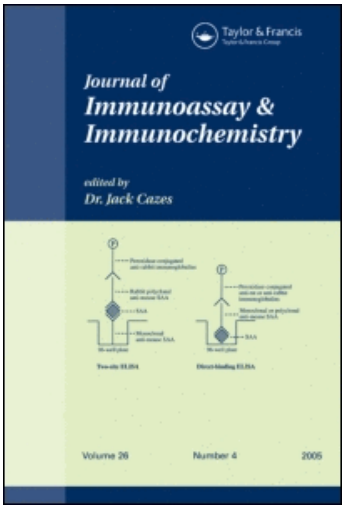

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ABSTRACT

Polystyrene latex beads were polymerized at two different pH (7.3 and 9.5) and their ability to create latex agglutination systems were studied. Sensitization of these latexes by DNA, antibodies to C-reactive protein, myoglobin, and human IgG. was conducted. Sensitivity and specificity of these systems were compared. Practical recommendations are given for preparation of such systems.

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INTRODUCTION

Polystyrene serves as a matrix for immobilization of various antigens/antibodies and is used widely in various types of immunoassays.[1] For instance, EIA (in different modifications) and latex agglutination are applied for rapid, specific and sensitive diagnosis of a number of diseases.[2,3] In particular, polystyrene latex agglutination test systems are applied for semi quantitative and quantitative determination of various antigens/antibodies.[4–6] The method provides a specific and nonexpensive detection of various substances. But, it is also well known that not all of the latexes are suitable for such reactions.[7] Moreover, there are some reports that latex agglutination systems give more false-positive results than erythrocytes sensitized by corresponding antigens/antibodies; the cause for this phenomenon is obscure.[8] In this approach, physical absorption of antigen/antibodies on polystyrene matrix or chemical coupling via amino or carboxyl groups of the protein globule with chemically modified polystyrene are used.[9–11] Both of the approaches have their advantages and disadvantages. Chemical coupling leads to a strong link between matrix and protein globule, and it prevents the removal of absorbed material. But, on the other hand, such binding may cause perturbation of protein structure and consequent changes of specificity of some epitopes. Physical absorption is a more mild process but, here, there is a problem of a possible removal of absorbed substances from latex beads. However, in the case of polystyrene, the binding has hydrophobic nature and a proper immobilization would lead to a prolonged stability of such systems. In this paper, data about polymerization and sensitization of two types of polystyrene latex beads, and their applicability for preparation of such test systems, are presented. Sensitization of these latexes by some antibodies/antigens (DNA, antibodies to C-reactive protein, human IgG, and myoglobin) is investigated. Effects of various factors on sensitivity and specificity of these systems are considered.

EXPERIMENTAL

Polystyrene Latex Bead Preparation

Polystyrene latex beads were prepared by polymerization at pH 7.3 and 9.5 using ammonium persulphate as an initiator of the reaction without an emulsifier. The latex bead sizes were estimated by turbidimetry.[12] Their concentrations were determined after drying the aliquots of the emulsion at 105°C.

Antigens and Antibodies

C-reactive protein from human pleural fluid, taken by puncture from patients with lung cancer, was purified to homogeneity according to Johnson.[13] Myoglobin from human heart, taken after autopsy, was purified by chromatography on CM-cellulose followed by gel filtration on Sephadex G-50. The immunoglobulin G fraction from human and rabbit sera were prepared according to Weir.[14] The methylated albumin was prepared from a commercial preparation (Gamma Biological Inc.) according to Mandel.[15] The native double stranded DNA was prepared from bovine liver by the phenol chloroform method, with cosequent fractionation on hydroxylapatite according to Becker.[16] The concentrations of these substances were determined by their exctinctions.[13,17,18] The purity of these proteins was estimated by electrophoresis according to Davis[19] (C-reactive protein and IgG in 6% PAAG and myoglobin in 15% PAAG).

Immunization Procedure

Rabbit antibodies to C-reactive protein, myoglobin, and human IgG were developed by hypodermic injections of pure antigens (100–200 μg) in Freund's complete adjuvant and then, after resting for 4 weeks, the rabbits were given the second hypodermic injection of antigen (200–300 μg) in Freund's incomplete adjuvant. After a week, a booster injection (800–1000 μg) was given. In the case of myoglobin, it was necessary to conduct an additional intravenous injection to obtain antibodies with high titer. The monospesificity of antibodies and their titers were determined by double immunodiffusion assay according to Weir.[14] Antibodies to CRP and myoglobin do not cross react with normal human serum. It was not nessecary to exhaust them.

Latex Agglutination Test

The latex agglutination tests were conducted on glass slides by mixing sensitized latex suspension with corresponding antigens/antibodies and, subsequently detecting the agglutination visually. For testing, we used pure antigens/antibodies for estimation of sensitivity of the system, as well as sera obtained from corresponding patients. For testing the DNA sensitized latexes, sera of patients with systemic lupus erythromathosis were used. Sera for testing were kindly given by the Laboratory of Immunology of the

Institute of Cardiology, Republic of Armenia. All of these sera were tested similarly by the following diagnostic test systems: C-reactive protein human IgG and antibodies to DNA (Human, Germany) myoglobin (Hexagon, USA).

RESULTS

The polystyrene latex beads were prepared at pH 7.3, (Latex 1) and at pH 9.5 (Latex 2) without emulsifier because there are data that indicate it prevents the binding of antigens/antibodies to latex beads.[20] In general, the polymerization compositions were the following: Latex 1, (50 mL of phosphate buffer 0.02 M, pH 7.3; styrene, 3 mL; ammonium persulphate, 50 mg; potassium chloride, 0.02 M), Latex 2, (50 mL of sodium pyrophosphate, 0.02 M pH 9.5; styrene, 3 mL; ammonium persulphate, 50 mg; potassium chloride, 0.02 M). The polymerization process was conducted at 60°C during 20–24 h. Prior to the polymerization, the system was bubbled with a nitrogen stream to remove oxygen from the suspension. During the polymerization process, the system was mixed periodically to prevent the polymerization of styrene itself. The increase of the concentration of styrene up to 10% leads to coagulation of system and decreases the final yield. The polymerized product was bubbled by a water stream to remove styrene traces then it was dialyzed extensively against distilled water to remove any side products. The latexes prepared by these methods have absorbance at 650 nm of about 80–100 and concentration of solids of about 10–12 mg/mL. The latex bead average sizes were estimated turbidimetrically from the relationship $\lg D$ versus $\lg \lambda$, where D is the optical density at the corresponding wavelength λ. This mean n^- correlates with sizes of latex beads. The value of n^- calculated from this relationship corresponds to latexes with average size about 1 μm. Linearity of the plots (not shown) demonstrates that the size scatter of the latex beads are minimal.

The latexes polymerized at pH 7.3 were subject to sensitization in phosphate buffer 0.02 M, pH 7.3 + 0.15 M NaCl, as well as in glycine–NaOH buffer pH 8.3, 0.1 M + 0.15 M NaCl. Moreover, for increase of sensitivity, the latex agglutination system for detection of myoglobin has higher ionic strength (0.1 M phosphate buffer pH 7.3 + 0.25 M NaCl) than usual. Figure 1 demonstrates sensitization of such latexes by various antigens/antibodies. For preparation of DNA-bonding latex beads, in any case, the methylated albumin was bonded to beads and then DNA was bonded to methylated albumin-latex beads by incubation at 37°C during 30 min. As is shown, concentrations of antigens/antibodies are very important for reliable

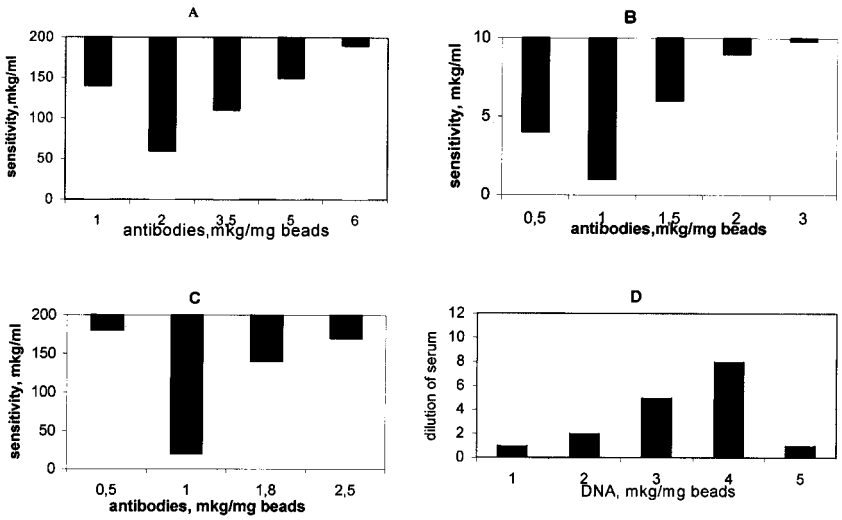

Figure 1. Sensitization of Latex 1 by antibodies to C-reactive protein (a), by antibodies to human myoglobin (b), by antibodies to human IgG (c) and by double stranded DNA (d). Concentration of latex beads, in any case, was 7 mg/mL. For testing DNA binding latexes, the sensitivities of Latexes 1 and Latexes 2 were estimated by dilution of sera of corresponding patients.

sensitivity. It should be noted that, in the case of sensitization of the Latex 1 by excess of antigens/antibodies, followed by centrifugation to remove the unbound molecules does not improve the results. Albumin, at concentrations of 0.1% and more, decreases the sensitivity of the system. The latexes polymerized at pH 9.5 are different by their properties (Fig. 2). In particular, sensitization takes place effectively in glycine–NaOH 0.1 M + 0.15 M NaCl pH 8.3–8.6. In the pH range of 7–8, spontaneous aglutination occurs. Here, the increase of the concentrations of sensitiziers leads to better sensitivity. Moreover, the excess of unbound molecules may be removed by centrifugation without affecting the sensitivity of the system. In comparison to Latex 1, here, the albumin addition upto 0.5% did not decrease the sensitivity of the agglutination system. Latex 1 gives agglutination reactions at low concentrations (0.3–0.8 mg/mL), but Latex 2 develops reliable sensitivity at concentrations of 10 mg/mL, and more. Evidently, Latex 1 has less stability than Latex 2.

We have conducted sensitization of both latexes at 37°C during 30–40 min and at 60°C during 10–15 min. Any differences in sensitivity and in specificity were not observed.

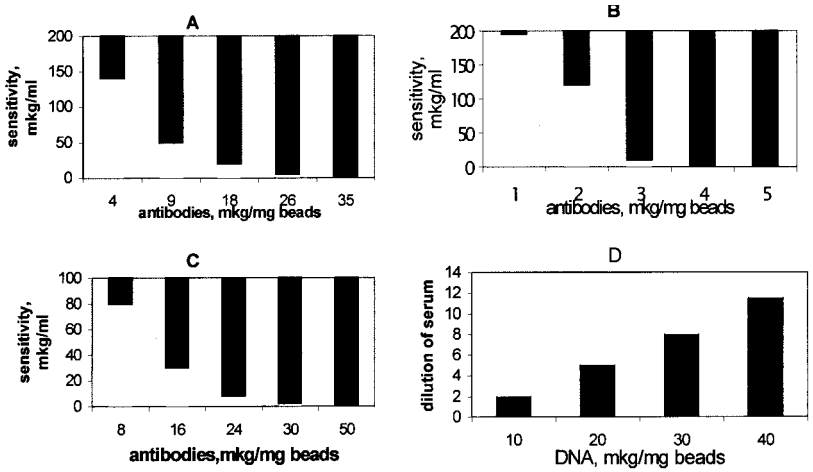

Figure 2. Sensitization of Latex 2 by antibodies to C-reactive protein (a), by antibodies to myoglobin (b), by antibodies to human IgG (c), and by double stranged DNA (d). Concentration of latex beads, in any case, was 12 mg/mL.

DISCUSSION

The data presented here show that latexes polymerized at the described conditions have different behaviour as carriers for the latex agglutination test systems. At first, protein binding capacity of these latexes are very different, (Figs. 1 and 2). Latex 2 has much more protein binding capacity than Latex 1. Moreover, sensitization of Latex 1 may be conducted at pH 7.3 and 8.3 but, in Latex 2, for antibody binding, the process takes place effectively only at pH 8.1–8.5. Further, after preservation, Latex 1 gives more positive results than Latex 2. It may be speculated that this is the result of the removal of sensitizer from latex beads, with consequent destabilization of the latex system. On the other hand, Latex 2 conserve their properties during long times (1 year or more). Apparently, these effects may be associated with surface properties of these latexes. It may be suggested that, in latexes polymerized at pH 7.3, absorption takes place not only by hydrophobic interactions but here there are some other ionic interactions that cause the removal of sensitizers. This was confirmed by the fact that the addition of albumin decreases the sensitivity of Latex 1. It may be the excess of albumin that possibly substitutes antigens/antibodies on latex beads. It is not excluded that traces of oxygen have such effects. The total charge of sensitizers is also crucial for effective binding. We have tried to prepare "sandwich" type immunoassay for C-reactive protein and myoglobin

(latex beads–CRP–antibodies to CRP, and latex beads–myoglobin–antibodies to myoglobin). So, for binding CRP, it was nessecary to conduct binding at pH 6.0 to decrease total charge of the protein molecule. In this case, very sensitive latex agglutination test systems for detecting CRP were prepared. But, for myoglobin, the results were not very good, apparently because there are only few antigenic epitopes on the small molecule of myoglobin by comparison with CRP which decreases agglutination capability of the myoglobin–antibody–myoglobin system. The data presented demonstrate that latexes polymerized at higher pH are more reliable for preparation of latex agglutination systems. Moreover, 0.5% albumin may be considered as an emulsion stabilizing agent that prevents any spontaneous non specific agglutination. Further, high stability of latex beads also prevents any non specific spontaneous agglutination. However, it should be noted that very high stability of latex suspension does not permit the effective agglutination of beads in the presence of antigens/antibodies. Our data demonstrate that nonsensitized latexes (concentration of solids 10 mg/mL) that are agglutinated by NaCl 0.7–0.9 M are suitable for preparation of such latex agglutination systems.

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