

PRELIMINARY STUDIES ON THE DETECTION OF HUMAN ALBUMIN USING ANTIGEN SPECIFIC PRECIPITATION OF MAGNETIC PARTICLES AND MAGNETIC PERMEABILITY MEASUREMENTS

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INTRODUCTION: A novel method for the specific detection of human albumin using magnetic permeability measurements and antigen-specific precipitation of magnetic particles, is presented. Earlier our group has presented the detection of proteins using magnetic permeability detection in direct and competitive sandwich assays [1]. Recently, we have reported the detection of Concavalin A [2,3] and DNA [4] using sandwich assays and nonspecific electrostatic interactions in combination with magnetic permeability measurements. Now we present the immunospecific detection of human albumin.

METHODS: Magnetic particles (100 nm) coated with protein A were obtained from Micromod GmbH in Rostock, Germany. Conjugation of polyclonal anti-human albumin IgG (goat) to the magnetic particles was carried out by incubating 0.45 ml magnetic particles with 90 μ l of a 1 mg/ml IgG solution in 162 mM PBS buffer, pH 7.4. Incubation was carried out for 3 hours at room temperature. Non-bound IgG was removed by washing the magnetic particles in PBS buffer using a high-gradient magnetic field device.

The measuring instrumentation used in the experiment, the MPM-100 (magnetic permeability meter), was obtained from the European Institute of Science in Lund, Sweden and is shown in Figure 1. The measuring principle is based on the fact that in the presence of human albumin, the magnetic particles will crosslink via antigen-antibody interactions, and these precipitates will sediment to the bottom of the vial in the presence of a magnetic field. The magnetic permeability of the precipitate is directly proportional to the amount of human albumin present in solution.

Precipitation studies for the detection of free human albumin in solution, were carried out by placing 200 μ l (0.25 mg/ml) of magnetic particles conjugated with polyclonal anti-human albumin IgG in a measuring vial. Subsequently, free human albumin was added to solution and the vial was allowed to stand at room temperature for 1 hour. After the 1-hour incubation, the vial was placed on

a permanent magnet for 30 minutes. Lastly, the vial was placed in the magnetic permeability meter, and the magnetic permeability of the precipitate was measured. Various amounts of human albumin (0.005, 0.01, 0.1, 0.5, 1.1, 2, 5, 10 and 15 μ g) were added and the samples were analyzed using this technique.



Fig. 1: The magnetic permeability meter used for the detection of human albumin.

RESULTS: The magnetic permeability of the precipitate formed by the magnetic particles in the presence of various concentrations (0.5 nM - 1.23 μ M) of human albumin was carried out. There is a linear increase in magnetic permeability of the precipitate up to about 840 nM. At 1.23 μ M nearly all the magnetic particles had been precipitated from solution. The linear range of the detection curve is shown in Figure 2. The equation of the line obtained is $y = 10.142 + 4.95 \times 10^6 x$, $R = 0.99015$. The limit of detection is 50 nM.

DISCUSSION & CONCLUSIONS: The detection of human albumin in solution was carried out using the antigen-specific precipitation of magnetic particles and magnetic permeability detection. We observed a linear response in the range of 0.5 - 840 nM human albumin. The limit of detection was 50 nM. Further studies on the kinetics of the system and the concentration of magnetic particles need to be conducted. However, our preliminary study

indicates that, for the detection of proteins, the antigen-specific precipitation of magnetic particles is a viable alternative to sandwich format assays.

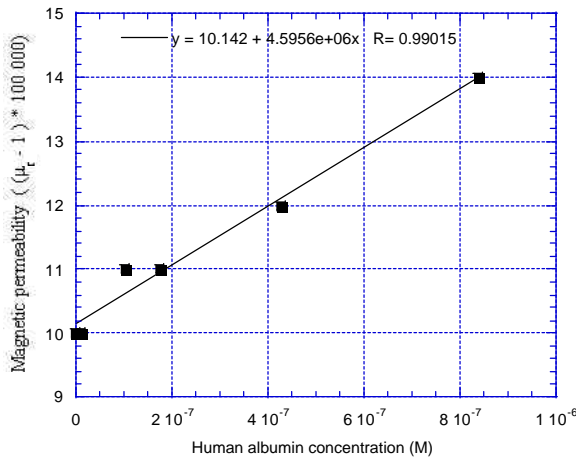


Fig. 2: The linear range (0.5 - 840 nM) for the detection of human albumin.

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