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Latex agglutination procedures in immunodiagnosis

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Abstract - Latex agglutination tests have been in use since 1956 to detect a wide range of analytes in the clinical laboratory. When spectrophotometers and nephelometers are used in place of the human eye to detect agglutination, it is possible to measure quantitatively and to develop sensitive particle immunoassays. Latex particles may be build from different organic materials to a desired diameter, and may be functionalized with chemical groups to facilitate attachment of molecules Proteins and other molecules may be passively adsorbed to the latex particles or covalently coupled to functional groups. Some described automated latex agglutination tests have sensitivities of a few picograms of analyte.

INTRODUCTION

Latex agglutination tests are very popular in clinical laboratories. These tests have been applied to the detection of over 100 infectious diseases, and many other applications are currently available. The first description of a test based in latex agglutination was the Rheumatoid Factor Test proposed by Singer and Plotz (ref. 1) in 1956. Since then, tests to detect microbial and viral infections, autoimmune diseases, hormones, drugs and serum proteins have been developed and marketed by many companies worldwide. New latex applications and technologies are still being devised and applied to new analytes.

In latex agglutination procedures, an antibody (or antigen) coates the surface of latex particles (sensitized latex). When a sample containing the specific antigen (or antibody) is mixed with the milky-appearing sensitized latex, it causes visible agglutination (Fig. 1). The degree of agglutination plotted as a function of agglutinant concentration follows a bell-shape curve similar to the precipitin one. Latex particles are used to magnify the antigen-antibody complex.

Many of the latex agglutination tests developed are performed manually and the agglutination is detected by visual observation. Although quite useful in the laboratory and cheap due to the absence of equipment needs, these manual assays suffer from lack of consistency in endpoint readouts. It has been stablished that about 100 clumps must be seen to determine agglutination, and that these clumps must be of about 50 μm in size to be seen by eye (ref. 2). Since the most common size for latex in these tests is 0.8 μm, about 10^6 latex particles will be required to make one visible aggregate, and about 10^7 particles will be needed to determine agglutination in a given test. Based on these calculations and assuming that about 10 bonds are required per particle to hold them together, Bangs (ref. 2) has evaluated the sensitivity of such a manual tests to be in the order of picograms.

During the last years, several new approaches to detect latex particles agglutination have been described using spectrophotometers and nephelometers which measure absorbed or scattered light, in place of the human eye. Some companies have devised particle counters appropiate to detect very small clumps. Angular anisotropy or quasi-elastic light scattering have also been applied to latex agglutination tests. Table 1 shows a possible classification of the main principles that have been used to detect latex agglutination.

Any of these new approaches allows to increase sensitivity and to improve standardization, and the procedures involved may be automated.

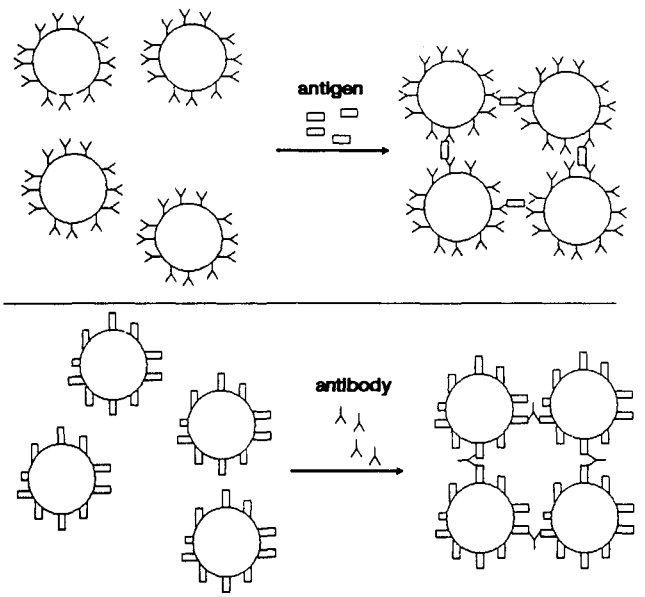

Fig. 1. Diagram of the agglutination of antibody-coated latex particles by antigen (upper) and of antigen-coated latex particles by antibody (lower).

TABLE 1. Principles used to detect latex agglutination

- Visual
- Turbidimetry
- Nephelometry
- Particle counting
- Angular anisotropy
- Quasi-elastic light scattering

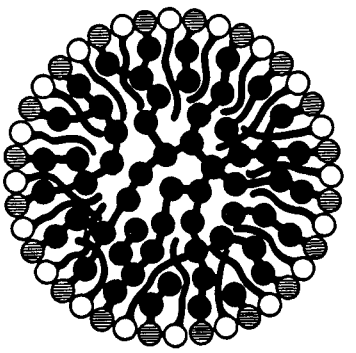

Fig. 2. Diagram of a polystyrene latex particle. Black ball chains represents polystyrene with sulfate free radicals (shaded balls). White balls denote the sulfonic acid group of the surfactant. Tail represents the hydrocarbon end of the surfactant.

THE LATEX PARTICLES

Latex particles are usually prepared by emulsion polymerization (ref. 3). Styrene is mixed into a surfactant (sodium dodecyl sulfate) solution, resulting emulsified in billions of micelles extremely uniform in diameter. Next, a water-soluble polymerization initiator such as potassium persulfate is added. When the polymerization is finished, polystyrene chains are arranged into the micelles with the hydrocarbon part in the center and the terminal sulfate ions on the surface of the sphere, exposed to the water phase (Fig. 2). Other hydrocarbons and derivatives have also been used in producing the so called "uniform latex particles", such as: styrene vinyl toluene, polyvinyl toluene, stryrene-divinylbenzene, polymethyl methacrylate, etc. (ref. 4). The term latex is used because the process evolved from synthetic rubber production and the emulsion has a milky appearance.

The latex particles may be build to a desired diameter by modifying the hydrocarbon, the surfactant, the initiator and the process of preparation (ref. 3). Latexes are available in particle sizes ranging from 0.05 μm to 2 μm from different manufacturers.

Latex particles have an inherent negative surface charge provided by sulfate and sulfonate groups. The particles can also be surface treated and functionalized to facilitate analyte attachment and to increase binding stability. Functional treatments include carboxilation, amidation, amination, hydroxylation and even magnetization (ref. 3,4). Latexes are also available in a variety of colors to facilitate visual read-out.

COATING OF THE LATEX PARTICLES

The simplest method of attaching proteins to the particles is by passive adsorption. Latex and proteins are mixed in an appropriate buffer solution, allowed to reach an equilibrium and washed several times until the latex is judged to be free of any residual unadsorbed protein (ref. 5-7). The main problem of passive adsorption is that proteins gradually desorb from the particles, modifying the agglutination characteristics of the sensitized latex over time. Also, some molecules do not adsorb onto the latex particles or adsorb in such a way that the binding site is inaccesible.

These problems are generally overcome by covalently coupling the molecule to the particle surface. Various methods have been developed for the covalent

linking of proteins and other molecules to particles via functionalized groups on the surface of the particles (ref. 8). Moreover, several companies offer a wide range of latex particles with different functional groups including: carboxylate, amino, chloromethyl, aldehydic, epoxy, hydroxylate, amide, etc. It is often necessary to use a difunctional reagent as glutaraldehyde, carbodiimides and others (ref. 9-12), and may be useful to put a short hydrocarbon chain (spacer) which provides a flexible linkage of ligands to the surface (ref. 3).

Some important hints have to be considered in any coating procedure:

1. The ligand should be as pure as possible. Impurities reduce both sensitivity and specificity of the test.
2. The amount of ligand attached to the surface of the particles should be carefully controlled. Agglutination may decrease if particles do not contain enough ligand and also if particles contain too much ligand.
3. Unbound ligand must be carefully removed after coating. Any aqueous ligand will modify the test sensitivity.
4. Once the particles have been coated with a molecule, their surface chemistry is different depending on the attached molecule and their stability could be altered.

AUTOMATED DETECTION OF LATEX AGGLUTINATION

Quasi-elastic light scattering (QELS)

This detection technique is also called "photon correlation spectroscopy", "laser doppler assay" or "dynamic light scattering". It is based on the fact that the intensity of light scattered from a latex suspension, when it is illuminated with a coherent light (i.e. laser), fluctuates with time, depending on brownian movement and therefore on the average diffusion coefficient, which could be correlated to the particle size.

This technique was first applied to a latex immunoassay for the detection of anti-bovine serum albumin by Cohen and Benedek in 1974 (ref. 13). The authors claimed a sensitivity of 10 ng/mL.

The main draw-backs of this technology are the sophisticated instrumentation required, the extremely delicate optical alignment, and the time required for each measurement. Furthermore, these instruments are very sensitive to vibration and noise.

Particle counting immunoassay (PACIA)

This technique is the only one which counts individual latex particles, using a flow cell. As the concentration of agglutinant increases, the latex particles aggregate and the number of individual monomers decreases. The number of monomers could be correlated with the concentration of analyte to be quantified (ref. 8). The detection limit of the technique is close to that of radioimmunoassays (RIA).

Automated instruments based in this technology have been marketed by Acade Diagnostic System (Belgium) and Sysmex (Japan).

Angular anisotropy

Angular anisotropy is a technique in which the ratio of the intensity of light scattered at two different angles is measured (Fig. 3). The anisotropy ratio reflects the increase in forward scattering and the decrease in backward scattering caused by particle agglutination.

This method was first described by Von Schulthess et al (ref. 14) applied to the detection of human chorionic gonadotropin (hCG). Latex particles coated with hCG, aggregated in the presence of the antiserum and the forward scattering increased while it was almost constant or even decreased at angles above 50°. The authors chose the ratio between 10° and 90° to maximize sensitivity and to skip source light fluctuations. The developed Latex Agglutination Inhibition method showed a detection limit as low as 2 IU HCG/L.

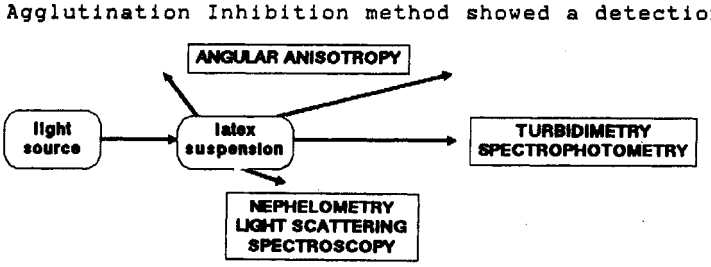

Fig. 3.
Angular anisotropy measures scattered light at two different angles. Nephelometry measures light scattered at just one angle. Turbidimetry measures the decrease of transmited light.

Nephelometry

Nephelometry refers to the measurement of light scattered at an angle from the incident beam (Fig. 3). The available instruments usually measure scattered light at 90°. The size of the latex particles commonly used is not very different from the wavelenght of the incident light, usually monochromatic and ranging from 200 to 800 nm. When particles agglutinate, the size of the aggregate is larger than the wavelenght and the light is scattered preferably in the forward direction as discussed in the previous paragraph. This is the reason why 90° is not the best choice, and why regular nephelometers are not suitable for latex agglutination tests (ref 15).

Some nephelometers have been specially designed to operate with latex agglutination and immunoprecipitation reactions. A good example is the Behring Nephelometer Analyzer with the following main features:

- Light source is a red diode. Wavelenght is 850 nm.
- The detector is a photodiode which measures the scattered light in the forward direction at small angles (13°-24°).
- Build-in computer able to store curves and to process and transform scattered light into analyte concentration.
- Detection limit as low as 10 ng/mL for some measuring systems.

Turbidimetry

This method measures the decrease of the intensity of the transmited light as latex particles aggregate (Fig. 3). The great advantage of this system is that measurements can be made in regular spectrophotometers. Today, clinical laboratories use to have fully-automatic spectrophotometers that not only measure transmitted light automatically at a desired time, but they also dilute, pipette and transfer to the cuvette the convenient volumes of reagents buffers and samples, incubate at a programmed temperature and make the necessary calculations using the selected algorithms and calibration curves. Finally, they print-out the results in the appropiate units or transfer data to computers. The possibility of running latex agglutination tests into these automatic analysers allows to process hundreds of samples in a short time without investment in new instrumentation or personnel.

CONCLUSIONS

Although latex particles have been used to amplify the antigen-antibody reaction since 1956 in manual slide tests, it has not been until recently when latex technology has been applied to other methodologies. New assay systems involving instruments to optically detect the agglutination converted latex agglutination technology into a highly reliable, more sensitive, and quantitative automatic immunoassay.

Nowadays, new assay techniques using latex particles are still coming, like the Cromatographic Strip Tests or the Membrane Particle Capture tests. Moreover, gene probes could also be developed using latex particles.

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