RAMA UNIVERSITY

www.ramauniversity.ac.in

DEPARTMENT OF BIOTECHNOLOGY
FACULTY OFENGINEERING & TECHNOLOGY

Precipitation reaction------ Principles & their applications

Content Outline

1. Precipitation principle
2. Types of precipitation reactions
3. Factors Affecting interactions
4. Cross reactivity

Precipitation reaction

•Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate. These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions.

•Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size in precipitation reactions.

•There is several precipitation methods applied in clinical laboratory for the diagnosis of disease. These can be performed in semisolid media such as agar or agarose, or non-gel Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size in precipitation reactions. There is several precipitation methods applied in clinical laboratory for the diagnosis of disease. These can be performed in semisolid media such as agar or agarose, or non-gel

support media such as cellulose acetate. Initially, the soluble Ag-Ab complex form within a few minutes; the formation of the visible precipitate occurs more slowly and often takes a day or two to complete. In the formation of an Ag-Ab lattice, the antibody molecule needs to be at least bivalent; monovalent Fab fragments cannot form a lattice. The antigen must either be bivalent or polyvalent; that is, it must have at least two of the same epitope or multiple of it.

Precipitation reactions can be broad of three types:

i. Precipitation in solution.

ii. Precipitation in agar.

iii. Precipitation in agar with an electric field.

Precipitation in solution

Ring test and flocculation test are examples of precipitation in solution. It is also called simple precipitation.

Ring test: In this test, a clear solution of the test antigen is layered slowly over the clear solution of antiserum in narrow test tube. Following a period of incubation, precipitation between antigen and antibodies in the antiserum solution is marked by the appearance of a white ring at the junction of two liquid layers. C-reactive protein (CRP), Lancefield grouping of β-haemolytic streptococci, Ascoli's thermoprecipitin test are the examples of the ring test.

Flocculation test: Flocculation test may be performed in a slide or tube. In the slide test, a drop of antigen solution is added to drop of serum solution in the slide and mixed well. Visible clumps appear in positive cases. VDRL test for the detection of reaginic antibodies in syphilis is an example of a slide flocculation test. Tube flocculation test for standardization of toxins and toxoids, Kahn test for syphilis is an example of a tube flocculation test.

Precipitation in agar

The precipitation test in agar gel is termed as an ***immunodiffusion*** test. Here, reactants are added to the gel and antigen-antibody combination occurs by means of diffusion. The rate of diffusion is affected by the size of the particles, temperature, gel viscosity, amount of hydration, and interactions between the matrix and reactants. An agar concentration of 0.3–1.5% allows for the diffusion of most reactants.

The reaction is visible in the form of a distinct band of precipitation.

When only antigen or antibody diffuse with a corresponding antibody or antigen being incorporated on agar gel, this is called single diffusion.

When both antigen and antibody diffuse in agar gel, it is double diffusion.

Oudin immunodiffusion: It is single diffusion in one direction (single diffusion of antigen in agar in one dimension). Here, the antibody is incorporated in agar gel in the test tube and the antigen is layered over it. Antigen diffuses and forms the line of a precipitate.

Oakley Fulthorpe immunodiffusion: It is double diffusion in one dimension. Here antibody is incorporated in agar gel in a test tube, above which a layer of plain agar is placed. The antigen is then layered on top of this plain agar. During the course of time, the antigen and antibody move toward each other through the intervening layer of plain agar and form band of the precipitate.

Radial immunodiffusion: Single diffusion in two dimensions is called radial immunodiffusion. Here antiserum solution containing antibody is incorporated in agar gel on a slide or Petri dish. The antigen is then applied to a wells cut into the gel. When the antibody present in the gel reacts with the antigen, which diffuses out of the well, a ring of precipitation is formed around the wells. The diameter of the precipitin ring formed is directly proportional to the concentration of the antigen.

Ouchterlony immunodiffusion: It is double diffusion in two dimensions. Both antigen and antibody diffuse independently through agar gel in two dimensions, horizontally and vertically. In this method, wells are cut in agar gel prepared in the slide. The antibody is placed in the central well and the antigen is placed on wells surrounding the central well. Antigen and antibody diffuse and precipitation bands are formed where they meet in optimal conditions

Types of Precipitation Reactions in agar with an electric field

1.Rocket immunoelectrophoresis

It is a combination of immunoelectrophoresis assay and Mancini assay. Rocket immunoelectrophoresis is a quantitative one-dimensional single electro-immunodiffusion technique. In this method antibody is incorporated in the gel at a pH value at which the antibodies remain essentially immobile. Antigen is placed in wells cut in the gel. Electric current is then passed through the gel, which facilitates the migration of negatively charged antigens into the agar. As the antigen moves out of the well and enters the agarose gel, it combines with the antibody to form immune complex which becomes visible. During the initial phase there is considerable antigen excess over antibody and no visible precipitation occurs. However, as the antigen sample migrates further through the agarose gel, more antibody molecules are encountered that interact with the antigen to form immune complex. This results in formation of a precipitin line that is conical in shape, resembling a rocket.

The greater the amount of antigen loaded in a well, the further the antigen will have to travel through the gel before it can interact with sufficient antibody to form a precipitate. Thus, the height of the rocket, measured from the well to the apex and area are directly proportional to the amount of antigen in the sample.

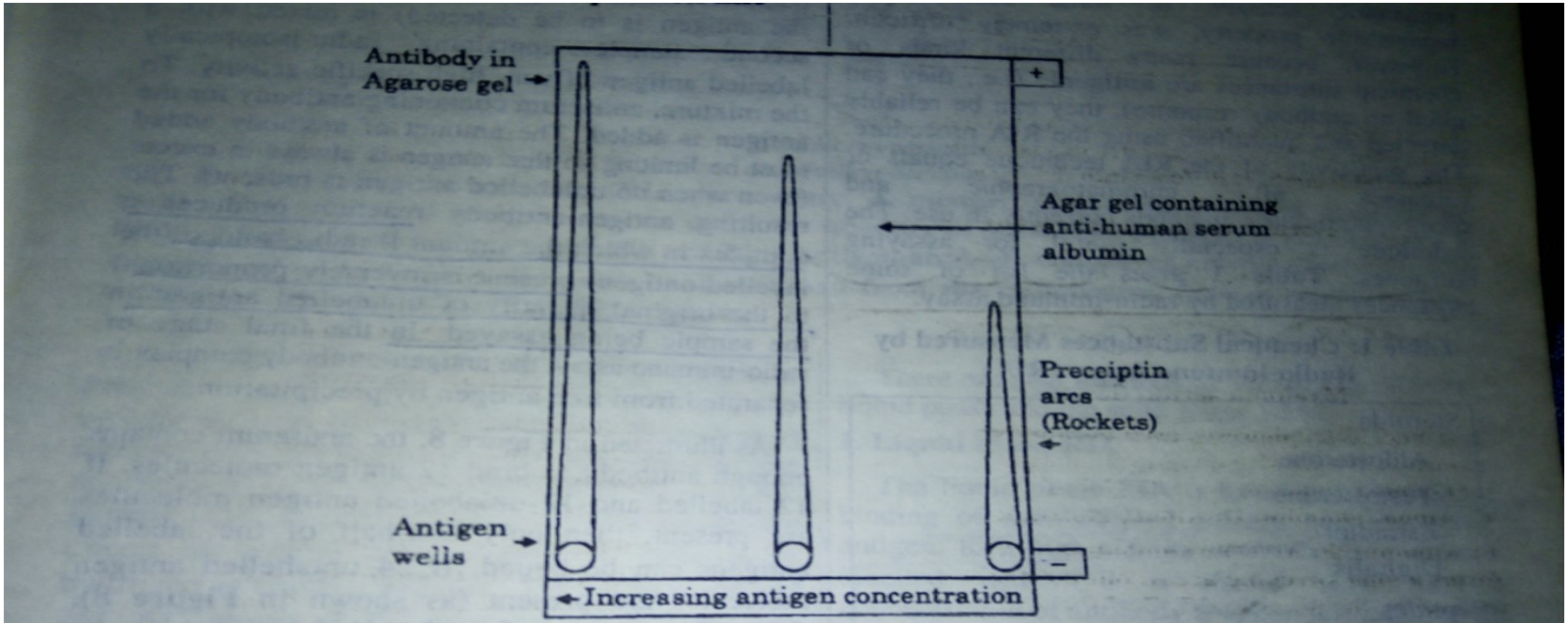

Fig. 7: Rocket electrophoresis. Antigen, in this case human serum albumin, is electrophoresed into gel containing antibody. The distance from the starting well to the front of the rocket-shaped arc is related to antigen concentration. In the example shown, human serum albumin is present at relative concentrations from left to right: 3 : 2 : 1.

Inference of rocket immunoelectrophoresis

•A precipitation ‘rocket’ spreading out from the loading well indicate positive reaction or specific antigen-antibody reaction due to the presence of antibody specific to the antigen.

•The absence of the precipitation indicates no reaction or the absence of any corresponding antibody – antigen.

•The height of the rocket, and its area are directly proportional to the amount of antigen in the sample, that is, the height of the precipitin peak depends on the concentration of antigens loaded in the corresponding wells.

•**Immunoelectrophoresis** refers to precipitation in agar under an electric field. It is a process of a combination of immuno-diffusion and electrophoresis. An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion. Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed. The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.

• The term “immunoelectrophoresis” was first coined by Grabar and Williams in 1953. When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size. Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

Important inferences drawn from Immunoelectrophoresis

•The presence of elliptical precipitin arcs represents antigen-antibody interaction.

•The absence of the formation of precipitate suggests no reaction.

•Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

Application

•The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.

•Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.

•The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).

References & Further reading

References

1. https://microbenotes.com/introduction-to-antigen-antibody-reactions/
2. https://www.sigmaaldrich.com/technical-documents/articles/biology/antibody-antigen-interaction.html
3. https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/42%3A_The_Immune_System/42.3%3A_Antibodies/42.3B%3A_Antibody_Functions
4. https://microbenotes.com/types-precipitation-reactions/

Further reading

1. Kuby, RA Goldsby, Thomas J. Kindt, Barbara, A. Osborne Immunology, 6th Edition, Freeman, 2002.
2. Brostoff J, Seaddin JK, Male D, Roitt IM., Clinical Immunology, 6th Edition, Gower Medical Publishing, 2002.
3. Janeway et al., Immunobiology, 4th Edition, Current Biology publications. 1999.
4. Paul, Fundamental of Immunology, 4th edition, Lippencott Raven, 1999.