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# Polyhydroxyethylmethacrylate-based magnetic DNA-affinity beads for anti-DNA antibody removal from systemic lupus erythematosus patient plasma

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**Abstract**

The aim of this study is to prepare magnetic poly(2-hydroxyethylmethacrylate) (mPHEMA) beads and to investigate their utility for the removal of anti-DNA antibodies from systemic lupus erythematosus (SLE) patient plasma. mPHEMA beads, in the size range of 80–120 μm, were produced by a modified suspension technique. Then, DNA was coupled onto mPHEMA beads by carbodiimide activation. The amount of ligand coupled was changed by changing the initial concentrations of carbodiimide and DNA. Human immunoglobulin G (HIgG) and anti-DNA antibody adsorption from aqueous solutions and human plasma were examined in a batch system. mPHEMA beads were characterized by swelling tests, electron spin resonance (ESR) and scanning electron microscopy. Important results obtained in this study are as follows: the swelling ratio of mPHEMA beads was 34%. The presence of magnetite particles in the polymeric structure was confirmed by ESR. The mPHEMA beads have a spherical shape and porous structure. Maximum DNA coupling of carbodiimide activated mPHEMA beads was 4.4 mg/g. Maximum HIgG adsorption from an aqueous solution was 47.5 mg/g. Anti-DNA antibody adsorption from SLE plasma was observed as 87.6 mg/g. Non-specific HIgG adsorption was 0.1 mg/g. More than 90% of the adsorbed HIgG molecules and anti-DNA antibodies were desorbed succesfully by using NaSCN solution. It was possible to reuse these DNA-affinity beads without significant losses in the antibody adsorption capacities. 

*Keywords:* Affinity beads; DNA; Polyhydroxyethylmethacrylate; Anti-DNA antibody; HIgG

## 1. Introduction

Antibodies against DNA (anti-DNA antibodies) were first described in the sera of patients with systemic lupus erythematosus (SLE) more than 20 years ago [1]. Since then, these antibodies have emerged as a central focus in the investigation of the pathogenesis of SLE and of autoimmunity in general. Antibodies against DNA serve as markers of diagnostic and prognostic significance in SLE, and there is compelling evidence for an association between anti-DNA antibodies and tissue damage [2]. Since many of the clinical manifestations of this disease can be attributed to immune complex deposition, the concept has arisen that antibodies against DNA

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mediate tissue damage by the formation of DNA–anti-DNA antibody immune complexes which localize throughout the body, most prominently in the kidneys [3]. However, this model, while consistent with many clinical and serologic findings, has been difficult to verify. For example, although there is suggestive evidence for DNA–anti-DNA antibody complexes in patient sera, such complexes have not been demonstrated either consistently or conclusively [4].

The level of anti-DNA antibodies correlates well with the disease activity and organ involvements, such as nephritis and cerabritis. In such cases the removal of anti-DNA antibodies from plasma may lead to a clinical improvement. For this reason application of DNA-affinity adsorbents has been considered as an effective therapeutic method. The first ex vivo application of DNA-attached adsorbents was achieved by Terman et al. [5]. They removed anti-DNA antibodies from the plasma of positively immunized rabbits by circulating their blood through an extracorporeal shunt containing coupled DNA. The first clinical trial of immunoadsorption was also done by Terman et al., in which they used DNA-affinity adsorbents to treat a female patient suffering from SLE [6]. Nicolaev et al. have successfully applied DNA-coupled activated carbon hemoperfusion column to the treatment of patients with psoriasis [7]. Letourneur and co-workers have succesfully utilized phosphorylated polystyrene derivatives acting as DNA like polymers for the removal of anti-DNA antibodies in in vitro systems [8,9]. Zhu et al. have used DNA-coupled non-woven poly(ethyleneterephthalate) fabric fibers for treatment of systemic lupus eryhtematosus [10]. Recently, Yu and He have prepared DNA-coupled hydroxyethyl cross-linked chitosan beads as immunoadsorbents for specific removal of anti-DNA antibodies in SLE serum [11]. Kato and Ikada have reported anti-DNA antibody adsorption using DNA-carrying poly(ethyleneterephthalate) microfibers [12].

The development of the magnetic carriers as an adsorbent in chromatography promises to solve many of the problems associated with chromatographic separations in packed bed and in conventional fluidized bed systems [13]. Magnetic carriers combine some of the best characteristics of fluidized beds (low pressure drop and high feed-stream solid tolerances) and of fixed beds (absence of particle mixing, high mass transfer rates, and good fluid–solid contact) [14]. Recently, there has been increased interest in the use of magnetic carriers in biomolecule coupling and nucleic acid purification [15]. Magnetic carriers can be produced using inorganic materials or a number of synthetic and natural polymers. High mechanical resistance, insolubility and excellent shelf life make inorganic materials ideal as carriers. The main disadvantage of inorganic supports is their limited functional groups for complexation with metal ions. Magnetic carriers can be porous or non-porous [16]. Magnetic carriers are more commonly manufactured from polymers since they have a variety of surface functional groups which can be tailored to use in specific applications. Polyvinylbutyral, polyethylene glycol, polyvinylalcohol (PVA), polyacrylamide, polymethylmethacrylate and alginate are typical polymeric carriers which are used in different applications [17–22].

In this study, we prepared a magnetic bioaffinity adsorbent carrying DNA for selective removal of anti-DNA antibodies from human plasma. We selected magnetic poly(2-hydroxyethylmethacrylate) (mPHEMA) beads as the basic solid phase, which is one of the most widely used hydrophilic polymers in biomedical applications, by considering possible applications of these sorbents in direct hemoperfusion extracorporeal therapy, in which blood compatibility is one of the main concerns [23–25]. Especially, when dealing with highly viscous mediums such as blood contact with the magnetic adsorbent in a magnetically stabilized fluidized bed is desirable because of high convective transport rates without cell damage (i.e., hemolysis). mPHEMA beads were produced by a modified suspension polymerization technique. The hydroxyl functional groups on these magnetic beads were activated by carbodiimide, and then bio-ligand DNA molecules were covalently coupled to the beads through the active sites. Human immunoglobulin G (HIgG) adsorption on the DNA-affinity beads from aqueous solutions containing different amounts of HIgG and at different pH values are reported here. Finally, anti-DNA antibody adsorption onto the DNA-affinity beads from the blood plasma of a patient with SLE is also discussed.

## 2. Experimental

### 2.1. Preparation of magnetic PHEMA beads

The mPHEMA, beads were prepared by modified suspension polymerization technique. 2-Hydroxyethylmethacrylate (HEMA), was purchased from Sigma (St. Louis, MO, USA), and was purified by vacuum distillation under a nitrogen atmosphere. The comonomer, ethyleneglycoldimethacrylate (EGDMA, Merck, Darmstadt, Germany) was used as the crosslinking agent. Magnetite particles ($Fe_3O_4$, diameter $<5$ μm) were obtained from Aldrich (USA). All other chemicals were obtained from Merck as analytical grade. A typical suspension copolymerization procedure of mPHEMA beads was given as below: the dispersion medium was prepared by dissolving 200 mg of PVA (molecular mass: 50000) in 50 ml of distilled water. The desired amount of 2,2′-azobisisobutyronitrile (AIBN) (0.06 g) was dissolved within the monomer phase 12.0/4.0/8.0 ml (EGDMA/HEMA/toluene) with 1.0 g magnetite particles. This solution was then transfered into the dispersion medium placed in a magnetically stirred (at a constant stirring rate of 600 rpm) glass polymerization reactor (100 ml) which was in a thermostatic water bath. The reactor was flushed by bubbling nitrogen and then was sealed. The reactor temperature was kept at 65°C for 4 h. Then the polymerization was completed at 90°C in 2 h. After polymerization, the mPHEMA beads were separated from the polymerization medium. The residuals (e.g., unconverted monomer, initiator and other ingredients) were removed by a cleaning procedure. Briefly, magnetic beads were transferred to a reservoir, and washing solutions (i.e., a dilute HCl solution, and a water–ethanol mixture) were recirculated through the system which includes also an activated carbon column, to be assured that the magnetic beads were clean. Purity of the magnetic beads was followed by observing the change of optical densities of the samples (wavelength 280 nm) taken from the liquid phase in the recirculation system, and also from the differential scanning calorimetry (DSC) thermograms of the magnetic beads obtained by using a differential scanning microcalorimeter (Mettler, Switzerland). The optical density of the uncleaned magnetic beads was 2.63, but after the cleaning operation this value was reduced to zero. In addition, when the thermogram of uncleaned beads was recorded, it had a peak around 60°C. This peak might originate from AIBN, but after application of the cleaning procedure, no peak between 30 and 100°C was observed on the thermogram.

### 2.2. Characterization of mPHEMA beads

#### 2.2.1. Swelling tests

Water uptake properties of the mPHEMA and mPHEMA/DNA beads were determined by the volumetric method. In this method, the dry beads of known amount (100 mg) were placed in two cylindrical glass tubes and top points of the tubes were marked. Afterwards the tubes were filled with distilled water and the beads were allowed to swell at room temperature. The height of the beads were marked periodicially (i.e., each 30 min). The height of the swollen beads in the tubes was used to calculate the swelling ratio from the following formula, where, $h_{\text{swollen}}$ is height of swollen beads, and $h_{\text{dry}}$ is height of dry beads:

$$\text{Swelling ratio (\%)} = \left[(h_{\text{swollen}} - h_{\text{dry}})/h_{\text{dry}}\right] \times 100 \quad (1)$$

#### 2.2.2. Analysis of magnetism

The degree of magnetism of the mPHEMA beads was measured in a magnetic field by using a vibrating-sample magnetometer (Princeton Applied Research, Model 150A, USA). The presence of magnetite particles in the polymeric structure was investigated with an electron spin resonance (ESR) spectrophotometer (EL 9, Variant).

#### 2.2.3. Scanning electron microscopy (SEM)

The surface morphology and internal structure of the mPHEMA beads were observed in a scanning electron microscope (JEOL, JEM 1200EX, Tokyo, Japan). mPHEMA beads were dried at room temperature and coated with a thin layer of gold (about 100 Å) in vacuum and photographed in the electron microscope with ×1000 magnification. The particle

size was determined by measuring at least 100 beads on photographs taken on an SEM system.

### 2.3. Carbodiimide activation

Prior to the activation process, the mPHEMA beads were kept in distilled water for about 24 h and washed on a glass filter with 0.5 *M* NaCl solutions and water in order to remove impurities. Carbodiimide was bought from Sigma. Carbodiimide aqueous solution (100 ml) with different initial concentrations (2.5–15.0 mg/ml) were prepared in carbonate buffer. The pH of each solution was quickly adjusted to 10.5 with 1.0 *M* NaOH while it was magnetically stirred. A 1.0 g amount of mPHEMA beads was then added to each solution and the activation procedure was continued for 24 h. The main problem in surface modification is to prevent deformation and/or destruction of beads. If there is any deformation, encapsulated magnetites (i.e., iron particles) can easily release from the polymeric structure. This decreases the magnetic properties of the polymeric adsorbent. In order to prevent structural destructions/deformations, coupling reactions were carried out under mild experimental conditions and a long reaction period. That is why carbodiimide was used for the incorporation of activated groups on/in to the mPHEMA structures under mild conditions. After the activation reaction, in order to remove the excess activation agent (i.e., carbodiimide), the mPHEMA beads were washed with 0.1 *M* $NaHCO_3$ and then with distilled water.

### 2.4. DNA coupling

DNA (from herring testes) was purchased from Sigma. The freshly carbodiimide-activated mPHEMA beads were magnetically stirred at a constant temperature of 20°C for about 20 h (i.e., equilibrium time) with 10 ml DNA solution. In order to observe the effects of pH and initial carbodiimide concentration on the covalent coupling of DNA, pH and the initial carbodiimide concentration were varied in the range of 4.0–8.0 and 2.5–15 mg/ml, respectively. The initial concentration of DNA in the medium was 1.0 mg/ml. To measure the effects of DNA concentration on coupling, the initial concentration of DNA was varied between 0.2 and 2.0 mg/ml.

After coupling, the DNA-affinity beads were washed with 0.1 *M* $CH_3COONa$ (pH 4.5)+0.15 *M* NaCl, and finally with 0.1 *M* $NaHCO_3$ (pH 9.5)+0.5 *M* NaCl. The amount of DNA-coupled on the carbodiimide-activated mPHEMA beads was determined by measuring the decrease of the DNA concentration and also by considering the DNA molecules adsorbed non-specifically (not through the carbodiimide activated sites), by spectrophotometry at 260 nm. Prepared mPHEMA beads were held at 4°C prior to use.

In order to estimate the amount of released DNA, the DNA-affinity beads (250 mg) were placed in test tube containing 10 ml of phosphate buffer solution (pH 7.4) and shaken on a rotary shaker for 24 h. The amount of DNA released into the medium was measured cumulatively as the absorption band at 260 nm by a bench-top spectrophotometer (Spectronic-21 Series, Bousch and Lomb, Germany).

### 2.5. Iron leach studies

In order to estimate the amount of leached iron from within the magnetic beads, the magnetic beads (250 mg) were placed in test tubes containing 10 ml of leach media and shaken on a rotary shaker for 24 h. The amount of iron leached into the medium was determined by a graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Germany). Three kind of release media were used: pH 2.0 buffer of acetic acid solution (50%, v/v), phosphate buffer solution (pH 7.0) and sodium citrate–NaOH buffer solution (pH 12.0).

### 2.6. Human IgG adsorption from aqueous solution

Adsorption of HIgG (Sigma, catalog No: 160101) on the DNA-affinity beads was studied batch wise. mPHEMA beads containing DNA were incubated with 50 ml of HIgG solution at 20°C for 4 h (i.e., equilibrium time). In order to study the effects of carbodiimide concentration and pH on coupling of HIgG to DNA-affinity beads, the carbodiimide concentration of the solution were varied between 2.5

and 15.0 mg/ml, and the pH of the solution was 7.4. The initial concentration of HIgG was 1.2 mg/ml solution.

To determine the effect of the amount of the DNA on adsorption of HIgG, the amount of the DNA on the mPHEMA beads was varied between 0.7 and 4.4 mg DNA/g, in which pH of the adsorption medium and the initial concentration of HIgG were 7.4 and 1.2 mg/ml solution, respectively. To observe the effect of the initial concentration of HIgG on adsorption, the initial concentration of HIgG was varied between 0.4 and 2.0 mg/ml. Here, the amount of DNA on the mPHEMA beads was constant (4.39 mg DNA/g), and pH of the medium was 7.4.

After the HIgG adsorption, in order to remove the non-specifically adsorbed HIgG molecules, the mPHEMA beads were washed with 0.1 *M* borate buffer + 0.15 *M* NaCl (pH 8.8), with 2.0 *M* urea + 0.15 *M* NaCl, and finally with 0.1 *M* $NaHCO_3$ (pH 9.5) + 0.5 *M* NaCl. The HIgG concentration in aqueous solutions was measured using the Folin–Lowry method [26]. The amount of adsorbed HIgG was calculated as:

$$q = [(C_i - C_t) \cdot V]/m \quad (2)$$

where, $q$ is the amount of HIgG adsorbed onto unit mass of the beads (mg/g); $C_i$ and $C_t$ are the concentrations of HIgG in the initial solution and in the supernatant after adsorption, respectively (mg/ml); $V$ is the volume of the aqueous phase (ml); and $m$ is the mass of the beads (g).

### *2.7. Anti-DNA antibody adsorption from SLE plasma*

Blood samples taken from a patient with SLE was used in these studies. Blood was centrifuged at 500 *g* for 30 min at room temperature to separate the plasma. 0.1 g of mPHEMA/DNA beads containing different amounts of DNA (between 0.7 and 1.81 mg DNA/g mPHEMA) were incubated with 2 ml of the plasma obtained from the SLE patient at 15°C for 20 min. The amount of anti-DNA antibody adsorbed on the mPHEMA/DNA beads was determined by radioimmunoassay (RIA) by measuring the decrease in the anti-DNA antibody concentration in the plasma of the patient.

### *2.8. Desorption and repeated use*

Desorption of HIgG was studied in 0.5 *M* NaSCN solution. The mPHEMA beads loaded with HIgG were placed in this desorption medium and stirred continuously (at a stirring rate of 600 rpm) for 1 h at room temperature. The final HIgG concentration in the desorption medium was determined by a solid-phase enzyme-linked immunosorbent assay (ELISA) method. The desorption ratio was calculated from the amount of HIgG adsorbed on the beads and the final HIgG concentration in the desorption medium.

Desorption of anti-DNA antibodies was carried out in 1.0 *M* NaSCN solution. The same procedure described above was applied. The final anti-DNA antibody concentration was determined by RIA by measuring the increase in the anti-DNA antibody concentration in the desorption medium. The desorption ratio was also calculated from the amount of anti-DNA antibody adsorbed on the beads and the final anti-DNA antibody concentration in the desorption medium.

In order to test the reusability of the DNA-affinity beads, the HIgG adsorption–desorption procedure was repeated six times using the same polymeric adsorbent. It should also be noted that, after desorption of HIgG, DNA leakage from the polymeric structure was also monitored continuously.

## 3. Results and discussion

### *3.1. Characteristics of mPHEMA beads*

mPHEMA beads used in this study are resistant to adhesion of blood proteins and blood cells [27]. They are rather hydrophilic and cross-linked structures, i.e., hydrogels. The simple incorporation of water weakens the secondary bonds within the hydrogels. This enlarges the distance between the polymer chains and causes the uptake of water. The equilibrium water uptake ratio of mPHEMA beads is 34% (w/w). It should be mentioned that the water-uptake properties of the mPHEMA beads did not change after carbodiimide activation and DNA coupling. It appears that carbodiimide activation and DNA immobilization had a negligible effect on the

swelling ration of these magnetic beads. The density of mPHEMA beads is 1.45 mg/ml.

The surface morphology and internal structure of mPHEMA beads are exemplified by the electron micrographs in Fig. 1. As clearly seen here (Fig. 1A), the magnetic beads have a spherical form and rough surface due to the abrasion of magnetite crystals (diameter $<0.1$ μm) during the polymerization procedure. The micrograph in Fig. 1B was taken with broken beads to observe the internal part. The presence of macropores within the microbead interior is clearly seen in this photograph. It can be concluded that the mPHEMA beads have a macroporous interior surrounded by a reasonably rough surface, in the dry state. The roughness of the microbead surface should be considered as a factor providing an increase in the surface area. In addition, these macropores reduce diffusional resistance and facilitate mass transfer because of high surface area. This also provides higher ligand coupling (i.e., DNA) and enhances the pathogenic antibody (i.e., anti-DNA antibody) removal capacity. On the other hand, non-magnetic PHEMA microbeads were in the uniform and spherical shape with smooth surface characteristics.

The presence of magnetite particles in the polymeric structure was confirmed by ESR. The intensity of the magnetite peak against magnetic field (Gauss) is shown in Fig. 2.

The application of an external magnetic field may generate an internal magnetic field in the sample which will add to or subtract from the external field. The local magnetic field generated by the electronic magnetic moment will add vectorially to the external magnetic field ($H_{ext}$) to give an effective field ($H_{eff}$); that is:

$$H_{eff} = H_{ext} + H_{local} \quad (3)$$

As seen in Fig. 2, mPHEMA beads have a relative intensity of 400. This value shows that polymeric structure has a local magnetic field because of magnetite in its structure.

The *g* factor given in Fig. 2 can be considered as quantity characteristic of the molecules in which the unpaired electrons are located, and it is calculated from Eq. (3). The measurement of the *g* factor for an unknown signal can be a valuable aid in the identification of a signal is origin. In the literature the *g* factor for $Fe^{3+}$ (low spin and high spin complexes) is determined between 1.4 and 3.1 and 2.0 and 9.7, respectively [28]. The *g* factor was found to be 3.2 for mPHEMA structure.

We also present the magnetic properties of the polymeric structure expressed in electron mass units (EMUs), showing the behavior of magnetic beads in a magnetic field using a vibrating magnetometer, in Fig. 3, and $H_r$ value, is defined as the external magnetic field at resonance. In the EMU spectrum and from the $H_r$ value, a 2250 Gauss magnetic field was found sufficient to excite all of the dipole moments present in 1.0 g mPHEMA sample. These values will be an important design parameter for a magnetically stabilized fluidized bed or for magnetic filtration using these magnetic beads. The value of the magnetic field required to stabilize the fluidized bed is a function of the flow velocity, particle shape, size, size distribution and magnetic susceptibility of beads. In the literature, this value was found to change from 8 to 20 kG for various applications [29,30], thus our magnetic beads will need less magnetic intensity in a magnetically stabilized fluidized bed or for a magnetic filter system.

Note that the leach of iron was measured in three different kinds of media including acetic acid solution (50%, v/v, pH 2.0), phosphate buffer solution (pH 7.0) and sodium citrate–NaOH buffer solution (pH 12.0). It should be noted that there was no measurable release.

### *3.2. DNA coupling*

DNA was used as the bio-ligand for specific binding of anti-DNA antibodies. Ligand leakage is a serious problem in column studies for biomedical applications. DNA leakage was investigated in phosphate buffer solution (pH 7.4). DNA leakage was not observed from DNA-affinity beads. In the first part of this study, the effects of medium pH, initial concentrations of carbodiimide and DNA on the coupling of DNA onto the mPHEMA beads were investigated in batch adsorption–equilibrium studies. In order to establish the optimal pH for DNA coupling, coupling studies were repeated at pH values between 4.0 and 8.0. The effect of pH on DNA coupling is significant. The saturation value of

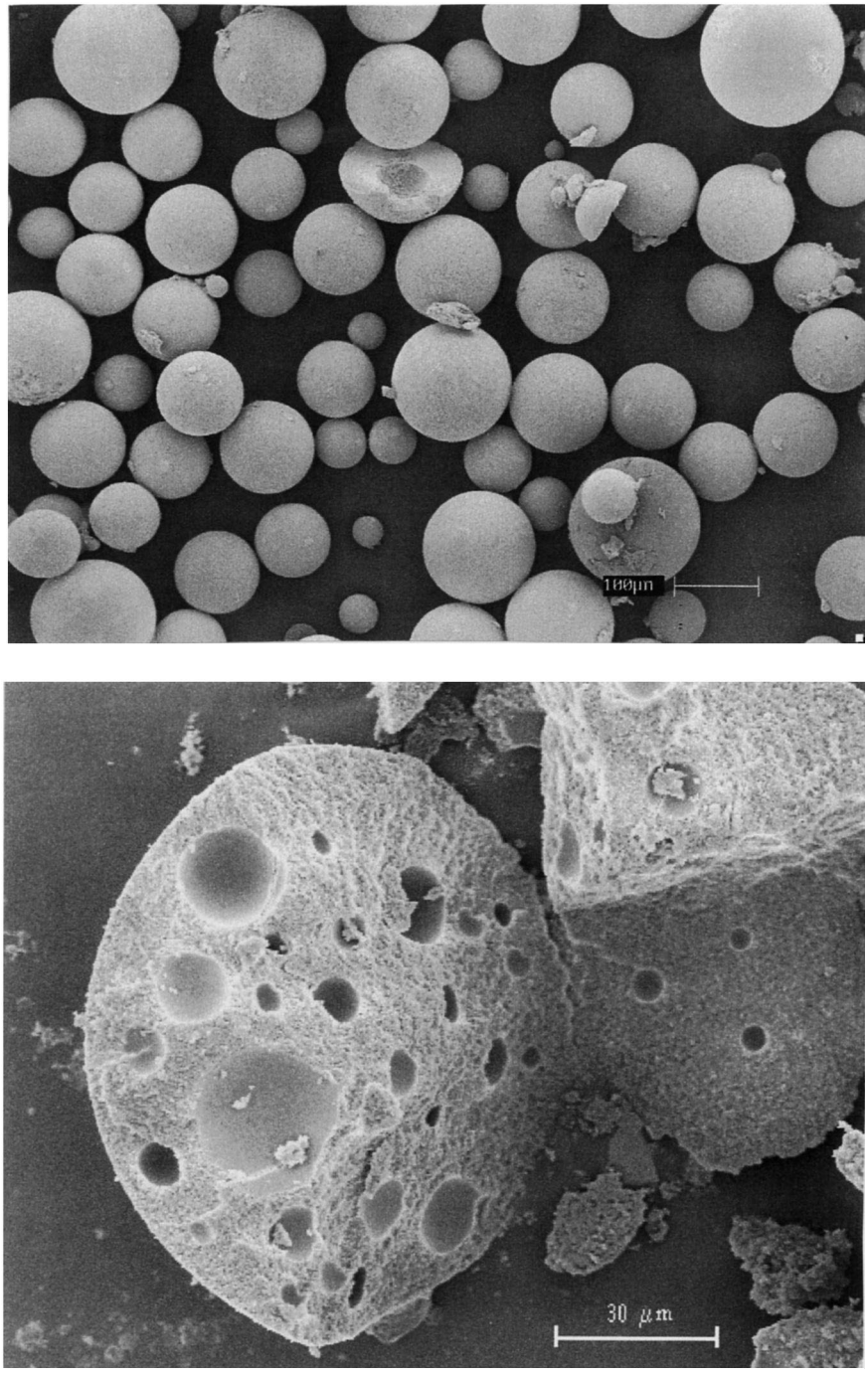


Fig. 1. SEM micrographs of mPHEMA beads: (A) surface; (B) cross-section.

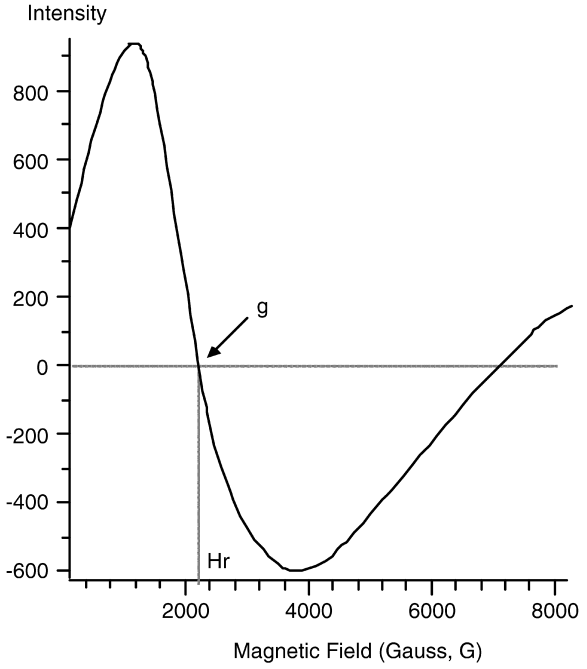


Fig. 2. ESR spectrum of mPHEMA beads.

DNA (4.39 mg DNA/g PHEMA) was observed around pH 6.0, which was assumed to be the optimal pH and used in the later part of this study. Note that both the three-dimensional structure and the degree of ionization of several groups on the DNA molecule can change with pH. Probably, DNA molecules have the most suitable structural properties for binding through carbodiimide active sites on the surface of PHEMA beads at pH 6.0. Significantly lower coupling capacities were obtained below and above pH 6.0. Similar pH values were proposed and applied for DNA coupling also by others [11,12].

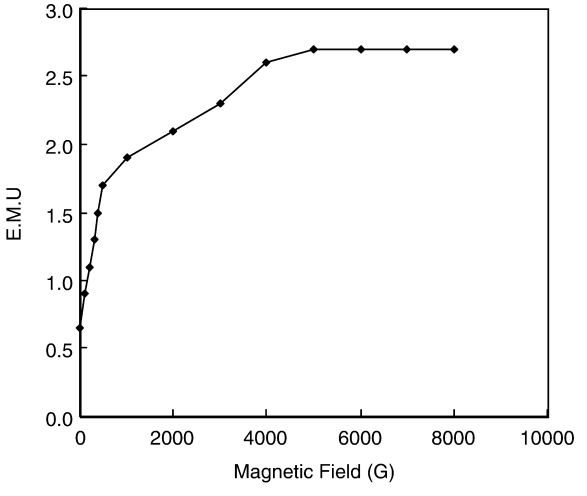


Fig. 3. The magnetic behavior of mPHEMA beads.

Yields of DNA coupling on affinity matrices reported in the related literature are in a wide range from about 0.08 mg double-stranded (ds)DNA per unit mass of adsorbent [10] up to 5.0 mg DNA/g (i.e., polyethyleneterephthalate microfibers) [12]. Recently, very high DNA coupling yields up to 290 mg/g of polyethyleneimine-coupled poly(*p*-chloromethylstyrene) beads were also reported [31]. In this study, the maximum ligand coupling achieved was 4.4 mg DNA/g over the experimental range of carbodiimide concentration, which was similar to coupling yields of conventional matrices.

The initial concentration of the activating agent, i.e., carbodiimide in the activation medium was changed between 2.5 and 15 mg/ml in order to change the number of the activated sites on the mPHEMA surfaces. These mPHEMA beads with different activation degrees were then incubated with DNA aqueous solutions at pH 6.0. Note that the non-specific adsorption (adsorption on the plain mPHEMA beads) was less than 0.1 mg DNA/g. The amount of DNA coupled on the beads increased by increasing the initial concentration of carbodiimide, up to 10 mg/ml, however, above this value, the effect was less pronounced. However, due to the size of the giant DNA molecules (molecular mass is approximately $1 \cdot 10^7$), it was not possible to immobilize more than 4.4 mg DNA/g PHEMA.

Note that an increase in the carbodiimide initial concentration corresponds to a larger number of activated sites on the surface of the carrier. Therefore, higher amounts of DNA are coupled on the carbodiimide activated mPHEMA beads with higher number of activated sites. It should be noted also that, there is always a saturation capacity which depends on the number of functional groups on the matrix and the size of the bio-ligand molecules. However, the key factor in the performance of ligands coupled on a solid surface is the ligand mobility after coupling rather than the total number of ligands available for coupling. Binding of DNA molecule tightly onto the adsorbent surface, multiple

interactions between the adsorbent and DNA molecule would certainly reduced effective utilization of active sites on the DNA molecule.

### 3.3. HIgG adsorption

The performance of an affinity matrix depends on the activity of the coupled ligand (i.e., DNA), which is not a linear function of ligand surface concentration. An optimal ligand surface concentration is expected in which the interaction ratio between DNA and immunoglobulin molecules is the highest. Solid-phase variables such as spacer arm length, ligand concentration, coupling method, and matrix porosity control the availability of the active sites on DNA molecule after coupling, while the liquid-phase variables such as pH and ionic strength provide an insight into the conformational state and degree of ionization of the immunoglobulin molecules, where favorable interaction with coupled DNA molecules will take place.

In this study, the effects of medium pH, surface concentration of the ligand (i.e., DNA), and initial concentration of HIgG in the incubation medium on the adsorption of HIgG molecules onto the plain and the DNA-affinity beads were studied in batch experiments.

In the first group of experiments we changed the pH of the incubation medium between 6.0 and 8.5, and looked at HIgG adsorption from aqueous solutions onto the DNA-affinity beads (containing 4.4 mg DNA/g). The initial concentration of HIgG in the incubation solution was 1.2 mg/ml solution. Fig. 4 shows the effects of pH on HIgG adsorption which is very significant. As shown in Fig. 4, the maximum HIgG binding capacity of DNA-affinity beads was found to lie at 47.5 mg HIgG/g, which was observed at pH 7.4. While, significantly lower adsorption capacities were obtained below and above of pH 7.4. The amount of HIgG coupled onto the DNA-affinity beads as a function of pH exhibits two adsorption domains, as shown in Fig. 4. HIgG is negatively charged at pH 7.4 (isoelectric point of HIgG: 6.2). However, it is interesting to note that the amount of HIgG coupled onto DNA containing beads shows a maximum at pH 7.4, with a significant decrease at lower and higher pH values. Specific interactions between HIgG and bio-ligand DNA molecules at pH

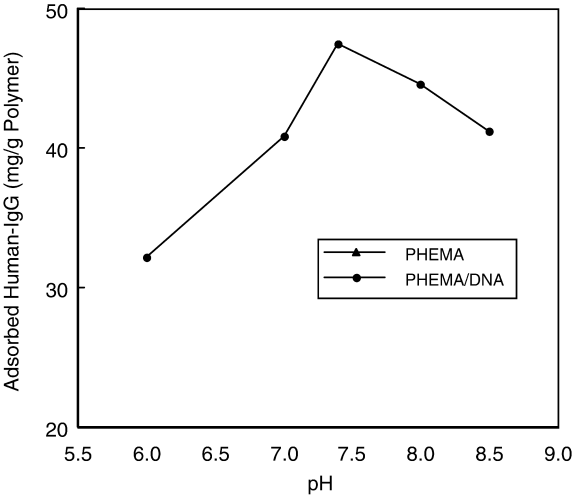


Fig. 4. Effect of pH on HIgG adsorption on DNA-affinity beads; DNA loading: 4.4 mg/g; HIgG concentration: 1.2 mg/ml; temperature: 25°C.

7.4 may result from the conformational state of both DNA and HIgG molecules (more folded structure) at this pH.

HIgG adsorption capacities of the mPHEMA beads containing different amounts of DNA (between 0.7 and 4.4 mg DNA/g) were investigated at pH 7.4. Table 1 shows the effects of bio-ligand (i.e., DNA) surface concentration on HIgG adsorption onto mPHEMA beads. When the surface DNA concentration (i.e., the number of DNA molecules per unit mass of the adsorbent) increased, the amount of HIgG adsorbed onto mPHEMA beads increased. The maximum HIgG adsorption capacity was 47.5 mg/g.

HIgG adsorption onto plain mPHEMA and DNA

Table 1
HIgG adsorption onto the DNA-affinity beads: HIgG concentration: 1.2 mg/ml; pH 7.4; and temperature: 25°C

| Coupled DNA (mg/g) | Adsorbed HIgG (mg/g) |
|---|---|
| 0.70±0.06 | 12.49±1.21 |
| 1.20±0.14 | 23.88±1.15 |
| 1.79±0.12 | 29.15±1.38 |
| 2.30±0.17 | 33.95±1.34 |
| 3.27±0.30 | 42.05±1.19 |
| 4.40±0.20 | 47.50±2.20 |

Results are the average and the standard deviation of three parallel studies.

carrying (4.4 mg DNA/g) mPHEMA beads from aqueous solutions containing different amounts of HIgG (between 0.4–2.0 mg/ml) was studied at a constant pH of 7.4. Fig. 5 gives the adsorption data on plain mPHEMA beads (0.1 mg HIgG/g). Specific adsorption (i.e., adsorption of HIgG molecules onto the mPHEMA beads through DNA molecules) was significant (up to 47.5 mg HIgG/g). Adsorption capacity increased with increasing the initial concentration of HIgG in the incubation medium. The amount of HIgG coupled to mPHEMA beads via DNA molecules reached almost a plateau value around 1.2 mg/ml, due to the possible steric hindrance effect mentioned before.

Coupled DNA proved useful also for the capture of anti-DNA antibodies directly from SLE patient plasma. The initial concentration of anti-DNA antibody within this patient plasma was 10.5 mg/ml. The mPHEMA beads carrying different amounts of DNA (between 0.7–1.8 mg/g) were incubated with the patient plasma. Table 2 gives the adsorption data. As seen here, there is very low non-specific adsorption (0.19 mg/g) of anti-DNA antibody onto the plain mPHEMA beads. There is a pronounced adsorption of anti-DNA antibody (up to 87.6 mg/g) on the DNA-derivatized magnetic beads. Adsorption increased with increasing surface concentration of DNA. However, too much DNA on the magnetic beads is not practical to work with several reasons. Note that anti-DNA antibody adsorption from the patient plasma was higher than HIgG adsorption from the aqueous solutions. There are two reasons to explain the difference in the adsorption values of HIgG and anti-DNA antibodies; (i) DNA molecules exhibit much higher affinity to anti-DNA antibodies than HIgG molecules. It should also be noted that affinity constants of DNA–anti-DNA antibody complex and DNA/HIgG molecules are $4.9 \cdot 10^{9}\ M^{-1}$ and $4 \cdot 10^{5}\ M^{-1}$, respectively. (ii) The anti-DNA antibody concentration (10.5 mg/ml) was much higher than HIgG concentration (1.2 mg/ml), which determines the extent of the adsorption.

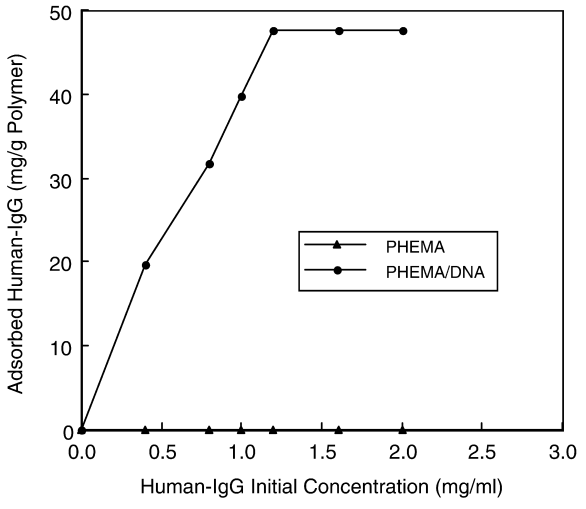


Fig. 5. Effect of HIgG initial concentration on HIgG adsorption through DNA-affinity beads; DNA loading: 4.4 mg/g; pH 7.4; temperature: 25°C.

Table 2
Anti-DNA antibody adsorption from SLE patient plasma: anti-DNA antibody concentration: 10.5 mg/ml; and temperature: 25°C

| Coupled DNA (mg/g) | Adsorbed anti-DNA antibody (mg/g) |
|---|---|
| 0.00 | 0.19±0.02 |
| 0.70±0.06 | 15.64±1.23 |
| 0.85±0.09 | 20.54±1.26 |
| 1.12±0.11 | 28.33±0.43 |
| 1.20±0.14 | 30.94±2.14 |
| 1.79±0.12 | 37.79±1.64 |
| 2.30±0.17 | 53.09±2.14 |
| 3.27±0.30 | 63.76±2.39 |
| 4.40±0.20 | 87.60±3.70 |

Results are the average and the standard deviation of three parallel studies.

### *3.4. Desorption and repeated use*

Desorption of HIgG from the DNA-affinity beads was also carried out in a batch experimental set up. The mPHEMA beads loaded with different amounts of HIgG were placed within the desorption medium, and the amount of HIgG desorbed in 1 h was determined. Up to 91% of the adsorbed HIgG was desorbed by using NaSCN as elution agent.

Desorption of anti-DNA antibody from the DNA-affinity beads was also monitored in a batch system. Very high desorption ratios were also obtained (up to 94%) for anti-DNA antibody. With the desorption data given above we concluded that NaSCN is a suitable desorption agent, and allows repeated use of the affinity adsorbents used in this study both for HIgG and anti-DNA antibody.

The addition of NaSCN might change the charge of the peptide side groups of the IgG due to their isoelectric points, resulting in the detachment of the IgG molecules from DNA. Note that there was no DNA release in this case which shows that DNA molecules are coupled covalently to carbodiimide activated mPHEMA beads. With the desorption data given above we concluded that NaSCN is a suitable desorption agent, and allows repeated use of the affinity adsorbents used in this study.

In order to show the reusability of the DNA-affinity beads, the adsorption–desorption cycle was repeated six times using the same DNA-affinity beads. There was no marked reduction both in the HIgG and anti-DNA antibody adsorption capacity of the mPHEMA beads. The HIgG and anti-DNA antibody adsorption capacity decreased only 8% after six cycles.

## 4. Conclusions

Pathogenic antibodies are becoming an important class of biomolecules for both the diagnosis and the treatment of a large variety of human diseases. The medical relevance of pathogenic antibodies has stimulated the development of cost- and time-effective removal techniques including polymeric carriers. Conventional immunoadsorption methods based on the use of a combination of classical chromatographic techniques are very time consuming. Magnetic carrier technology enables the use of magnetic processing for rapid and selective separation. Magnetic separation has several potential advantages over conventional approaches. Magnetically stabilized fluidized bed cartridges require high flow-rates with a much lower operating pressure than a microbead-packed bed column. In this technique, the biomolecule to be separated can be directly transported by convection to the ligand coupled on the surface of the beads, higher throughput and faster processing times onto the DNA-affinity beads can be achieved. Magnetic PHEMA beads, were produced by modified suspension polymerization of HEMA. A bioligand, DNA was then coupled to these beads to reach a loading up to 4.4 mg/g, which resulted a HIgG adsorption of 47.5 mg/g from aqueous solutions. A remarkable increase in the HIgG adsorption capacities were achieved from SLE patient plasma (up to 87.6 mg/g). Successful desorption ratios (more than 90% of the adsorbed HIgG) were achieved by using 0.5 *M* NaSCN. It was possible to reuse these DNA-affinity beads without marked reduction in the adsorption capacities.

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