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The use of coated paramagnetic particles as a physical label in a magneto-immunoassay

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Abstract

An ideal label for use in an immunoassay would require no further chemical or electromagnetic stimulation prior to its detection and would be free from interference from the sample matrix. Micron sized paramagnetic particles are able to perturb magnetic fields. This perturbation can be directly detected using a suitable electronic device and is independent of the sample matrix. In this study coated paramagnetic particles were used as a physical label in a non-competitive solid phase 'sandwich' assay for the detection of human transferrin. The transferrin acted as a 'biological bridge' allowing a dose dependant immobilization of the paramagnetic particles to a polyethylene terephthalate solid phase. Quantitation of the paramagnetic label was achieved using an electronic detection system allowing a linear dose response with a femtomolar detection limit (260 fmol). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Magneto-immunoassay; Paramagnetic particles; Transferrin, Magnetometer; Polyethylene terephthalate; Solid phase

1. Introduction

In immunoassay techniques, a label is conjugated to the antibody or its antigen. The label has to be easily measured and used to detect the presence of antibodies bound to antigens. Traditionally, radioisotopes were the most common labels used, but these have been largely replaced by enzyme, fluorescent and chemiluminescent labels. The newer labels offer a greater sensitivity in assay design and remove any concern over the handling and storage of radioactive substances (Gosling, 1990; Schall and Tenoso, 1981). In many immunoassay techniques, the analyte is quantified by removing the unreacted (free) label in the system from the reacted (bound) label. This entails either physically absorbing the unreacted labelled compound or precipitating the bound label using another antibody. Modern procedures use solid phase techniques where the immune-reaction takes place on a solid surface, allowing the unreacted components to be easily washed away, thereby leaving only bound label to be detected. Many different types of solid phases are employed including microtitre plates, plastic test tubes, plastic beads, latex microparticles and plastic films (Gosling, 1990). In many automated assay systems paramagnetic particles have been used as the solid phase in the separation stage (Clements et al., 1992; Brun et al., 1990). The paramagnetic particles are first coated with a polymer layer and then with proteins allowing them to be used as the solid phase on which the immune reaction takes place. The coated paramagnetic particles (about 1–5 μm in diameter) are not permanently magnetized but are attracted to permanent magnets therefore washing the coated paramagnetic particles does not require filtration or centrifugation. Following the washing steps, the immobilized label on the surface of the coated paramagnetic particles is detected in the conventional manner.

In a new generation of immunoassays, magnetic or paramagnetic material is being used as a label, which is directly detected. Methods for detection previously described include, a force amplified biosensor (Baselt et al., 1997), a Maxwell bridge (Kriz et al., 1998) and a super-conducting-quantum-interference-device (SQUID; Enupuku et al., 1999). At UWE we have developed a novel magnetometer that is based on mea-

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suring a change in frequency (Richardson et al., 2000). In this paper the novel magnetic detector is used to quantify the number of paramagnetic particles bound to the polyethylene terephthalate (PT) solid phase in a magneto-immunoassay for human transferrin.

2. Materials and methods

2.1. Materials

All reagents and antibodies were prepared in 0.05 M phosphate buffered saline (PBS) pH 7.4.

The solid phase polyethylene terephthalate (PT) (Xerox™ transparencies laser/copier type A) was purchased from Rank Xerox, UK.

Rabbit anti-human transferrin (Dako® A/S, Denmark) was diluted 1:1000 in PBS and mixed with 1% glutaraldehyde v/v (Sigma-Aldrich, UK), giving a solution of 60% antibody and 40% glutaraldehyde. This was prepared immediately prior to use.

Blocking solution was PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich, UK) and 1 M glycine (BDH, Poole).

The analyte human holo-transferrin (Sigma-Aldrich, UK) was diluted in the blocking solution, with concentrations ranging from 0.5 to 4 μg ml^{-1}.

A polyclonal sheep antibody against human transferrin (The Binding Site, UK) was diluted 1:1000 in the blocking solution. Biotinylated donkey anti-sheep polyclonal antibody (Chemicon International Inc., CA) was diluted 1:120 in PBS.

Streptavidin coated M-280 paramagnetic particles were purchased from Dynal® UK Ltd.

2.2. Preparation of solid phases

Strips of PT measuring 5 mm in width and 25 mm length were fixed to plastic sticks using Loctite superglue (Loctite Ireland Ltd), with a final working area of 5 × 5 mm.

2.3. Preparation of paramagnetic particles

Using a magnetic particle concentrator (Dynal™ UK Ltd) streptavidin paramagnetic particles were washed 3 times with 1 ml PBS and resuspended in biotinylated donkey anti-sheep immunoglobulin (1.1 μg biotinylated antibody per 10^7 paramagnetic particles). After 30 min incubation at 4 °C with bidirectional mixing the paramagnetic particles were washed 5 times with 1 ml of PBS/BSA, using the magnetic particle concentrator to aid removal of the supernatant. The biotinylated donkey anti-sheep-streptavidin paramagnetic particles were then resuspended in PBS containing 1% BSA.

2.4. Magneto-immunoassay procedure

A sandwich assay for human transferrin was developed as shown in Fig. 1.

Unless otherwise stated all incubations were performed in a damp chamber at room temperature. The washing procedures consisted of 1 × 3 ml distilled water followed by 3 × 3 ml PBS.

The PT strips were coated with 35 μl rabbit anti-human transferrin/1% glutaraldehyde mix for 1 h. After washing, the strips were incubated in the blocking solution for 1 h and washed. 20 μl of each human transferrin standard was applied (resulting in the application of 0, 10, 20, 40 and 80 ng of human transferrin) and incubated for 30 min. The strips were washed again and incubated with 30 μl of sheep anti-human transferrin for 30 minutes, washed and incubated with 30 μl biotinylated donkey anti-sheep-streptavidin paramagnetic particles overnight (16 h) at 4 °C. The strips were then washed and allowed to dry at room temperature for 5 mins prior to presentation to the magnetic detector (see Fig. 2). The strips were placed into the coil, as shown in Fig. 3, for 10 s and the change in frequency was recorded. Five replicates of each transferrin concentration were performed.

3. Results and discussion

We have demonstrated that using a suitable magnetic detector we can use commercially available, micron sized paramagnetic particles as a label in an immunoassay for human transferrin. Paramagnetic particles were captured onto a PT solid phase, by the analyte transferrin, in a dose dependant manner. The concentration range 1.0–4 μg ml^{-1} equates to 20–80 ng of transferrin in the 20 μl applied to the solid phase. Our detector measured a change in frequency induced

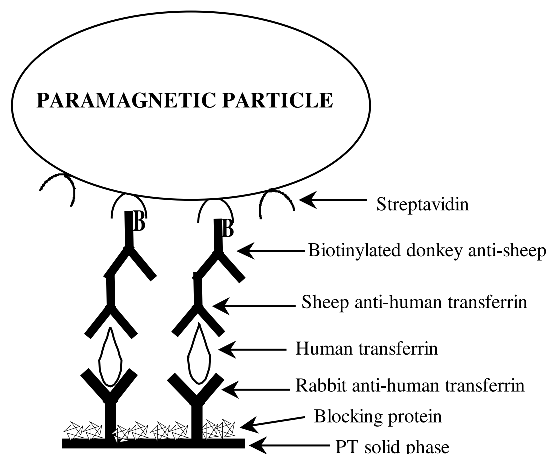

Fig. 1. Diagrammatic representation of the magneto-immunoassay for human transferrin.

Fig. 2. Magnetic detector (on the left) with power pack and frequency meter.

when the paramagnetic particles were introduced into the detector coil. The observed frequency change ranged from 29 to 62 Hz depending upon the amount of transferrin captured on the solid phase. The coil design is important for maximum sensitivity (Richardson et al., 2000), and the detector described in this paper used an oval coil configuration.

Other techniques for detecting magnetic and paramagnetic material have been described using a Maxwell bridge (Kriz et al., 1998), a Superconducting Quantum Interference Device, SQUID (Enupuku et al., 1999) and a Force Amplified Biological Sensor (Baselt et al., 1997). Kriz et al. (1998) reported using ferromagnetic nanoparticles to measure concanavalin A as a model analyte. This resulted in a detection limit of 250 nM with a coefficient of variation of 6.2% (Kriz et al., 1998). Although this was not strictly an immunoassay, relying on simple adsorption of reactants onto silica carrier particles, the authors developed this as a sandwich type assay, stating that an immunoassay could be easily developed at a later date. A magneto-immunoassay for interferon β was described by Enupuku et al. (1999) also using magnetic nanoparticles. They showed a detection limit of approx. 0.5 units/ml, which could not be compared in molar units. This assay involved using a SQUID as the detection device, and was quoted as being able to detect 600 pg of magnetic material. The authors were confident that they would soon be able to detect as few as 10 pg of magnetic particles. A third group has demonstrated that they could detect micron sized paramagnetic particles (Dynabeads) using a cantilever beam force transducer, which they developed (Baselt et al., 1997). Although they have shown they can detect very low numbers of paramagnetic particles they have not yet demonstrated their use in an immunoassay.

In this report we describe a magneto-immunoassay using paramagnetic particles as the label. This was a sandwich assay using transferrin as a model analyte. The paramagnetic particles used, 2.8 micron diameter streptavidin coated Dynabeads™, were commercially available. The paramagnetic particles were used in excess (approx. 6.7×10^8 per test stick) in order to provide an excess of binding sites available for the immune reaction. In this assay the paramagnetic particles were used as a label in an indirect sandwich assay. Biotinylated anti-sheep IgG was attached to streptavidin paramagnetic particles enabling them to be used as a universal reagent, recognizing antigen-specific sheep IgG. This could be used in a wide range of different assays. For simplicity, a biotinylated antigen specific antibody could be attached to the paramagnetic particles, but would require a different labelled antibody for each technique.

Following the use of glutaraldehyde in the solid phase coating procedure the unreacted functional groups were blocked by glycine contained in the blocking reagent. The inclusion of BSA in all further reagents was sufficient to prevent non-specific binding.

As shown in Fig. 4 the model assay showed good linearity ($r^2 = 0.9767$) over the amounts of human transferrin studied, 0–80 ng (equivalent concentration 1.0–4 μg ml^{-1}). At higher transferrin concentrations we would expect to reach saturation on the solid phase and hence a maximum signal. The detection limit of our magneto-immunoassay was found to be 20 ng, this was calculated as 2 standard deviations of the intercept of the y-axis above the zero standard (from Fig. 4). The 10 ng standard was below the calculated detection limit and mathematically there is no significant difference for the points between the zero and 10 ng standards. The calculated detection limit of 20 ng in a 20 μl sample equates to 0.5 μg ml^{-1} or 260 fmol, comparable with detection limits seen using radio labels (Vanderwalle et al., 1989) or nephelometric techniques (LeVine et al., 1999). The precision profile shows that the best precision is seen at higher concentrations of transferrin, as expected, with the coefficient of variation being equal to 6.4%, comparable to other magneto-assays described (Kriz et al., 1998).

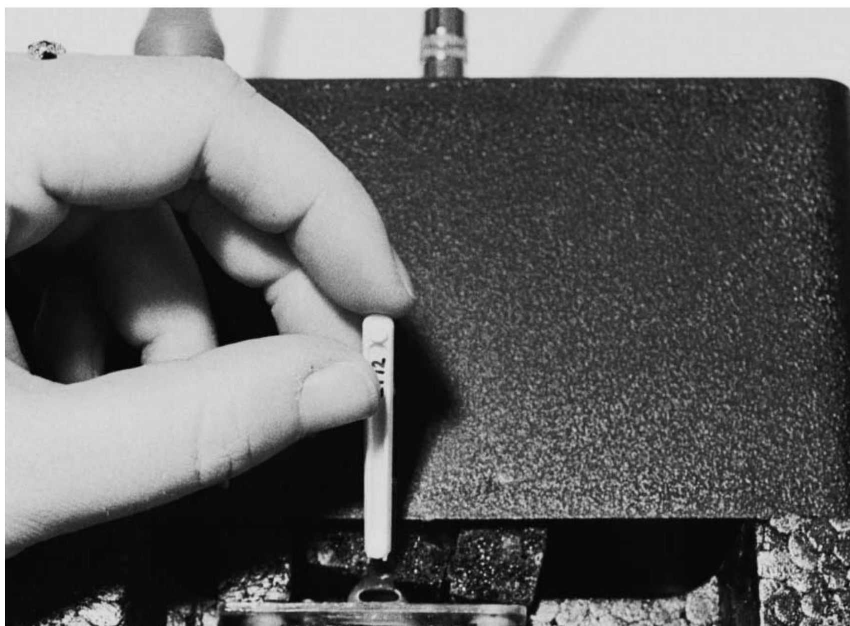

Fig. 3. Presentation of the test strip to the coil of the magnetic detector.

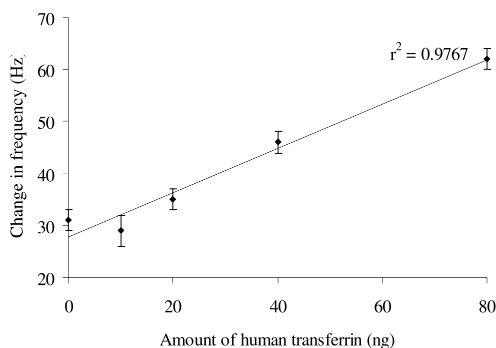

Fig. 4. The relationship between the amount of human transferrin and change in frequency following the magneto-immunoassay for human transferrin.

Micron sized paramagnetic particles used as labels in an immunoassay have considerable advantages over traditional labels such as radioisotopes, enzymes and fluorescent labels. Paramagnetic particles are physical labels of great mass, do not decay or degrade, and cannot be denatured or neutralized. Detection of paramagnetic particles is related to a physical measurement derived from their structure, with minimal background signal and no sample interference. The signal from the label is rapidly measured (requiring just 10 s) and does not require pre-treatment, any further chemistry or any excitation. In addition to these advantages the use of paramagnetic particles on a solid phase allows the 'sample stick' to be kept indefinitely and if required, re-measurement can be performed in the future without loss of signal.

The biggest difference between conventional labels and the paramagnetic particles used in this transferrin assay is their size. Paramagnetic particles are massive in comparison to the analyte. In the assay described in this paper the analyte acted as a biological bridge, cross-linking and immobilizing the label to the solid phase via an immune reaction. In reality it is thought that many cross-links are formed by many analyte molecules, holding the particle on the solid phase. In this application this will limit the practical detection limit, but in other applications with other detection technologies it is possible to detect a single particle which could reflect a single antigen interaction giving an immunoassay technique with extreme sensitivity.

The detector developed at the University of the West of England is small ($15 \times 9.5 \times 4.5$ cm) and constructed from conventional electronic components. This will allow the construction of a small hand-held device. In conjunction with a rapid immunoassay employing paramagnetic particles as the label, we foresee a simple, robust analytical device for use in diagnostic situations such as Point of Care and Near Patient Testing. In addition, this technology with suitable magneto-immunoassays could be used for other applications where a hand-held device would be required such as field testing in environmental or agri-food applications and where sensitive 'one-off' tests are required.

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References

Baselt, D.R., Lee, G.U., Hansen, K.M., Chrisey, L.A., Colton, R.J., 1997. A high-sensitivity micromachined biosensor. Proc. IEEE 85, 672–680.

Brun, A., Gaudernack, G., Sanderg, S., 1990. A new method for isolation of reticulocytes: Positive selection of human reticulocytes by immuno magnetic separation. Blood 76, 2397–2403.

Clements, J.A., Forrest, G.G., Jeffery, M., Kemp, P.M., Kjeldsen, N.J., Rattle, S.J., et al., 1992. The Anagen system for automated fluorometric immunoassay. Clin. Chem. 38, 1671–1677.

Enupuku, K., Minotani, T., Gima, T., Kuroki, Y., Yamashita, M., Katakura, M., et al., 1999. Detection of magnetic nanoparticles with superconducting quantum interference device (SQUID) magnetometer and application to immunoassays. Jap. J. Appl. Phys. 38, L1102–L1105.

Gosling, J.P., 1990. A decade of development of immunoassay methodolgy. Clin. Chem. 36, 1408–1427.

Kriz, K., Gehrke, J., Kriz, D., 1998. Advancements towards magneto immunoassays. Biosens. Bioelectr. 13, 817–823.

LeVine, S.M., Lynch, S.G., Ou, C., Wulser, M.J., Tam, E., Boo, N., 1999. Ferritin, transferrin and iron concentrations in the cerebrospinal fluid of multiple sclerosis patients. Brain Res. 821, 511–515.

Richardson, J., Hill, A., Luxton, R., Hawkins, P., 2000. A novel measuring system for the determination of paramagnetic particle labels for use in immunoassay and DNA analysis, Biosens. Bioelect., submitted for publication.

Schall, R.F., Tenoso, H.J., 1981. Alternatives to radioimmunoassay: labels and methods. Clin. Chem. 27, 1157–1164.

Vanderwalle, B., Hornez, L., Revillion, F., Lefebvre, J., 1989. Secretion of transferrin by human breast cancer cells. Biochem. Biophys. Res. Commun. 163, 149–152.