separate isolate, and identify related components of complex mixtures. The purification of biomolecules is the most popular use of gel chromatography due to the ability of a gel to fractionate molecules on the bases of size [1]. Carbohydrate antigen 125 (CA 125 antigen) has been characterized as a high molecular mass glycoprotein aggregate. It has wide molecular weight heterogeneity from 50 – 300 Kda [2]. Many researchers tried to purify it from many sources like sera of ovarian cancer patients, ovarian cancer cell line and human milk using size exclusion chromatography [3, 4]. There was also a study to isolate (CA 125) antigen from amniotic fluid and fetal membrane by gel filtration and then by SDS electrophoresis [5]. Since there were no studies dealing with the isolation, purification, or characterization of (CA 125) antigen from breast cancer tissues, accordingly the aim of this study deals with the isolation of (CA 125) from malignant breast tumor tissues (rather than the blood of breast cancer patients) and then the optimum conditions for its binding to the labeled antibody were carried out.

PATIENT & METHODOLOGY:-

In the present work three groups of patients of breast cancers were selected according to the investigation of a histopathologist. The first group includes the malignant post – menopausal breast patients (GI), the second contains the malignant pre – menopausal tumor patients (GII) and the third group comprises of patients with benign breast tumor patients (GIII). The fourth group consists of 16 apparently healthy individuals and considered as a control group. (Table 1 – 1) describes the host information for patients and control individuals in this study. All selected patients were admitted for treatment at [Al – Yarmook Teaching Hospital & Al – Kadhimiyah Teaching Hospital]. All surgical operations of breast tumor were carried out under the supervision of surgeons.

COLLECTION OF SPECIMENS:-

The tumor was surgically removed from breast tumor patients by either mastectomy (cancer patients) or lumpectomy (benign tumor patients). The specimens were immediately kept in a saline solution and stored at - 20°C to the time of homogenization.

HOMOGENIZATION OF BREAST TUMOR TISSUES:-

The frozen tissue was sliced finely and scalped in petridish standing on ice, and then homogenized with three fold volumes of buffer pH 7.2 using manual homogenizer [6]. The homogenate was filtered through four layers of nylon gauze to eliminate fiber connective tissues. The filtrate was centrifuged at 400 rpm for 30 minutes at 4°C in order to precipitate the remaining intact cells and the intact nucleus. The supernatant and precipitate fractions were separated and frozen at - 20°C until used.

DETERMINATION OF PROTEIN CONCENTRATION:-

Total proteins were determined by Lowry method [7] using bovine serum albumin (BSA) as a standard. The standard curve of protein concentration was constructed by measuring the absorbance of the different standards at 750 nm. The straight line equation for this standard curve was found and used for determination of homogenate protein content.

PREPARATION OF THE GEL:-

The gel was prepared by allowing the pre – swollen gel to swell again in tris – buffer (0.05 M) pH 7.2 then left to settle and the excess of buffer was decanted. The step was repeated several times. The gel was degassed using evacuation pump and slurry was left for 24 hrs to equilibrate with buffer. The swollen gel was suspended and carefully poured into a vertical glass column (0.94 X 27 cm) down the wall using a glass rod. After the gel has settles, the column was equilibrated with tris –buffer for 24 hrs.

VOIDVOLUMEDETERMINATION:

The void volume of column was determined by using blue dextran 2000 at concentration of 2 mg / ml dissolved in tris – buffer pH 7.2, and then the elution was carried out with the same buffer at a flow rate of 9 ml / hr. Faction of 1 ml were collected and their absorbance was measured at 600 nm.

SEPARATION OF CA 125 FROM MALIGNANT TISSUE:-

Postmenopausal Homogenate: The sample of tissue homogenate (444 ul) containing 8 mg protein was applied to the surface of the gel, and then equilibrated with 0.05 M tris – buffer pH 7.2. The sample was eluted by using the same buffer with a flow rate of 9 ml / hr and fractions volume of 1 ml each were collected, and the gel filtration was carried out at 10°C. The protein content of each fraction was determined using Lowry method and absorbance of each fraction was measured at 280 nm. The fraction that contained CA 125 was identified by the assay method. The binding of each fraction was calculated and plotted against the fraction number. The same procedure was repeated for standard CA 125 antigen equipped with the IRMA kit. The purification fold of CA 125 was calculated from the following equation:

Purified fold = [bound of purified fold CA 125 / mg protein] / [bound of crude CA 125 / mg protein]

SEPARATION OF (^{125}I – Anti CA 125 Ab / CA 125 Ag) COMPLEX:-

CA 125 reacts with ^{125}I – Anti CA 125 antibody at their optimum conditions. At the end of reaction 444 ul of the reaction mixture was applied to the surface of the gel, equilibrated with tris – buffer pH 7.2, a flow rate of 9 ml / hr was used and 1 ml fractions were collected. The radioactivity of each fraction was counted. This experiment was repeated for standard CA 125 equipped with the kit. At the same time, the free ^{125}I – Anti CA 125 antibody has been poured into the column and the radioactivity for each fraction was recorded.

DETERMINATIONOFMOLECULAR WEIGHT FOR ISOLATED CA 125:-

The molecular weight of the isolated CA 125 was determined by using gel filtration technique (Sepharose CL – 6 B) and calibrated with Pharmacia Calibration kit. Highly purified high molecular weight proteins (thyroglobulin, catalase, ferritin and aldolase) as standard proteins. Thyroglobulin

plateau region where the binding is almost constant inspite of an increase in protein concentration. The shape of the curve is similar to that generated for the crude CA 125, but the amount of purified protein was less than that used in crude CA 125 antigen (17 ug for purified AgI, 14 ug for AgII, 402 ug for crude).

Optimum ^{125}I – anti CA 125 Antibody Concentration: (Figure 9 A&B) describe the effect of increasing ^{125}I – anti CA 125 antibody concentration of binding with both AgI respectively. The curves were of saturation type in which the binding increases to a point after which it will stay constant. The same behavior been seen with respect to that generated for crude CA 125, but the concentration for ^{125}I – anti CA 125 antibody is less (2.9 mg for AgI, 2.1 mg for AgII and 2.6 mg for crude).

Optimum pH: The pH effect on binding of partially purified CA 125 was studied. (Figure 10 A&B) shows the binding of AgI & AgII to the ^{125}I – anti CA 125 antibody. The optimum pH for AgI was 7.4 and 7.6 for AgII. An increase of about 0.2 – 0.4 unit of pH in comparison to that crude CA 125 of GII. This change may be due to the polar groups of the amino acids residues present in the binding medium that may be lost by exclusion process during gel filtration.

Time Course of AgI and AgII of the Binding of CA 125 to ^{125}I – anti CA 125 Antibody: The optimum time and temperature for the purified CA 125 (AgI & AgII) (Figure 11 A&B) show a change in their values relative to that found for crude CA 125 IN GII. For AgI the optimum time was 1 hr and optimum temperature was 5°C where the time was shortened 1 hr to that for crude CA 125, while 5°C is still the maximum binding temperature for both. On the other hand, the optimum time for AgII was also 1.5 hr, while the temperature was 25°C. These changes may relate to the isolation of CA 125 from many proteins by size exclusion process that results in changing the medium of reaction.

Table (1) Describe the host information for patients and control individuals in this study

Group	Patients	No.	Age range	Type of tumor
GI	Post – menopausal breast tumor	23	55 – 80	Infiltrative ductal carcinoma
GII	Pre – menopausal breast tumor	17	23 – 48	Infiltrative ductal carcinoma
GIII	Benign breast tumor	25	19 – 52	Fibroadenoma
Control	Healthy individuals	16	23 - 36	None

Table (2) Purification of the isolated CA 125 antigen from post – menopausal breast cancer homogenates (GI) using gel filtration chromatography

CA 125	Protein (mg)	Specific binding	Specific binding / mg protein	Purification fold
GI	0.361	9.5	26.3	1.0
AgI	0.017	7.8	458.8	17.5
AgII	0.014	5.5	393	15.0

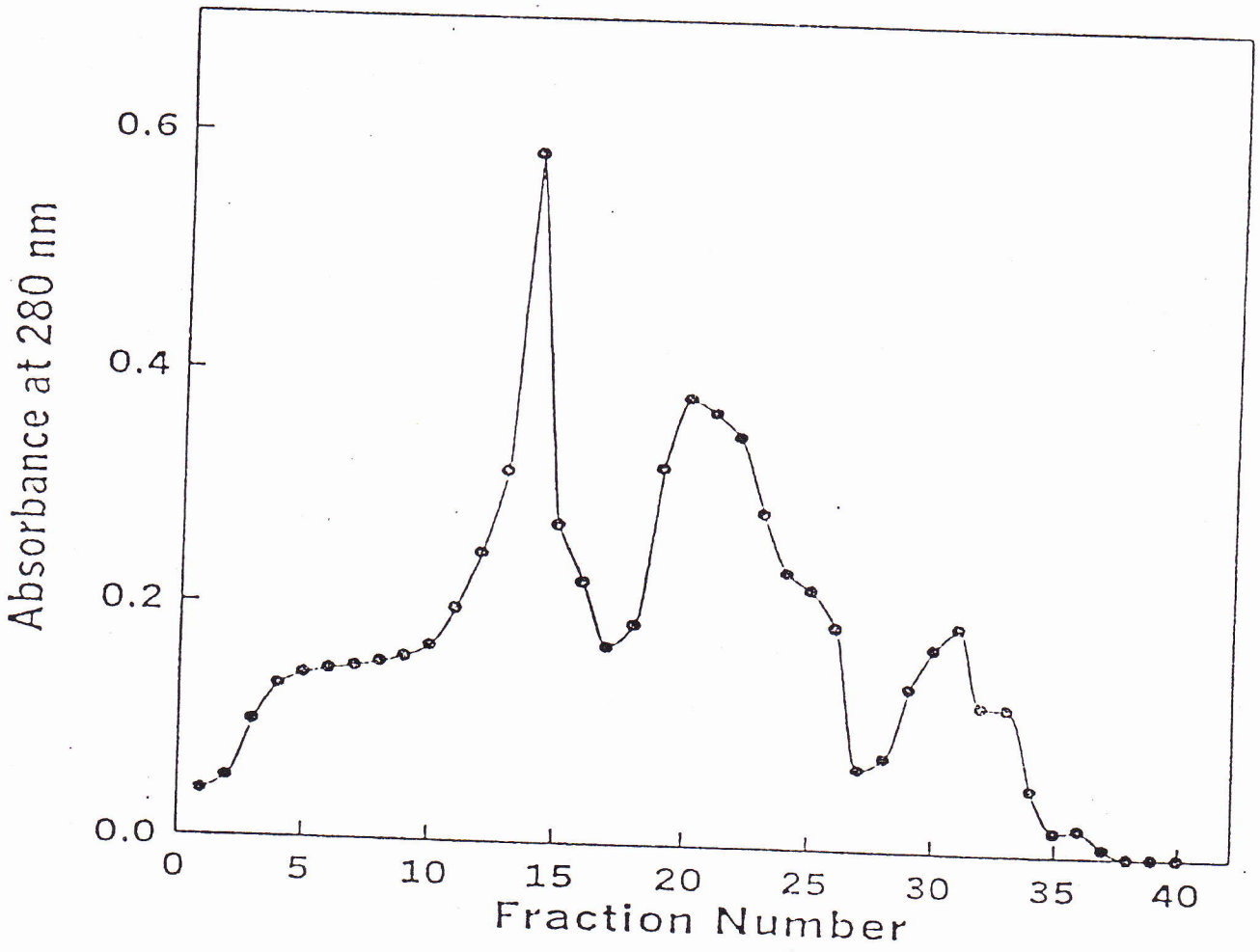

Figure 1: The elution profile of GI homogenate, using Sepharose CL – 6B gel, 9 ml / hr flow rate, tris – buffer pH 7.2, 0.02% sodium azide at 25°C.

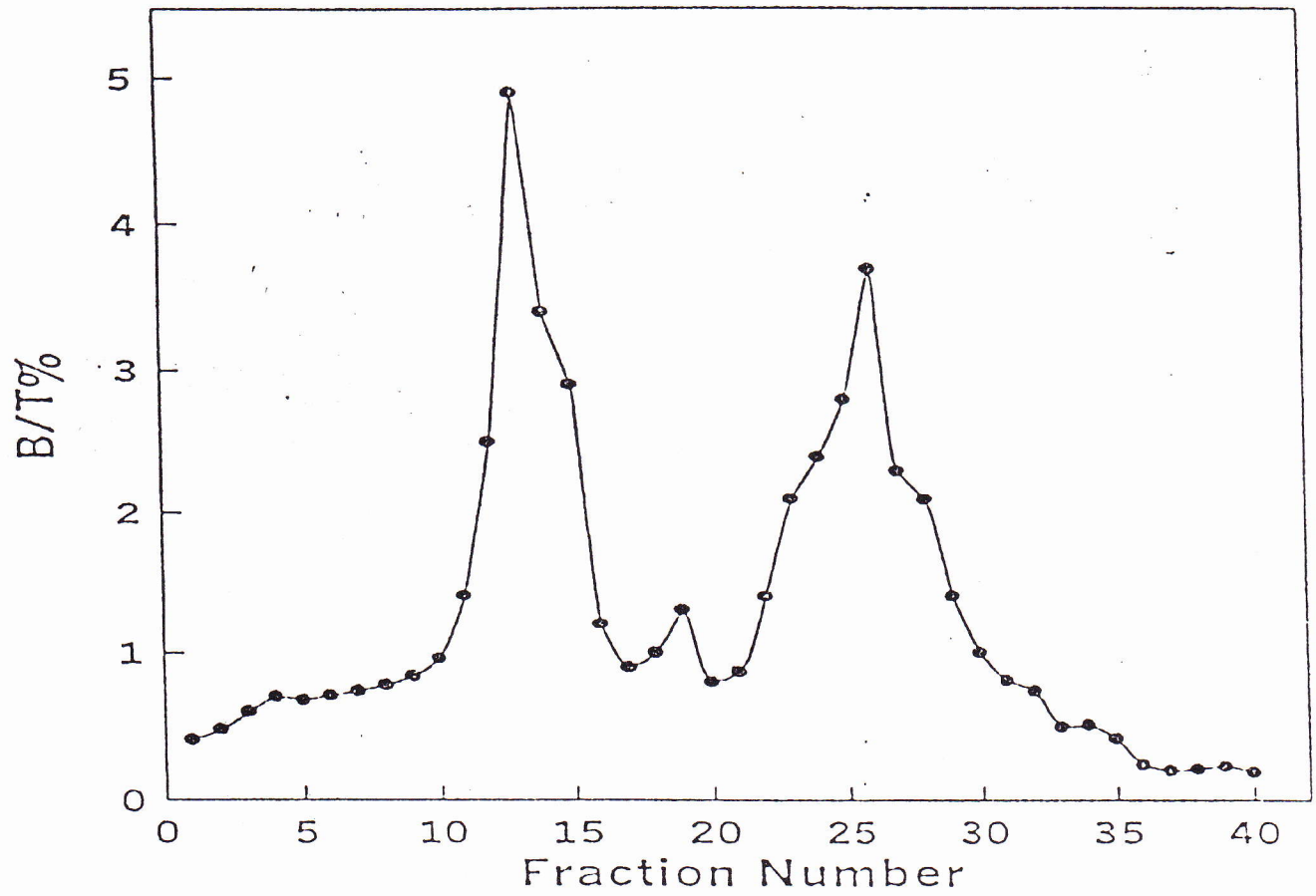

Figure 2: The binding of the eluted fractions of GI homogenate at the optimum reaction conditions of GI.

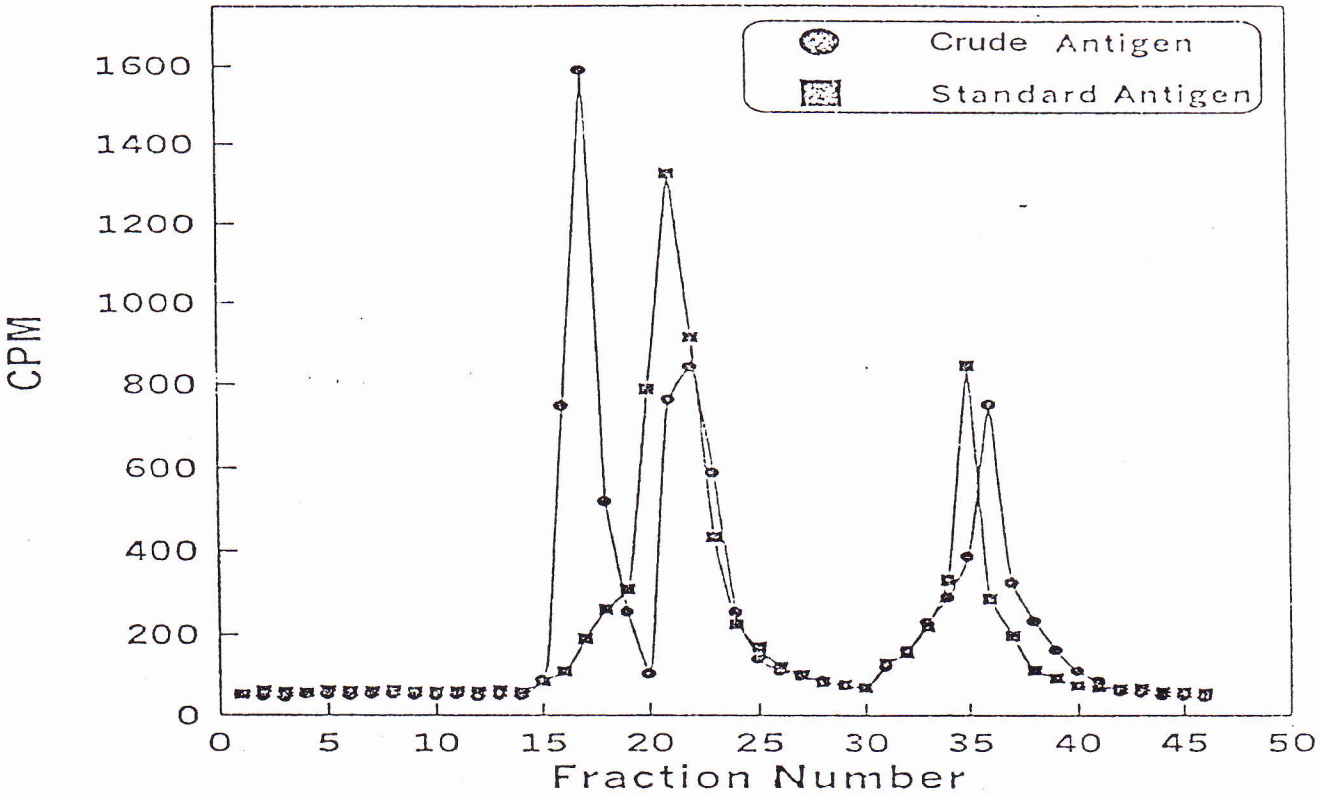

Figure 3: The elution profile of complexes prepared from GI homogenate and standard CA 125 antigen, using Sepharose CL – 6B gel, 9 ml / hr flow rate, tris – buffer pH 7.2, 0.02% sodium azide at 25°C.

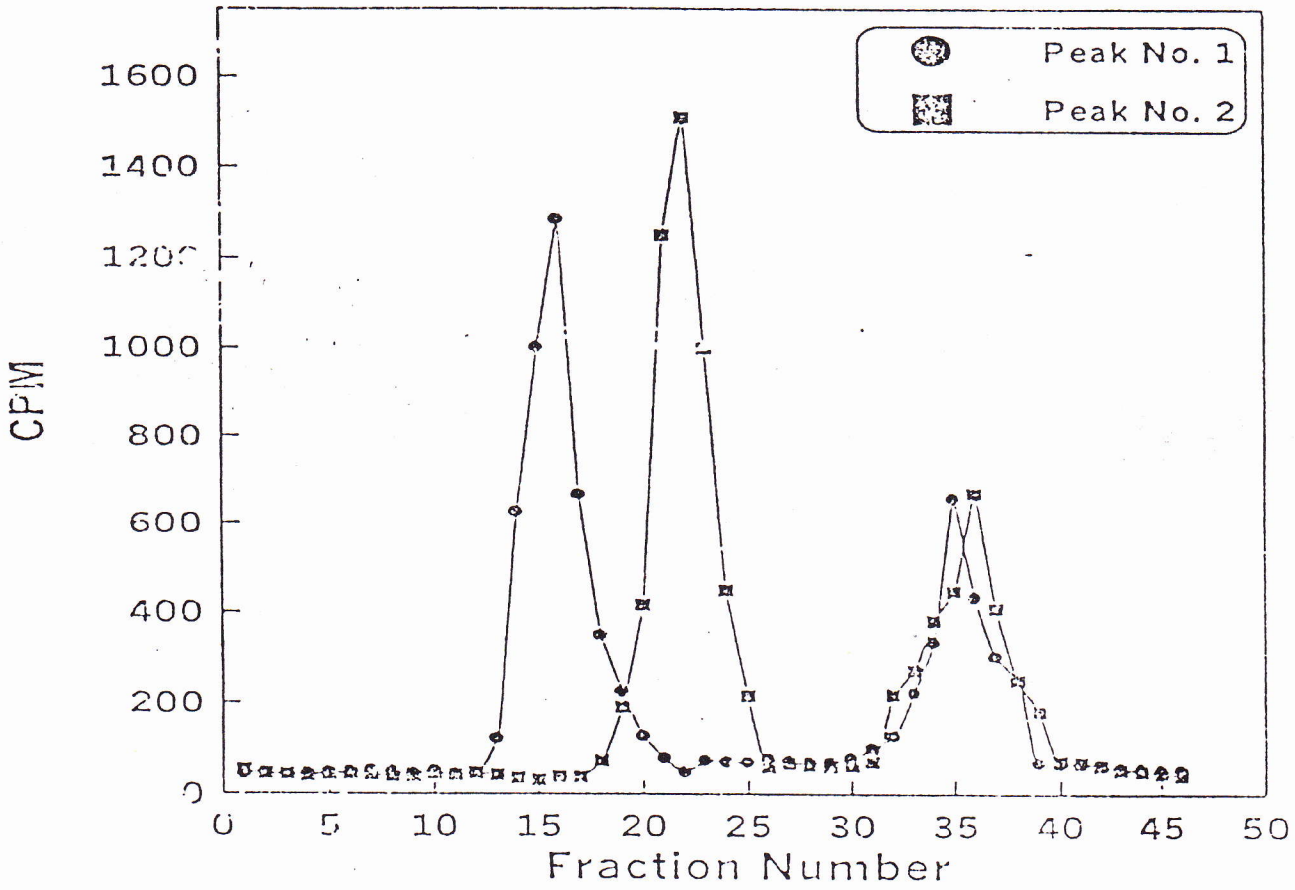

Figure 4: The elution profile of complexes prepared from pooled peaks separated by gel filtration from the GI homogenate, using sepharose CL – 6B gel, 9 ml / hr flow rate, tris – buffer pH 7.2, 0.02% sodium azide at 25°C.

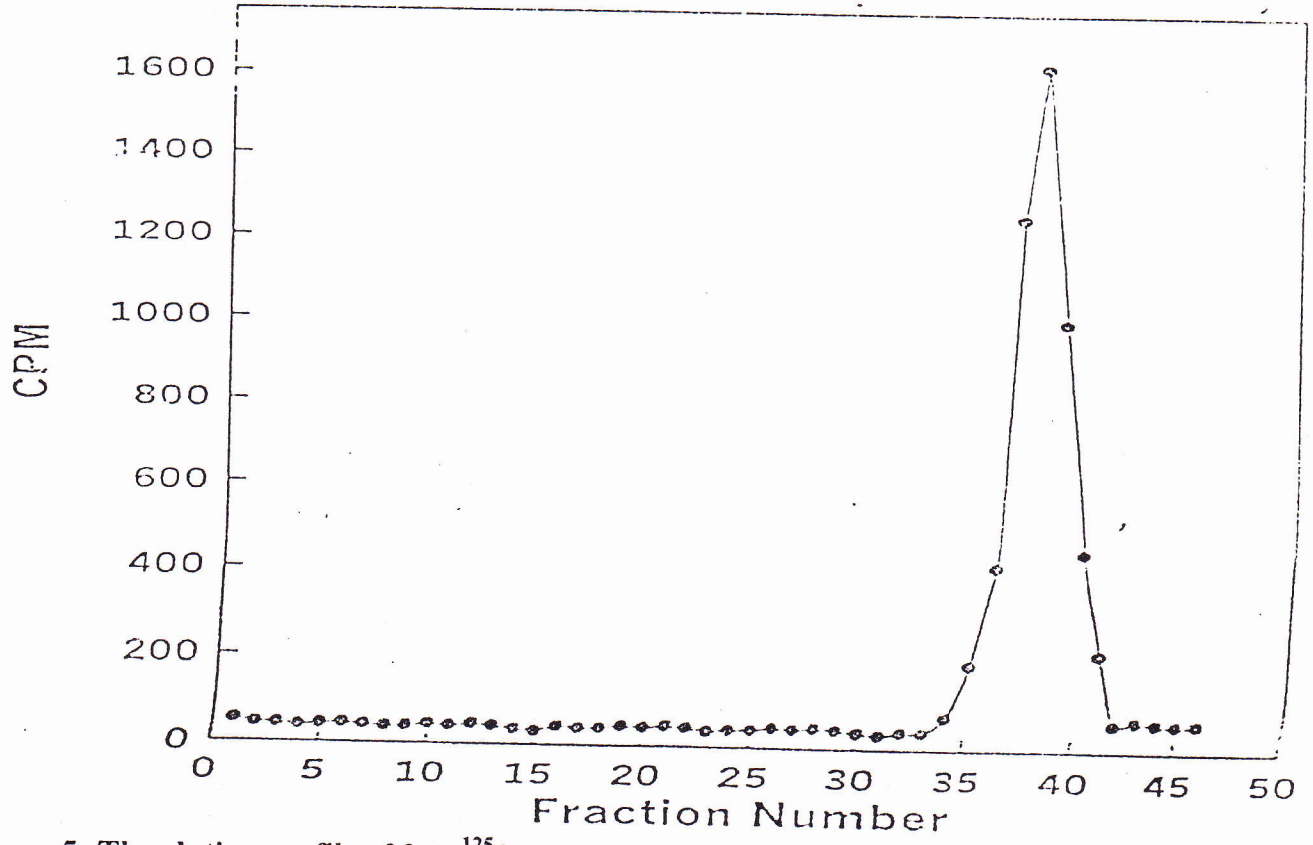

Figure 5: The elution profile of free ^{125}I – anti CA 125 antibody, using Sepharose CL – 6B gel, 9 ml / hr flow rate, tris – buffer pH 7.2, 0.02% sodium azide at 25°C.

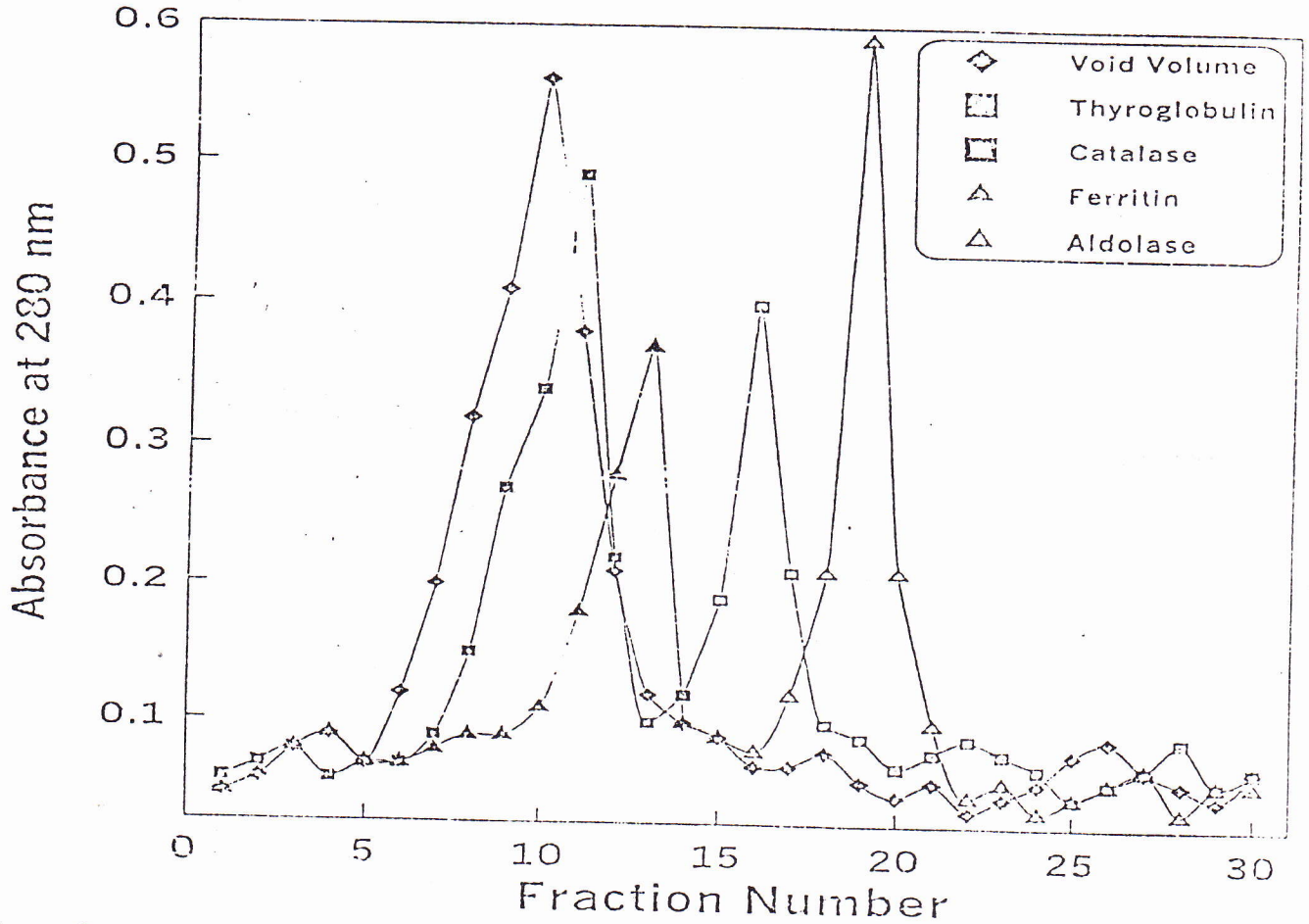

Figure 6: The elution profile of standard proteins, using Sepharose CL – 6B gel, 9 ml / hr flow rate, tris – buffer pH 7.2, 0.02% sodium azide at 25°C.

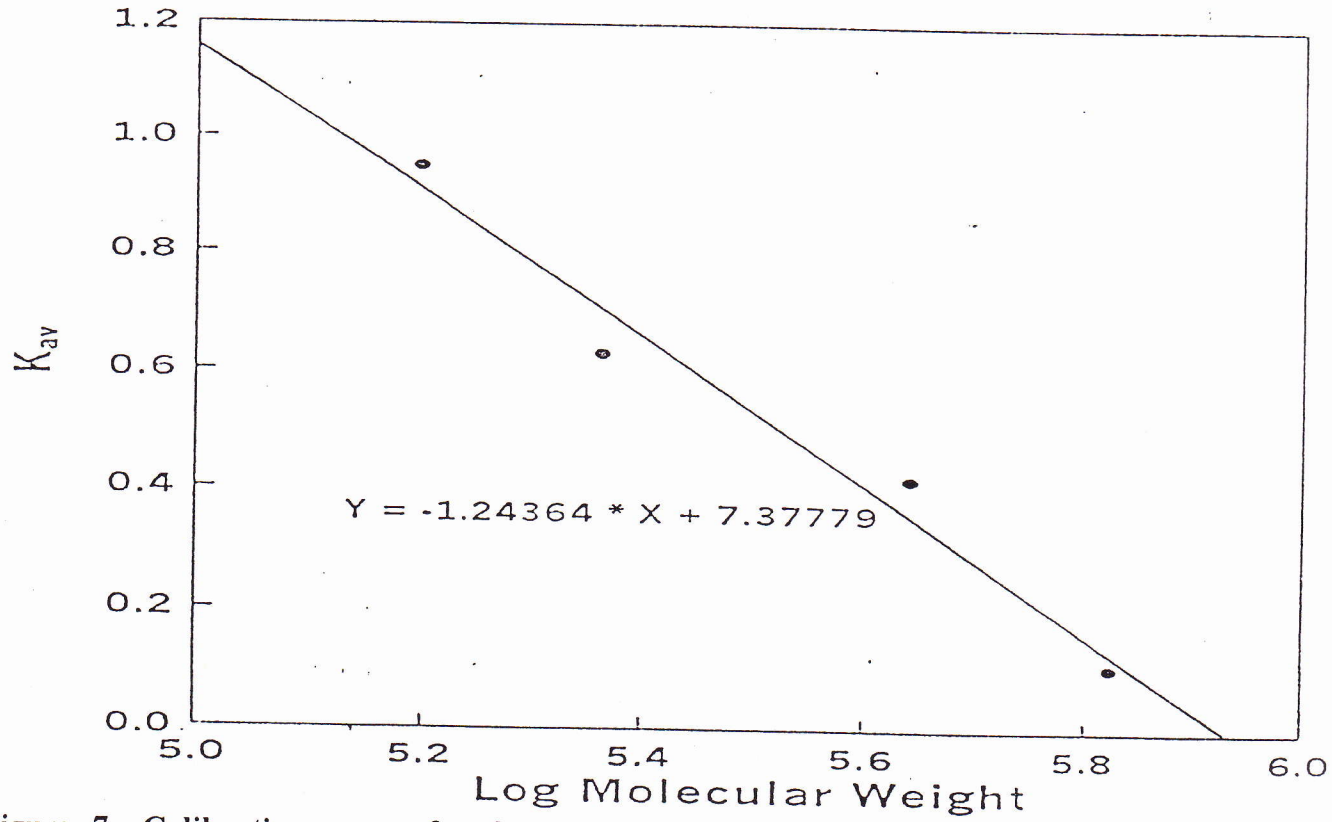

Figure 7: Calibration curve for determination of molecular weight by gel filtration chromatography using high molecular weight Pharmacia Calibration curve Kit.

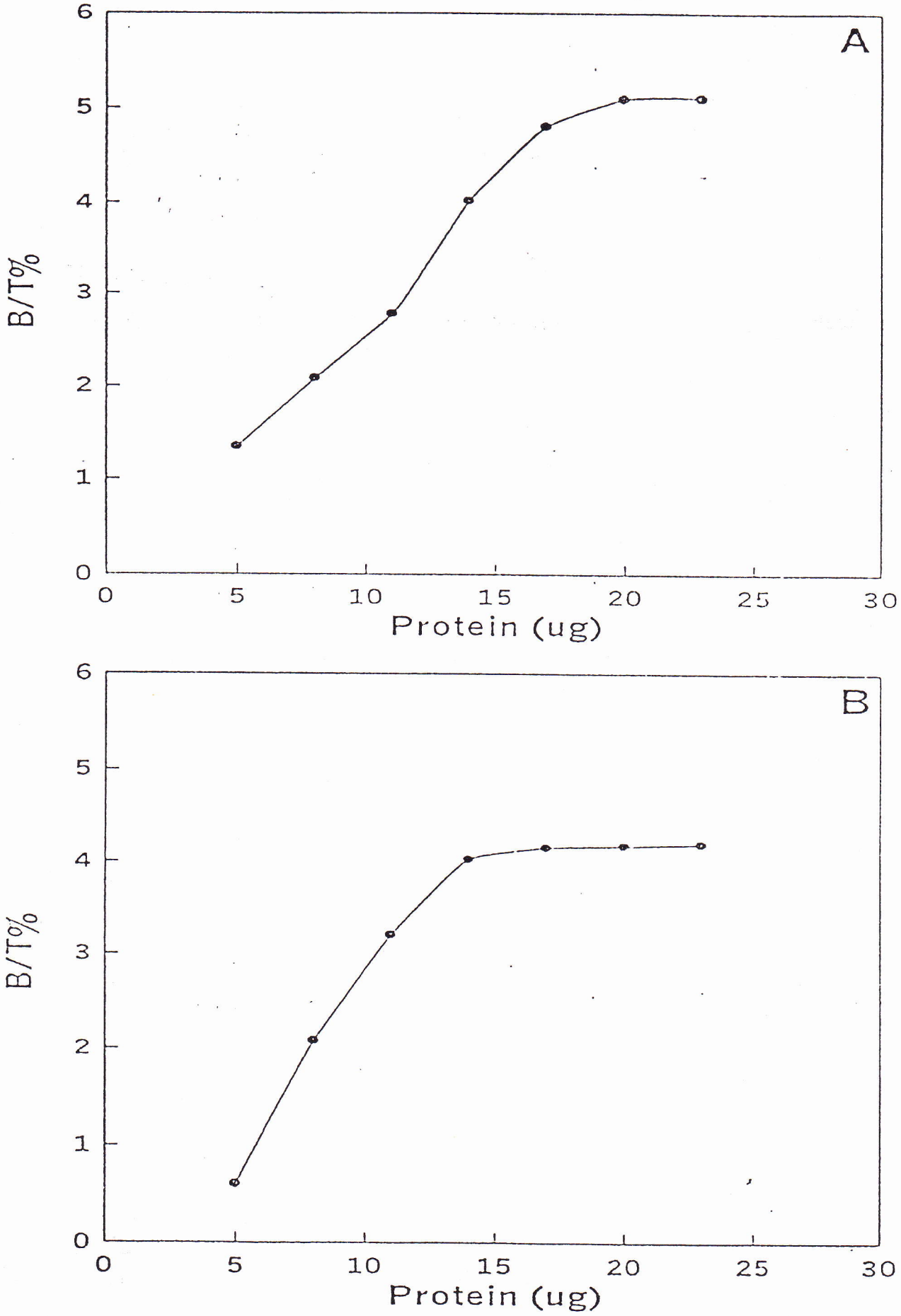

Figure 8: The effect of protein concentration on the binding of the isolated CA 125 to its ^{125}I – anti CA 125 from GI homogenates, A) AgI, B) AgII.

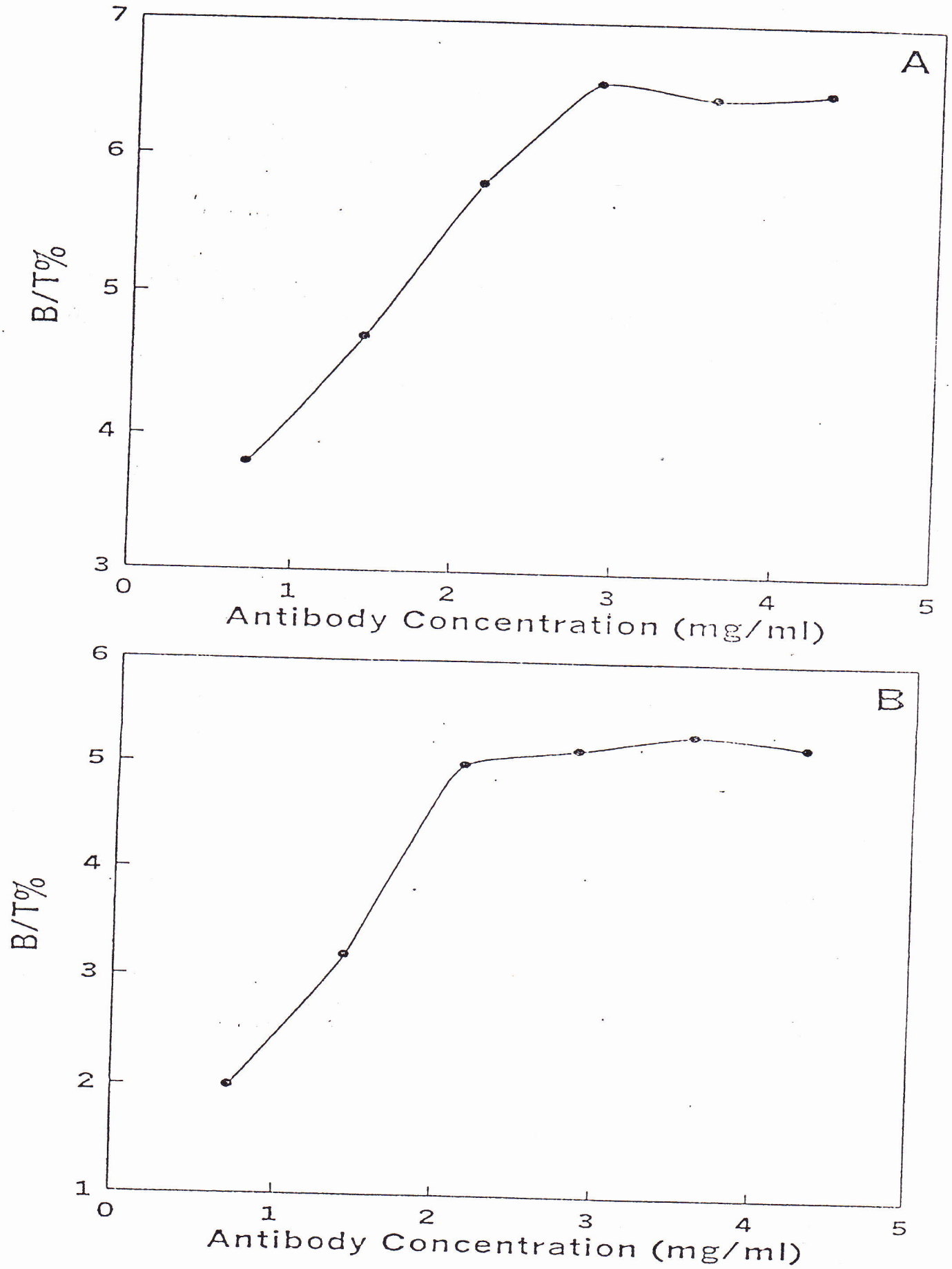

Figure 9: The effect of ^{125}I – anti CA 125 antibody concentration on the binding with isolated CA 125 from GI homogenates, A) AgI, B) AgII.

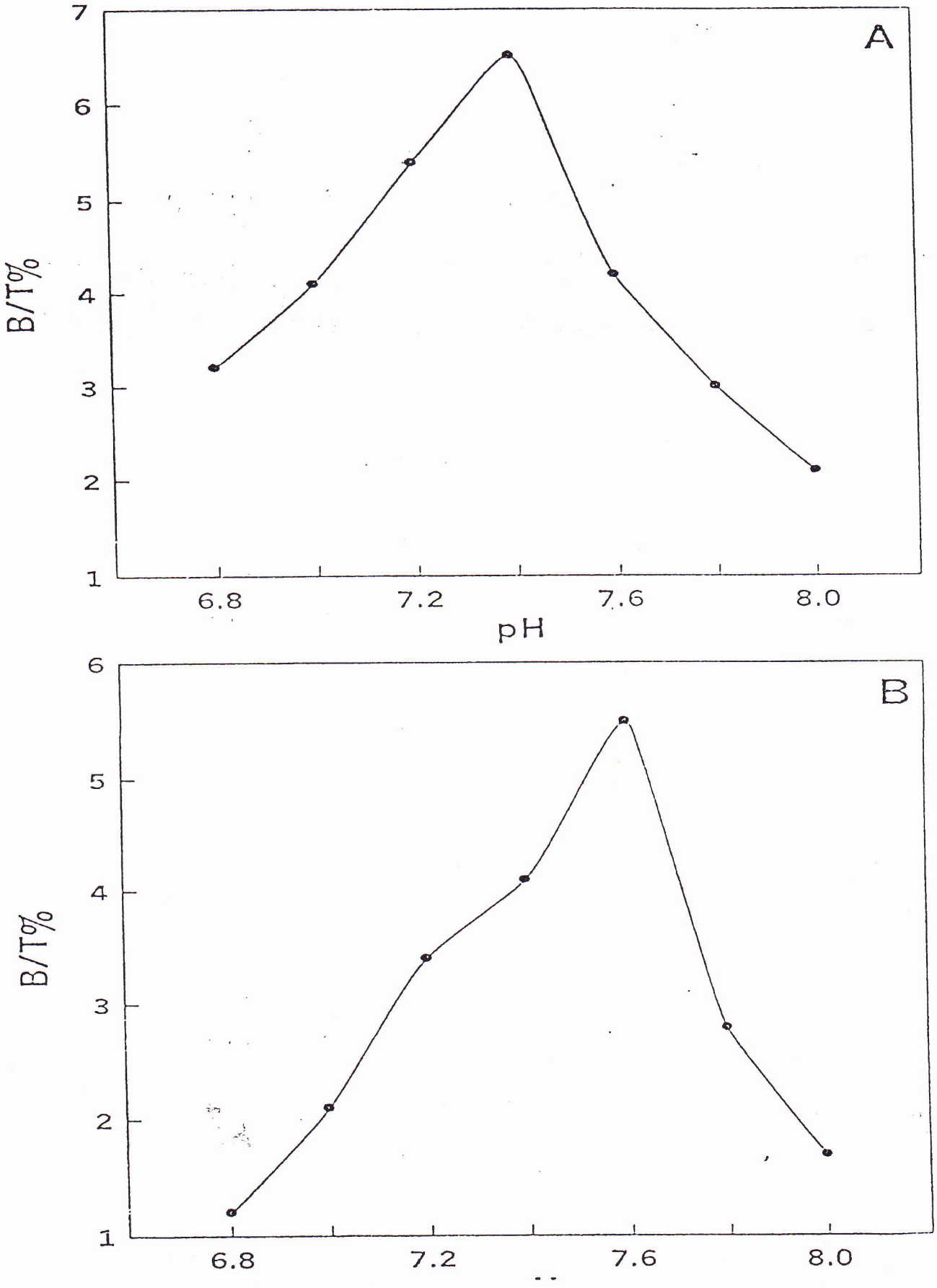

Figure 10: The effect of pH on the binding of the isolated CA 125 to its ^{125}I – anti CA 125 from GI homogenates, A) AgI, B) AgII.

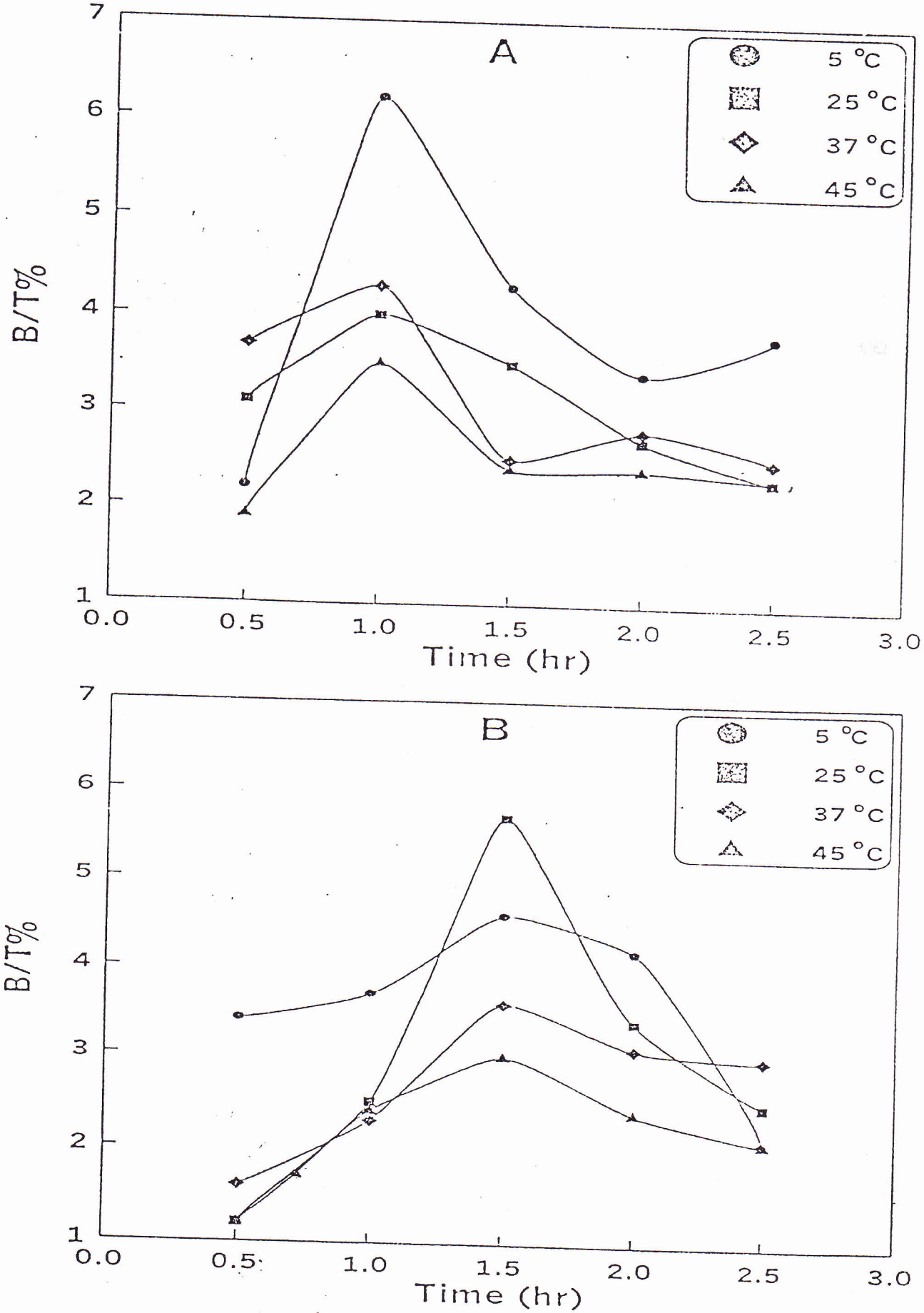

Figure 11: Time course of the binding of the isolated CA 125 antigen to its ^{125}I – anti CA 125 from GI homogenates, A) AgI, B) AgII.

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