

A SIMPLE AND ECONOMICAL PROCEDURE FOR THE PURIFICATION OF IMMUNOGLOBULIN Y FROM EGG YOLK BY T-GEL CHROMATOGRAPHY

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ABSTRACT

The use of immunoglobulin Y (IgY) instead of polyclonal antibodies prepared in mammals has been reported recently. These antibodies are found to have better properties in terms of specificity and ease of large scale production. In this study, proteins from egg yolk were extracted with acid and chloroform. The result of cellulose acetate electrophoresis at pH 9 showed some extra bands which were further purified with sodium sulfate precipitation (20%). The purified band was observed in the γ globulin region. SDS-PAGE electrophoresis of the product showed impurities which were removed by T-gel chromatography. Following that only H-chain of immunoglobulin Y with a molecular weight of about 66 KD was observed. Using the above procedure, a yield of around 40.7 mg IgY / egg was obtained.

Key words: IgY , T-Gel Chromatography, Egg Yolk

INTRODUCTION

Antibody in egg yolk has been termed IgY (yolk immunoglobulin). Molecular weight and isoelectric point of this immunoglobulin are different from immunoglobulin G (IgG) obtained in mammals (1-3). Chicken egg yolk antibodies (IgY) play an increasing role as an alternative to mammalian

polyclonal antibodies. They are recently used in biomedical research for diagnostic, prophylaxis, and treatment of diseases (4-6).

It is known that due to phylogenetic distance between mammals and birds, antibodies against mammalian proteins are produced easier in chicken compared to rabbits (1). Since IgY is transferred from blood to egg yolk, therefore it can be collected daily. In this way without using an invasive procedure such as bleeding, the antibody is

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collected and can be stored at 4°C for longer period of time (7). Reports also indicated that much higher amounts of specific immunoglobulin could be obtained in the eggs of immunised chicken than from the serum of rabbits (7). It is also interesting to know that IgY does not bind to mammalian complement, FC-receptors and rheumatoid factors (8). However the large amount of lipids present in egg yolk and the failure of staphylococcus protein A and streptococcus protein G to bind IgY resulted in problems in the purification of chicken IgY (1). Several procedures for the isolation and purification of IgY have been reported (9-13). The first step in the IgY purification is the isolation of water soluble components which contain IgY, followed by precipitation of IgY with sodium sulfate, ammonium sulfate or polyethylene glycol (PEG). The final step includes a chromatography procedure, such as ion-exchange, gel filtration and hydrophobic (9-13). An alternative and efficient procedure named as thiophilic interaction chromatography (TIC) was discovered by Porath et al (13). They observed that a divinyl-sulfone activated gel, coupled to 2-mercaptoethanol (the T-gel) showed selective binding of immunoglobulins in a salt-dependent manner. Thiophilic chromatography differs from hydrophobic chromatography in that, different proteins are bound to the gels and thiophilic interactions are temperature independent (14). It is also observed that NaCl reduces the thiophilic interactions, while promoting hydrophobic interactions (14-15). Although the mechanism of protein binding to T-gel up till now is not well understood, nevertheless it has been postulated that the interaction could be through the formation of an electron donor-acceptor complex between adjacent electron rich and deficient regions situated within a hydrophobic cavity. T-gel chromatography has also been used in purification of human, rat, rabbit and cow immunoglobulins (13,16) and the purification of monoclonal antibodies from hybridoma supernatant and ascitic fluid (17). There have also been reports on the use of the T-gel in the purification of antibody fragments (18). In recent years, use of T-gel chromatography for IgY purification has been in focus (1). The difficulties involved in producing specific antibodies and their use in immunotherapy

prompted us to investigate the easy and economic purification of IgY from chicken eggs for possible application in case of specific and large scale antibody purification. In this study, we used acid-chloroform extraction of proteins followed by sodium sulfate and T-gel chromatography for IgY purification from chicken egg yolk.

MATERIALS AND METHODS

Chloroform, sodium sulfate, divinylsulfone (DVS), 2-mercaptoethanol and sepharose CL-4B were obtained from Merck Chemical Company, Germany. Other reagents were obtained from Sigma Chemical Company, St. Louis. Mo, U.S.A. All other reagents were obtained from standard sources.

Extraction of yolk proteins

IgY and other water-soluble proteins were extracted following the methods described by Akita and Nakai (19) and Ntakarutimana et al (9) with a minor modification as follow. Yolk from three eggs was separated and passed through a nylon mesh into a measuring cylinder. To a 3 ml of egg yolk was added 15 ml of 3 mM HCl and the pH was adjusted to five using a 10% solution of acetic acid. The suspension was incubated at 4°C for three hours and centrifuged at 3500 rpm for 20 min. An equivalent volume of chloroform was added to supernatant and incubated at 4 for 12 h and centrifuged (15 min, 3500 rpm). The supernatant was separated and was treated with 2.6 g of sodium sulfate. The mixture was stirred at room temperature for 15 min, centrifuged (15 min, 3500 rpm) and precipitate was dissolved in 4 ml of tris buffer saline (TBS, 10mM, pH 7.3, containing 0.15 M NaCl and 0.05% NaN₃) and 2 ml of 36% Na₂SO₄ were then added, incubated for 1 h and centrifuged as above. The precipitate was collected and dissolved in 2 ml of TBS (containing 0.5 M Na₂SO₄). The product was electrophoresed and stored until future treatments.

T-gel chromatography

T-gel was prepared following the method described by Scoble and Scops (14). Sepharose CL-4B (1.8 ml) was added to 1.8 ml of Na₂CO₃ (0.5 M, pH 12) and kept at room temperature for

equilibration (30 min). The suspension was added with 150 μ l of DVS and incubated at room temperature for another 6h. The activated gel was washed with sodium carbonate solution (0.5 M, pH 11) and suspended in 3.6 ml of the same solution. Mercaptoethanol (50 μ l) was added and the mixture was incubated at room temperature for 1h. A column (5x0.55 cm) was packed with a final volume of the gel (1.7 ml), washed and equilibrated with

TBS (containing 0.5M sodium sulfate). The sample was applied at a flow rate of 0.5 ml/min, diluted with TBS and electrophoresed.

RESULTS

From three eggs, 37 ml of yolk (average 12.3 ml from each egg) was obtained. After chloroform extraction, three phases formed (figure 1) in which immunoglobulins were found in aqueous phase at

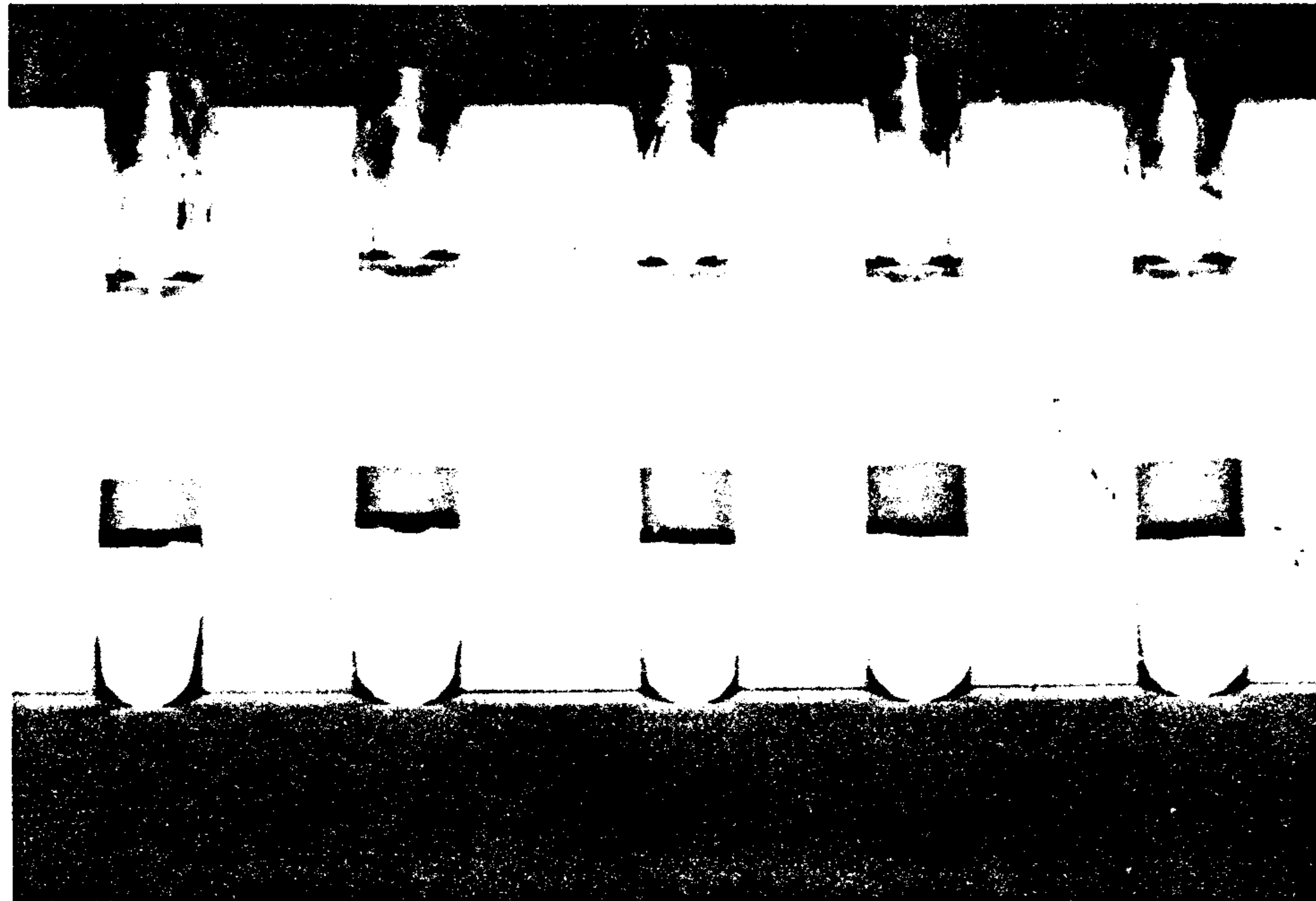


Figure 1. Chloroform extraction of egg yolk. The upper phase contained water soluble proteins, the middle phase solid material (lipids) and the lowest phase chloroformic extracts.

the top. Sodium sulfate precipitation of this solution resulted into one band in the Y-region (figure 2) which could be further purified by T-gel chromatography. Figure 3 indicated a typical chromatogram in which the IInd peak indicated TBS diluted proteins. The cellulose acetate electrophoresis of this peak is shown (figure 4) where there exists only one band. The SDS-PAGE of this compound is shown (figure 5) and it is found that before T-gel chromatography three bands are obtained while after T-gel chromatography only one band about 66KD remains. Finally it was found that the concentration of IgY in the pool fractions of IInd peak was 9.91 mg (i.e. 40.6 mg/egg).

DISCUSSION

Yolk immunoglobulins (IgY) are found to have better properties than mammalian antibodies, since they do not interact with rheumatoid factors (RF) and are produced in larger quantities (40.6 mg/egg in our procedure) than mamalian serum output (10), and are cheaper and compatible with modern animal protection regulation. Nevertheless, IgY antibodies have not been used as frequently as one might expect. This may be due to unsatisfactory isolation and purification procedure.

Reports on purification of IgY are available. The different strategies used are acidification (1,19), chloroform extraction (9,11) and dextran sulfate-CaCl₂ precipitation (7,20) for lipid removal. In all

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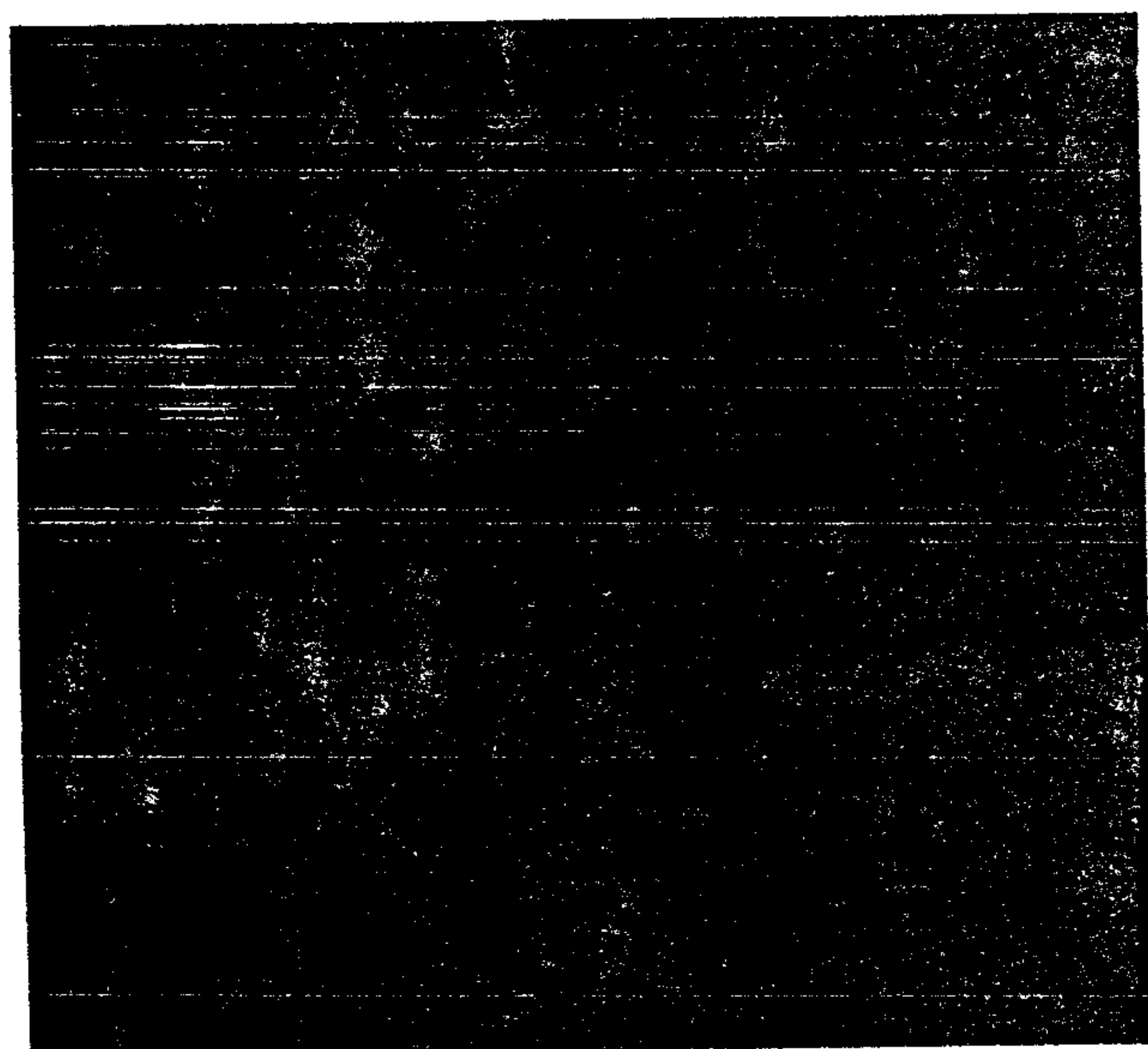


Figure 2. Cellulose acetate electrophoresis after sodium sulfate precipitation. Egg yolk diluted with TBS(I) aqueous phase after chloroform extraction (II) and sodium sulfate precipitation product (III, IV).

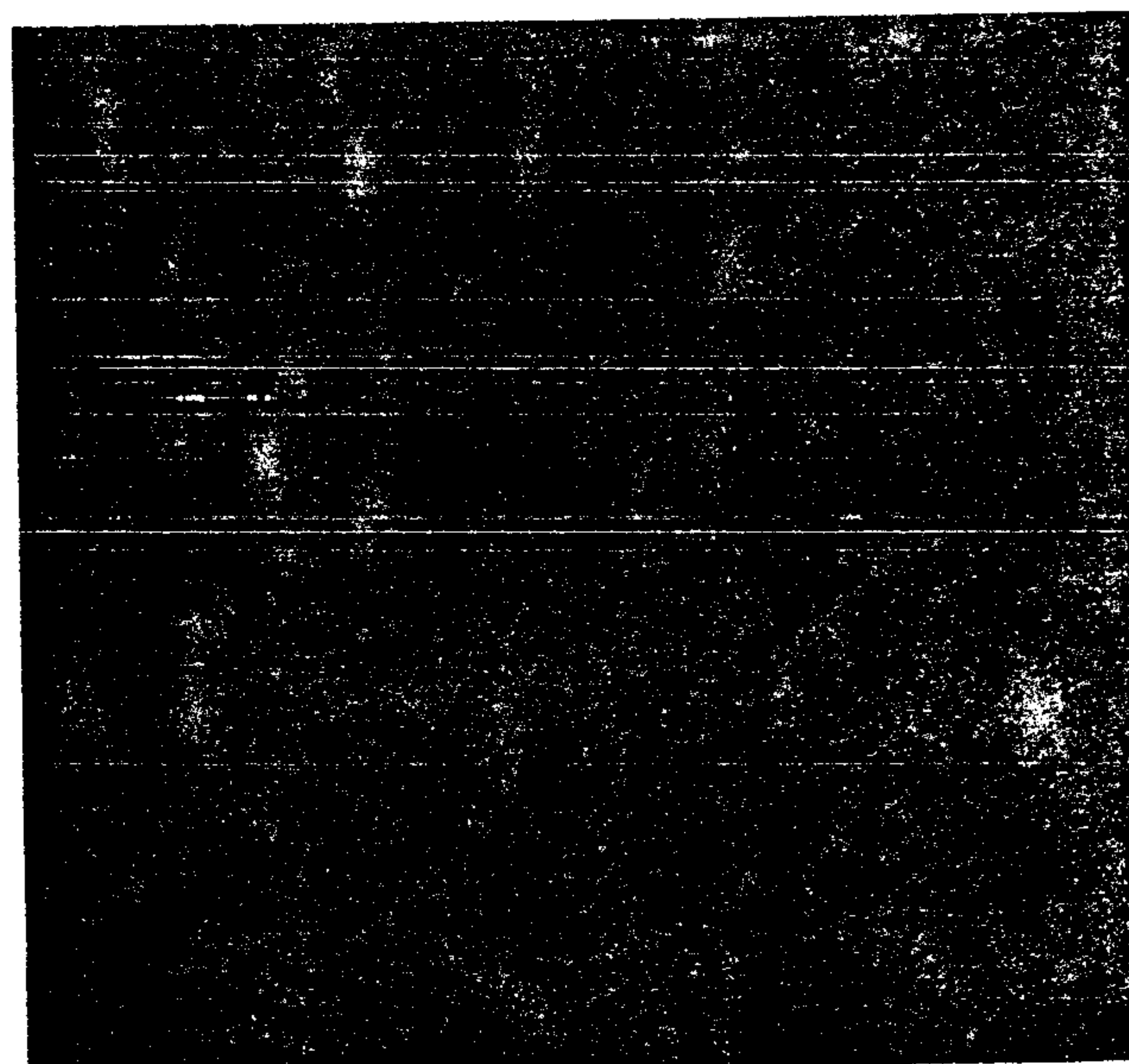


Figure 4. Celulose acetate electrophoresis of egg yolk after dilution with TBS (I) and fractions of IIInd peak after T-gel chromatography (II, III).

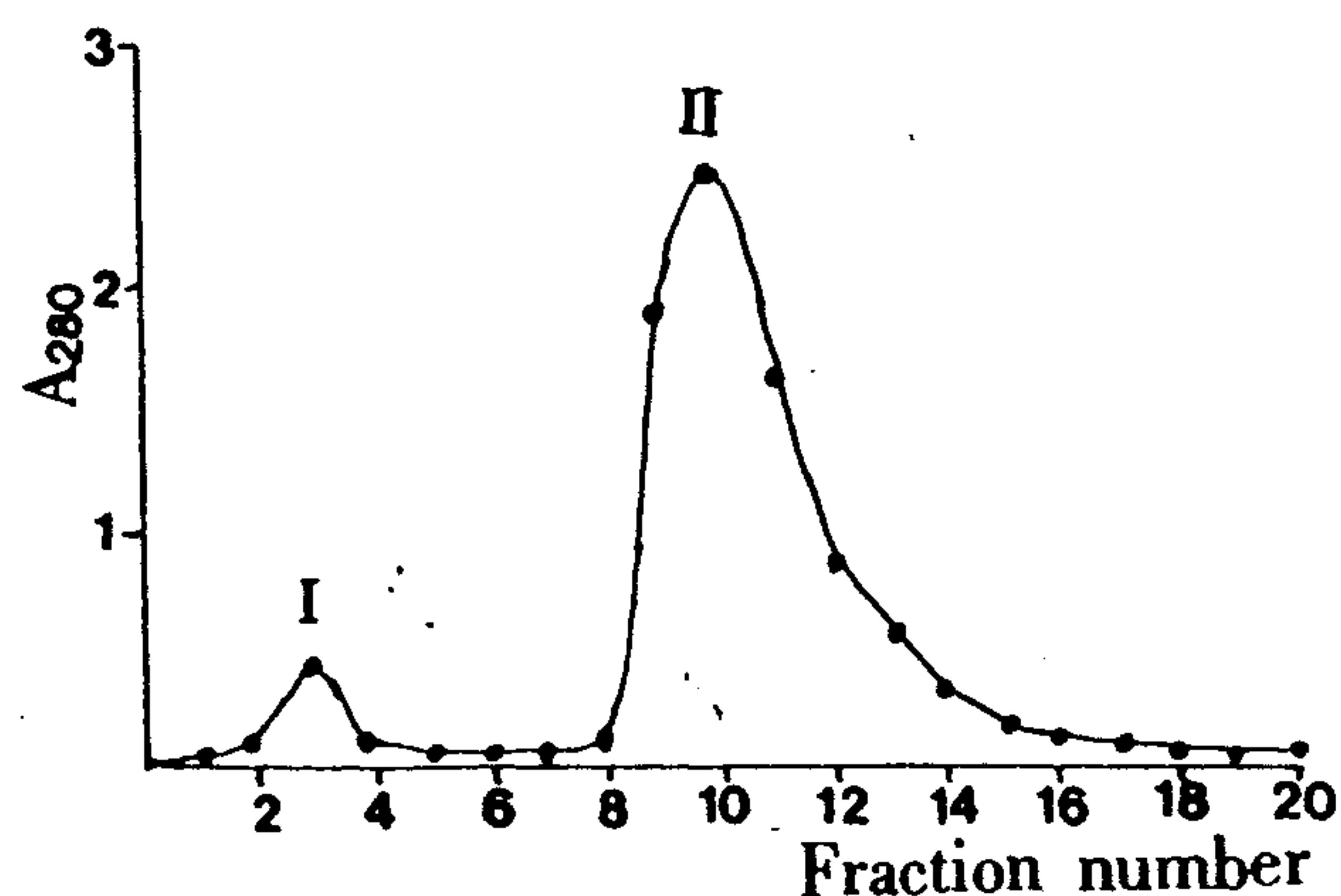


Figure 3. A typical chromatogram after T-gel chromatography. Peak II contained IgY. (Column dimensions: 5 0.55 cm, flow rate=15 ml/hr).

these procedures, high speed centrifugation (with a speed of around 10000×g) or else expensive compounds are required. We developed an easy and inexpensive method for IgY purification. A sodium sulfate precipitation resulted into one band in the region of gamma globulin. However this product was found to contain some impurities which could only be removed by T-gel chromatography. Two peaks in chromatography have been reported by others (1). We also found that the first peak contained proteins with no T-gel affinity which was

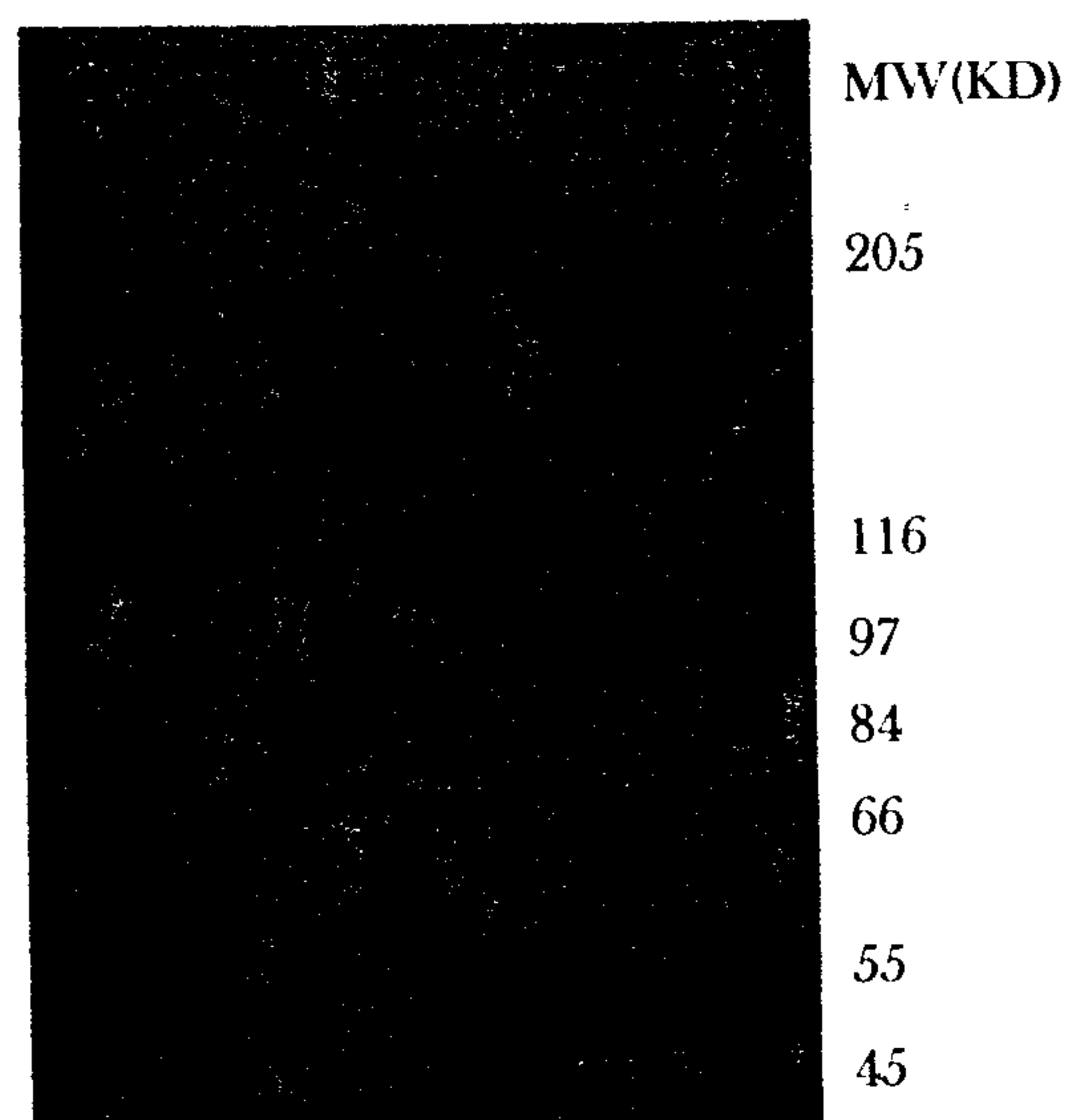


Figure 5. SDS-PAGE electrophoresis of samples after precipitation with sodium sulfate (I), pooled fractions of IIInd peak after T-gel chromatography (II, III), and standard molecular weight proteins (IV).

diluted with TBS (containing 0.5M Na₂SO₄) and the second peak was shown to contain the yolk IgY, which was only diluted with TBS. These observations were also reported by others (1,17).

Presence of about 66 KD band in SDS-PAGE indicates a IgY H-chain which is dissociated from L-chain using 2-mercaptoethanol. The yield of IgY obtained in this study from 3 ml of yolk was around 9.91 mg. Considering 12.33 ml of yolk from each egg, the yield was calculated to be about 40.7 mg/egg. Others also reported a yield of 14.5 mg upto 72 mg IgY from each egg (1, 9, 10, 21) and 26.8-51 mg with T-gel purification (1). Hence it is concluded that the antibodies can be extracted from the egg yolk on a preparatory scale by means of a simple and economical procedure.

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