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# Preparation and use of immunoglobulin-binding affinity supports on Emphaze beads

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**Abstract**

The Emphaze support couples to nucleophiles with a leak-resistant linkage resulting from the reaction with intrinsic azlactone functionalities. Protein A, protein G, and protein A/G were coupled to the Emphaze support and to an agarose matrix at various concentrations of ligand. Mannan Binding Protein was similarly coupled to the Emphaze matrix and agarose. These supports were investigated as to their binding characteristics toward immunoglobulins, both in a gravity separation format and using a medium pressure chromatography system under high linear flow. In addition, the lectin jacalin was coupled both to Emphaze and agarose and compared for total binding capacity toward human IgA. There were significant differences in the binding characteristics of human IgG for the Emphaze and agarose supports. Mouse monoclonal IgG1 showed a binding optimum at pH 5 on Emphaze protein A, A/G and G as compared to an optimum of pH 8 for the agarose supports. Similar binding pH optimums were observed for human IgG. Slightly higher capacities were observed for the Emphaze IgG binding than for the corresponding agarose supports. Emphaze Mannan Binding Protein had an almost identical binding capacity for mouse IgM as did a similarly loaded agarose support.

## 1. Introduction

Affinity chromatography is a powerful technique based on the specific attraction of a ligand for its target molecule(s). The ligand typically is covalently attached to an insoluble matrix and is subsequently used to purify its target from a complex mixture. The matrix composition and the ligand coupling chemistry are very important parameters for the specific binding of target molecules. The ideal chromatography support has several characteristics critical to its performance. The matrix should be a beaded, spherical, porous support with narrow particle size distribution and good chromatographic qualities. The non-specific binding character of the matrix should be low. In addition, the potential for using high linear flow rates with the support is advantageous, because it allows the chromatographic operation to be done in shorter periods. Finally, the support must be easily derivatized with the affinity ligand, resulting in a stable linkage.

Activated agarose is often used for the immobilization of ligands for affinity purification. There are several activation schemes commonly used: CNBr, reductive amination, N,N′-carbonyldiimidazole (CDI) coupling and NHS-ester coupling (for review, see Hermanson et al. [1]). A new support material, the 3M Emphaze Biosupport Medium AB 1, has recently been used in the preparation of a number of immobil-

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ized proteins useful for the purification of immunoglobulins. The polymeric Emphaze matrix is well suited for affinity chromatography operations, having large pores (1000 Å) and an exclusion limit in excess of 2 000 000 relative molecular mass. The rigid nature of the beads (average particle size 50–80 $\mu$m) allows linear flow-rates up to 3000 cm/h, permitting use of the support in automated chromatography systems.

The Emphaze support is prepared by the copolymerization of vinyldimethylazlactone and the cross-linking monomer, methylenebisacrylamide [2]. Due to the presence of the azlactone monomer incorporated during the manufacturing process, the support contains amine-reactive groups. The Emphaze matrix is supplied dry to prevent hydrolysis of the azlactone groups during storage. The hydrated volume of the support is approximately 8–10 ml/g of dried beads.

An azlactone can react with an amine-containing ligand in a ring-opening reaction to form a leak-resistant amide-bond linkage (Fig. 1). This process creates an indigenous 5-atom spacer arm terminating in an amide bond with the ligand. After coupling, the resulting gel surface environment is completely hydrophilic, displaying very low non-specific binding character.

Amine-containing molecules can be immobilized onto the beads simply by dissolving the ligand in a suitable coupling buffer and adding the dry support to the solution with mixing. Coupling is usually complete in 1 h. The remaining active sites are quenched with ethanolamine.

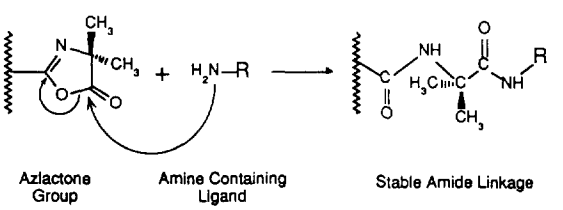


Fig. 1. The reaction of an amine-containing ligand with the azlactone groups on the surface of the Emphaze beads proceeds by nucleophilic attack on the electron-deficient carbonyl. The ring-opening process results in the creation of a stable amide-bond linkage with the ligand and the formation of an indigenous 5-atom spacer arm.

One idiosyncrasy of the coupling of proteins to the Emphaze support is the necessity for the presence of a high molar concentration of a lyotropic salt in the coupling buffer [3]. A protein with its charged outer surface has difficulty getting near enough to the azlactone polymer to react. The lyotropic salt drives the protein close enough to the bead to couple efficiently.

Previous studies investigated the immobilization of protein A or antibodies on the Emphaze support with special emphasis on obtaining high binding capacity or activity [4,5]. In this study, the immobilization and performance of five different immunoglobulin-binding proteins is investigated: native protein A, recombinant protein G, recombinant protein A/G, jacalin and rabbit-serum mannan binding protein (MBP). The use of protein A and protein G immobilized on agarose and other support materials for immunoglobulin purification is well documented in the literature [6–13]. Protein A/G is a recombinant fusion protein which combines the binding characteristics of protein A with the binding properties of protein G [14,15]. Jacalin is a lectin that can be used to purify human IgA1 from human serum [16]. MBP has binding specificity for mouse IgM and can be used to purify monoclonal IgM antibodies from ascites [17]. A comparison of the coupling yields for immobilizing these proteins on Emphaze is investigated versus a typical reductive amination coupling procedure using periodate-oxidized agarose (CNBr coupling for MBP). In addition, the use of some of these affinity supports for subsequent purification of immunoglobulins from various sources is discussed and compared to their agarose counterparts.

## 2. Experimental

### 2.1. Reagents

3M Emphaze Biosupport Medium AB 1, buffer salts, BCA protein assay reagent, native protein A, recombinant protein A/G, recombi-

nant protein G, jacalin and AminoLink coupling gel were obtained from Pierce (Rockford, IL, USA). Rabbit serum was obtained from Pel-Freeze (Rogers, AK, USA). Mouse IgG1 (clone MOPC-21) and human IgA were obtained from Sigma (St. Louis, MO, USA).

The supports that were prepared on Emphaze beads and the prepacked columns that were used can be obtained from Pierce.

### 2.2. Chromatographic equipment

Waters 650E protein purification system with Waters 996 PDA detector was used for all automated chromatography separations. The fraction collector was a Retriever II from ISCO. The spectrophotometer used was a Hitachi U-2000.

### 2.3. Preparation and analysis of immobilized protein A, protein A/G and protein G

Native protein A, recombinant protein A/G and recombinant protein G were coupled to 3M Emphaze Biosupport Medium AB 1. Two coupling buffers were compared for coupling efficiency: 0.1 *M* sodium phosphate, 0.6 *M* sodium citrate, pH 7.5 and 0.1 *M* sodium carbonate, 0.6 *M* sodium citrate, pH 9. For the preparation of each ml of Emphaze affinity support, either 3, 6 or 9 mg of protein A, protein A/G, or protein G were dissolved in 2 ml of the appropriate coupling buffer and added to 0.125 g of dry Emphaze beads. The solution was fully mixed and reacted for 1 h at room temperature. Excess protein solution was drained, and the gel was washed with water and 1 *M* NaCl to completely remove uncoupled protein. Next, 2 ml of 3 *M* ethanolamine, pH 9 were added to quench unreacted azlactone sites. The reaction was continued for 2 h at room temperature. The resulting affinity support was washed thoroughly with water, 1 *M* sodium chloride and another water wash. The gel was stored in 0.02% sodium azide at 4°C until used.

Coupling efficiency was determined by comparing the amount of protein in the reaction prior to coupling with the amount of protein left in the solution after coupling. It is necessary to use a protein assay system such as BCA instead of absorbance at 280 nm because of the interference of a small amount of Triton X-100 remaining from the manufacture of the beads. The detergent is added to allow sufficient hydration of the support without introducing bubbles.

Native protein A, recombinant protein A/G and recombinant protein G were coupled using AminoLink coupling gel according to the manufacturer's protocol. AminoLink is periodate oxidized agarose which couples amines using reductive amination. The support was charged with both 3 and 6 mg of the appropriate immunoglobulin binding protein.

### 2.4. Capacity of human IgG under gravity flow conditions

To determine human IgG binding capacities for each of the prepared supports, 2 ml columns of Emphaze immobilized protein A, protein A/G and protein G and the corresponding agarose supports were prepared. Emphaze and agarose immobilized protein A and protein A/G were equilibrated in binding buffer 1 (0.1 *M* potassium phosphate, 0.15 *M* NaCl, pH 8.0). An overload binding capacity analysis was performed on each support by applying 100 mg of human IgG in binding buffer 1 to each column and washing with binding buffer 1 until the absorbance at 280 nm reached baseline. Elution was performed by applying 2-ml aliquots of 0.1 *M* glycine, pH 2.8, collecting fractions and monitoring absorbance at 280 nm.

Columns (2 ml) of Emphaze and agarose immobilized protein G were equilibrated in binding buffer 2 (100 m*M* sodium acetate, pH 5.0). Overload total binding capacity analysis was performed for each column by applying 100 mg of human IgG in binding buffer 2 to each 2-ml column. The columns were washed with binding buffer 2 until the absorbance at 280 nm reached baseline. Elution was performed by applying 2-ml aliquots of 0.1 *M* glycine, pH 2.8,

collecting fractions and monitoring the absorbance at 280 nm.

### 2.5. Investigation of binding conditions for IgG binding supports

Studies were performed to determine the effect of varying buffer pH conditions on the binding of human IgG to the Emphaze and agarose protein A, A/G and G columns. The binding buffers used were: 0.1 *M* acetic acid, pH 4.0; 0.1 *M* citric acid, pH 4.5; 0.1 *M* sodium acetate, 0.15 *M* sodium chloride, 0.05% sodium azide, pH 5.0; 0.1 *M* 2-(N-morpholino)-ethanesulfonic acid (MES), 0.15 *M* sodium chloride, 0.05% sodium azide, pH 6.0; 0.1 *M* sodium phosphate, 0.15 *M* sodium chloride, 0.05% sodium azide, pH 7.2; 0.1 *M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.15 *M* sodium chloride, 0.05% sodium azide, pH 8.0; 0.1 *M* sodium carbonate, 0.15 *M* sodium chloride, 0.05% sodium azide, pH 9.0; 0.1 *M* 3-cyclohexylamino-1-propanesulfonic acid (CAPS), 0.15 *M* sodium chloride, 0.05% sodium azide, pH 10.0. The elution buffer used was 0.1 *M* glycine, pH 2.8.

The supports were packed into 50 mm × 3.0 mm columns (0.353 ml R-PAC (rapid protein affinity chromatography) columns (Pierce) for automated chromatography. The columns were equilibrated in the appropriate binding buffer for 10 min at a flow-rate of 1 ml/min. Human IgG was dissolved at a concentration of 20 mg/ml in deionized water. The human IgG solution was diluted 1:1 in the corresponding binding buffer. At time = 0 min, 2.0 ml of the diluted human IgG sample were injected onto the column using a flow-rate of 1 ml/min of binding buffer. At time = 6 min, 100% elution buffer was introduced and at time = 14 min, the buffer was changed to 0.02% sodium azide in water. The absorbance was monitored at 280 nm.

The binding of mouse IgG1 (clone MOPC-21) to Emphaze immunoglobulin binding supports was also investigated using the same protocol above, except that an injection of 0.2 ml of 20 mg mouse IgG1/ml of gel was used.

### 2.6. Preparation and analysis of immobilized jacalin

Emphaze immobilized jacalin was prepared by coupling 3 mg of purified jacalin per ml of gel in carbonate/citrate buffer, pH 9.0 as described above. Agarose-jacalin was prepared using AminoLink coupling gel according to manufacturer's instructions and charged with 4.5 mg of jacalin per ml of gel. The prepared supports were tested for binding capacity of human IgA. A 1-ml column of each support was equilibrated in 0.1 *M* sodium phosphate, 0.15 *M* sodium chloride, pH 7.2 (PBS) under gravity flow. Human IgA (1 mg/ml) was prepared in PBS. Approximately 6 ml of sample were applied to a 1-ml column of both Emphaze immobilized jacalin and agarose jacalin. The columns were washed until the absorbance at 280 nm reached baseline. Elution was performed by adding 0.1 *M* melibiose in PBS to each column. Fractions (1 ml) were collected and monitored by absorbance at 280 nm.

### 2.7. Preparation and analysis of immobilized mannan binding protein

The preparation of immobilized mannan and its subsequent use for the isolation of MBP from rabbit serum is based on a method described by Kozutsumi et al. [18] and modified as by Nevens et al. [17]. The immobilization of MBP on agarose is as described by Nevens et al. [17].

Coupling of mannan binding protein to Emphaze was performed according to the immunoglobulin binding protocol described above except a carbonate–citrate buffer of pH 8.5 instead of 9.0 was used. The coupling reaction was carried out overnight for convenience.

### 2.8. Purification of IgM on immobilized MBP

A 1-ml column of the Emphaze MBP was packed and equilibrated with 10 m*M* Tris, 1.25 *M* sodium chloride, 20 m*M* calcium chloride, pH 7.4 (binding buffer). The equilibration and binding steps were performed at 4°C. Purified mouse IgM solution (4 ml) was applied to the column

and 2 ml fractions were collected. The columns were washed with binding buffer until baseline was reached. After warming the columns to room temperature, bound IgM was eluted with 10 m*M* Tris, 1.25 *M* sodium chloride, 2 m*M* EDTA, pH 7.4 (elution buffer). Fractions were collected and $A_{280}$ was measured.

## 3. Results and discussion

### *3.1. Coupling efficiencies of Emphaze protein A, protein A/G and protein G*

The coupling efficiency of amine-containing ligands to the Emphaze support is affected by the pH of the reaction buffer. As in many nucleophilic coupling mechanisms, increasing the pH from physiological to more alkaline values results in higher reaction rates. Since potential increases in the hydrolysis rate at elevated pH may overshadow any reaction rate enhancements, it is instructive to determine the coupling characteristics of the azlactone functionality as the pH is increased. Table 1 shows the results of coupling the immunoglobulin-binding proteins, protein A, protein G and protein A/G, at two different pH conditions. Both buffer compositions contained the same amount of lyotropic salt (0.6 *M* sodium citrate) to eliminate any potential variation in coupling yield due to that component. The results indicate that coupling of protein A to the Emphaze support was the only one not dramatically influenced by pH. The immobilization of protein A/G and protein G was more efficient at pH 9 than pH 7. This finding correlates to the results obtained coupling other proteins (such as avidin and streptavidin) to the Emphaze support. In general, to obtain the maximum degree of coupling with proteins that are stable under such conditions, performing the Emphaze immobilization reaction in an elevated pH environment will result in better yields than coupling at physiological pH.

### *3.2. Binding capacities of immobilized protein A, protein A/G, and protein G*

The capacities of the three IgG-binding supports were determined for purifying human IgG under gravity conditions. The results of this study are found in Table 2. All capacities are expressed as mg human IgG bound per ml of gel.

Using overload analysis, the capacities of the IgG binding supports for human IgG prepared on the 3M Emphaze Biosupport Medium were equal to or greater than those prepared under similar conditions on an agarose support. Since the same amount of each protein was charged to the immobilization reactions in both the Emphaze and agarose preparations, the increases in capacity for the Emphaze supports may be due to better coupling yields in the azlactone reaction. Possibly the immobilized protein remains in a more active state on Emphaze than when

Table 1
Coupling efficiency of various proteins to the Emphaze matrix at two different pH values

| Ligand | Coupling efficiency (%) | | | | | |
|---|---|---|---|---|---|---|
| | Phosphate, pH 7.5 | | | Carbonate, pH 9.0 | | |
| | Loading 3 mg/ml | Loading 6 mg/ml | Loading 9 mg/ml | Loading 3 mg/ml | Loading 6 mg/ml | Loading 9 mg/ml |
| Protein A | 88 | 89.6 | 94 | 88 | 89 | 96 |
| Protein G | 58.8 | 76 | 74.7 | 93 | 95 | 92.6 |
| Protein A/G | 86 | 90.8 | 87.4 | 94.6 | 96.6 | 97.3 |
| Avidin | 81.3 | 81 | 80 | 90 | 89 | 92 |
| Streptavidin | 43 | 70.4 | 97.8 | 91 | 97.8 | 98 |

Table 2
IgG binding capacities for protein A, protein G, and protein A/G immobilized on Emphaze Biosupport Medium or an agarose matrix

| | IgG Binding capacity (mg IgG bound per ml gel) | | | | | |
|---|---|---|---|---|---|---|
| | Protein A | | Protein G | | Protein A/G | |
| | Ligand loading 3 mg/ml | Ligand loading 6 mg/ml | Ligand loading 3 mg/ml | Ligand loading 6 mg/ml | Ligand loading 3 mg/ml | Ligand loading 6 mg/ml |
| Capacity on Emphaze | 24 | 37 | 22 | 31 | 20 | 35 |
| Capacity on agarose | 17 | 35 | 10 | 18 | 14 | 26 |

coupled to agarose under reductive amination conditions. In addition, since the binding studies for protein A and protein A/G were done at the pH optimum determined for the two proteins when coupled to agarose supports (pH 8.0), the Emphaze matrices would show an even greater differential of binding capacity if the separations were done at pH 5.0 (see the results of the pH study, below).

### *3.3. Binding of human IgG on prepacked columns of Emphaze and agarose protein A, A/G and G using various binding buffer pH values*

Note: In this study, the prepacked columns of agarose required that considerable caution be used to prevent collapse of the support when used on automated chromatography systems. To condition the columns, the flow-rate was brought up very slowly to 1 ml/min. The agarose could not withstand the high flow-rates achievable with the Emphaze support, therefore all comparative studies were done at a flow-rate compatible with the agarose matrix.

Purification of human IgG using various pH binding buffers under medium pressure conditions yielded some surprising results. Optimal binding for protein A is typically observed at approximately pH 8 [8]. By contrast, Emphaze protein A displayed a maximum binding point at pH 5. Even the binding at pH 4 and 4.5 for the Emphaze protein A was higher than that observed at pH 8. Agarose protein A displays minimal binding at pH 4.5; significant binding at pH 5, 6 and 7.2, and a maximum binding point at pH 8. The human IgG applied to the columns was a purified, lyophilized preparation of IgG and was highly soluble in all binding buffer conditions. To determine if the support was merely non-specifically binding protein at the lower pH values, bovine serum albumin was applied to the column. There was no detectable binding of the albumin to the column under the conditions described in the Experimental section. Figs. 2 and 3 illustrate the results of this comparison.

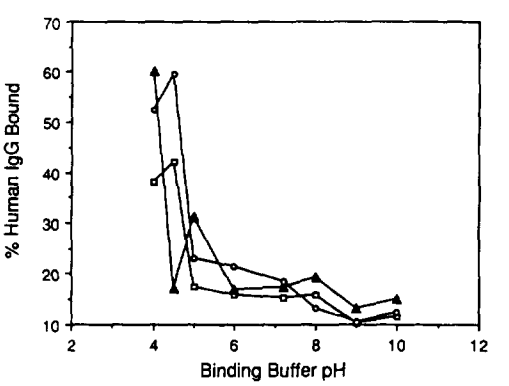


Fig. 2. Binding capacity for human IgG as a function of binding buffer pH using Emphaze protein A (▲), G (○) and A/G (□).

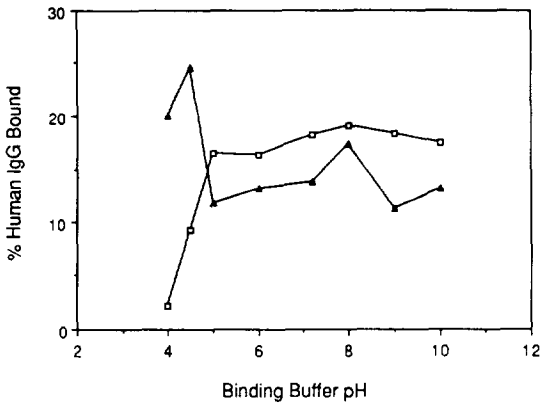


Fig. 3. Binding capacity for human IgG as a function of binding buffer pH using agarose protein A (□) and protein A/G (▲).

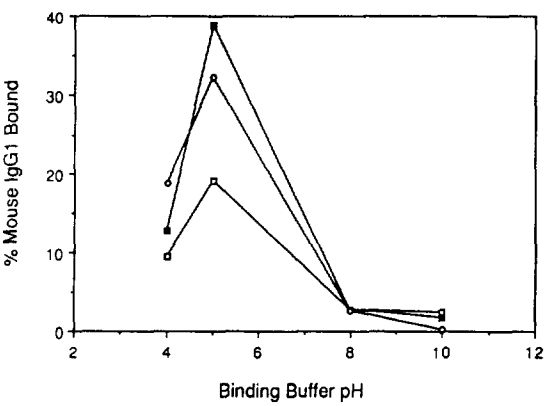


Fig. 4. Binding capacity for mouse monoclonal IgG1 as a function of binding buffer pH using Emphaze protein A (□), G (○) and A/G (■).

Emphaze protein A/G demonstrated a similar binding pattern as Emphaze protein A with an optimal percent human IgG bound at pH 4.5. Binding of human IgG was efficient on protein A/G from pH 4–8. The percent binding of human IgG dropped significantly at pH 9. Protein A/G immobilized on agarose also showed a binding optimum for human IgG at pH 4.5. The binding dropped significantly at pH 5, 6 and 7.2 and showed an increase at pH 8. Albumin was again applied to determine if there was non-specific binding occurring, but no binding was observed.

Protein G displayed significantly higher binding for human IgG at pH 4 and 4.5. Typically, the optimal pH for binding IgGs to protein G is described as pH 5 [11]. A large decrease in the binding of human IgG at pH 5 and another decrease at pH 7.2 was observed.

Prepacked columns of Emphaze protein A showed very surprising pH binding characteristics for the mouse monoclonal tested (IgG1 clone MOPC-21) (Fig. 4). Optimal binding was observed at pH 5. Binding was also enhanced at pH 4. Significantly, binding was very inefficient at pH 8 and 10. Prepacked columns of Emphaze protein A/G and protein G had optimal binding for this monoclonal at pH 5. There was also excellent binding at pH 4 and minimal binding at pH 8 and 10. The monoclonal studies were not duplicated on the agarose prepared supports.

### *3.4. Binding of human IgA to immobilized jacalin*

Emphaze jacalin was charged with 3 mg of jacalin, while agarose jacalin was charged with 4.5 mg of the lectin. Nevertheless, Emphaze jacalin had a binding capacity for human IgA of 2.6 mg/ml gel versus 1.5 mg/ml gel for agarose jacalin.

### *3.5. Binding of mouse IgM from ascites on MBP immobilized on agarose and emphaze beads*

Nevens et al. [17] showed that mouse monoclonal IgM can be purified in a single step using rabbit MBP immobilized on agarose. Although the IgM obtained was greater than 95% pure based on gel filtration analysis, the speed of the gravity separation was limiting. The necessity for equilibration and binding at 4°C, extensive washing steps, and subsequent warming of the column to room temperature for elution of the bound fraction resulted in an 8–10-h chromatography procedure.

Because the Emphaze support can withstand higher linear flow-rates than the agarose matrix, the Emphaze MBP gel was investigated for use in a rapid protein affinity chromatography (R-PAC) format to decrease the time of IgM purification. Instead of doing the separation in a cold

room for the binding step, the binding buffer was merely kept on ice and the sample chilled until needed. The elution buffer was maintained at room temperature to facilitate the rapid elution conditions required under increased linear flow. It was determined that the Emphaze MBP support could separate IgM from mouse ascites in minutes instead of hours. Typical separation times of 2–15 min were obtained, depending on the instrument used and the void volume of buffer in the lines. A representative separation is shown in Fig. 5. Attempts to use the agarose MBP support under similar high-flow conditions failed due to collapse of the matrix and increases in back-pressure that essentially shut down the instrument.

The bound and not-bound peaks from the separation on Emphaze MBP were collected to analyze the composition of the fractions. HPLC gel filtration separations were done using a program that identifies the major components of ascites fluid based upon standards. The results of these separations are shown in Figs. 6 and 7. Analysis of the not-bound fraction indicates that a slight amount of IgM was not completely removed by the Emphaze MBP column under the sample size and flow conditions used. Also present in the not-bound fraction were a minor amount of mouse IgG and albumin. For the bound fraction, the only significant peak present in the gel filtration analysis was IgM. Analysis by area percent indicates a relative IgM purity of about 98% in the Emphaze MBP bound peak.

To investigate whether Emphaze MBP could be used to purify IgM from serum samples, similar affinity separations were done with serum samples from a number of different animals, and the bound and not-bound peaks analyzed by gel filtration. In all the serum samples tested, significant binding of a 150 000 molecular mass component and several low-molecular-mass species completely masked any capacity for binding pentameric IgM (data not shown). While dialysis of the samples effectively eliminated the low-molecular-mass contaminants from binding, the

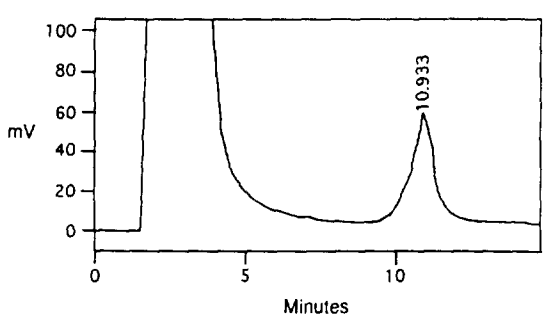


Fig. 5. Purification of mouse monoclonal IgM from ascites fluid using Emphaze MBP according to the Experimental section.

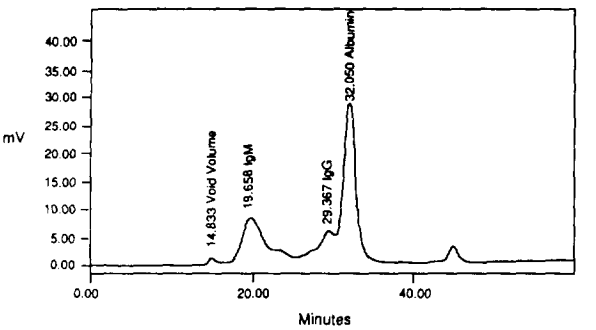


Fig. 6. Gel filtration profile of the not-bound fractions from the purification of mouse monoclonal IgM (from ascites) on Emphaze MBP. The ascites fluid normally contains IgG and albumin components. The peaks were identified by the retention time of standards applied to the column.

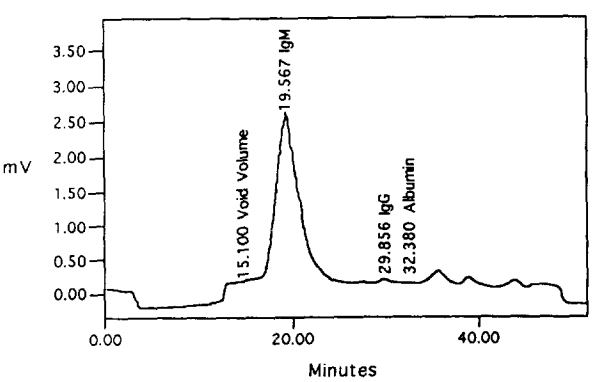


Fig. 7. Gel filtration profile of the bound fractions from the purification of mouse monoclonal IgM (from ascites) on Emphaze MBP. Note the removal of contaminating IgG and albumin normally present in the ascites fluid.

150 000 molecular mass component could not be so easily removed. The 150 000 molecular mass component co-elutes with typical IgG. There are two possible explanations for this. It is possible that the 150 000 molecular mass component is a monomeric form of IgM. However, the most plausible explanation is that in species other than mouse and human, IgG binding occurs. Further studies need to be done to determine the identity of this component. Since MBP is a calcium-dependent lectin, it most probably binds carbohydrate residues on antibody molecules. In ascites fluid containing IgM monoclonals, the predominate contaminating protein is albumin, not IgG. In serum samples, however, many polysaccharide-containing molecules are present in larger quantities than IgM, some of which have sugar residues that may be able to compete for binding sites on the affinity support. For this reason, the utility of MBP affinity supports appears to be limited to IgM purification from ascites and cell culture supernatant.

When purified IgM was applied to the Emphaze MBP column, a 97% recovery of protein was observed. The column had a capacity of 1.03 mg/ml of gel for purified mouse IgM. Agarose MBP had a nearly identical capacity. Emphaze MBP offers significant time savings; minutes vs. 8–10 h. The same prepacked column of Emphaze MBP was used for all purifications. Over 20 purifications were done on the same support material with no loss of binding capabilities.

## 4. Conclusions

The 3M Emphaze Biosupport Medium is a novel chromatographic material that comes preactivated with azlactone groups, making it suitable for coupling amine-containing ligands. The ease of immobilization and lack of hazardous materials necessary for performing the chemistry make it an attractive alternative to more traditional coupling methods.

The immobilization of immunoglobulin binding proteins to the Emphaze matrix resulted in affinity supports which displayed high binding characteristics. There were, however, discrepancies between binding capacities at various pH values of the Emphaze IgG-binding supports versus the agarose IgG-binding supports. Especially interesting was the binding characteristics of the supports using lower pH binding buffers. Emphaze protein A had maximum binding capacity for human IgG at pH 5 versus a pH optimum of 8 for agarose protein A. Chromatography of mouse IgG1 showed significant binding to Emphaze protein A at pH 5 and 4. Mouse IgG1 does not normally display such strong binding characteristics to protein A supports, especially in the absence of high-salt binding buffers. These data suggest that perhaps a binding optimization for each monoclonal could validate the use of Emphaze protein A for the purification of mouse IgG1 monoclonals with greater efficiency than when using other protein A matrices. Particularly, higher yields of mouse IgG1 may be achieved by using lower pH binding environments. Emphaze protein G and A/G, which also bound this monoclonal optimally at pH 5 could also be considered for purification. The large fluctuation in binding due to binding buffer pH, regardless of the support being employed, should be carefully considered in each purification strategy, especially in large-scale optimizations.

In the case of coupling jacalin to the Emphaze and agarose supports, the polymeric matrix again displayed increased binding capacities. Although less lectin was charged to the Emphaze matrix compared to agarose, the Emphaze support showed higher total binding of human IgA1. Again, it may be that the azlactone coupling chemistry is more efficient than reductive amination or that the immobilization results in a superior binding support.

The time savings that can be realized using the Emphaze support was most dramatically illustrated in the purification of IgM. Agarose MBP separation takes 8–10 h to yield >95% pure IgM. The same purification on Emphaze MBP in an automated medium pressure system was accomplished in 2–15 min with remarkable recovery and purity. The durability of the support was also demonstrated in that there was no loss of binding capability even after 20 uses.

In summary, the 3M Emphaze support shows several distinct advantages over agarose: the Emphaze support can be used in small columns for analytical methods development on an automated medium pressure or HPLC system, allowing rapid optimization of affinity chromatography conditions under high linear flow. Since the support has a particle size of approximately 60 $\mu$m, the separation can then be scaled up directly to large process columns utilizing the information developed on the automated system. With regard to antibody purification, the Emphaze support yielded better binding characteristics than an agarose matrix, especially under low pH conditions. The potential for increased yields for the purification of mouse monoclonal IgG1 antibodies makes the support deserving of consideration for process separations.

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