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### *Chapter 3*

## **COAGULATION, FLOCCULATION, AGGLUTINATION AND HEMAGGLUTINATION: SIMILAR PROPERTIES?**

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### **ABSTRACT**

Coagulation, flocculation and agglutination are terms that usually cause confusion. Coagulation is a process of making colloidal matter dispersed/suspended in a liquid to join in a coherent mass. Flocculation is a physical process of contact and adhesions wherein the aggregates form larger-size clusters called flocs being excluded from suspension. These processes have several remarkable applications such as water treatment. The agglutination phenomena can be defined as the linkage of particles or cells in a liquid resulting in formation of clumps. In detail, aggregation/agglutination is the outcome of connections established by agglutinating agents among different components of the particulate materials. Antibodies and lectins, proteins which bind to specific targets (epitopes or carbohydrates, respectively) can be highlighted as agglutinating agents. The interaction can be used to identify microorganisms, proteins and carbohydrates; when agglutination involves erythrocytes, the technique is called hemagglutination. The aim of this chapter is to clarify differences about the important phenomena coagulation, flocculation, agglutination, and hemagglutination; characteristics and applications of molecules and substances able to exert these processes are also discussed.

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## 1. INTRODUCTION

The processes of coagulation, flocculation and agglutination involve the union of substances, particles or cells dispersed or suspended in a liquid forming aggregates which remain or not in suspension. These processes have several applications such as water treatment in which synthetic coagulants are used; the latter compounds are often toxic to animals and harmful to human health. As an alternative environmentally friendly, synthetic coagulants may be substituted for natural coagulants from plants.

Aggregation/agglutination is the outcome of connections established by agglutinating agents among different components of particulate materials. Agglutination is a term broadly used in medical-clinical area to designate the formation of visible aggregates as a result of interaction among specific antibodies and insoluble particles containing antigenic determinants; agglutination interactions are employed for laboratory diagnosis of diseases.

Lectins, carbohydrate recognition and surface interactive proteins have a plethora of applications and can contribute as useful coagulants and agglutinants in distinct research areas.

## 2. COAGULATION AND FLOCCULATION

Coagulation and flocculation are related phenomena which can usually occur together and involve the clumping of particles with consequent destabilization and coming out of the aggregates from suspension. In a chemical perspective, coagulation is defined as the process of making the colloidal matter dispersed/suspended in a liquid to join in a coherent mass. The flocculation is an essentially physical process of contact and adhesions wherein the aggregates form larger-size clusters called flocs which are excluded of the suspension (IUPAC). Flocculation is widely used as synonymous with agglomeration, aggregation, and coagulation.

Coagulants and flocculants are useful in food and beverage industries to remove microscopic particles that affect water taste, appearance and texture (Wong et al., 2007). Nevertheless, these processes are mainly known due to their great importance in the water treatment, which is discussed below.

### 2.1. Coagulation and Flocculation in Water Treatment

Water is a natural element essential to life; freshwater comprises only 3% of the total water on Earth and only a small percentage (0.01%) of this water is available for human use (Hinrichsen and Tacio, 2002). It is worryingly that this small fraction of freshwater is under stress due to the exponential population growth, increasing and disordered urbanization, and unsustainable consumption by industry and agriculture (Azizullah et al., 2011).

The water, due to its physical-chemical properties, is not found in pure state in nature but accompanied by foreign dissolved substances as well as particles and microorganisms in suspension. Microbial pathogens (bacteria, virus and protozoa), inorganic pollutants (acids, salts and toxic metals), ions (nitrates, phosphate, sulphates,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{F}^-$ ) and toxic products (detergents, disinfectants, pharmaceuticals, chemical reagents, radioactive elements) are often present contaminating water leading to problems at public health, economics and environmental levels (Zhicong et al., 2011, Jean et al., 2012). In addition, pesticides and organic compounds like oils are also threats to water quality (Azizullah et al., 2011). Then, there are many sources of water contamination, primarily substances derived from agricultural and industrial activities, as well as those present in soil (Gopal et al., 2007).

The conventional water treatment includes the steps coagulation, flocculation, sedimentation, filtration, and disinfection. Coagulation and filtration are the most critical processes determining the success or failure of water treatment system (Ghebremichael, 2004). Coagulation has been employed to decrease turbidity, color and to remove pathogens (Matilainen et al., 2010). When properly operated, the other processes such as flocculation and sedimentation, may not be required (Conley, 1961) and the role of disinfection can be significantly reduced (Ghebremichael, 2004). Figure 1 shows the stages of water treatment, detailing the steps of coagulation and flocculation.

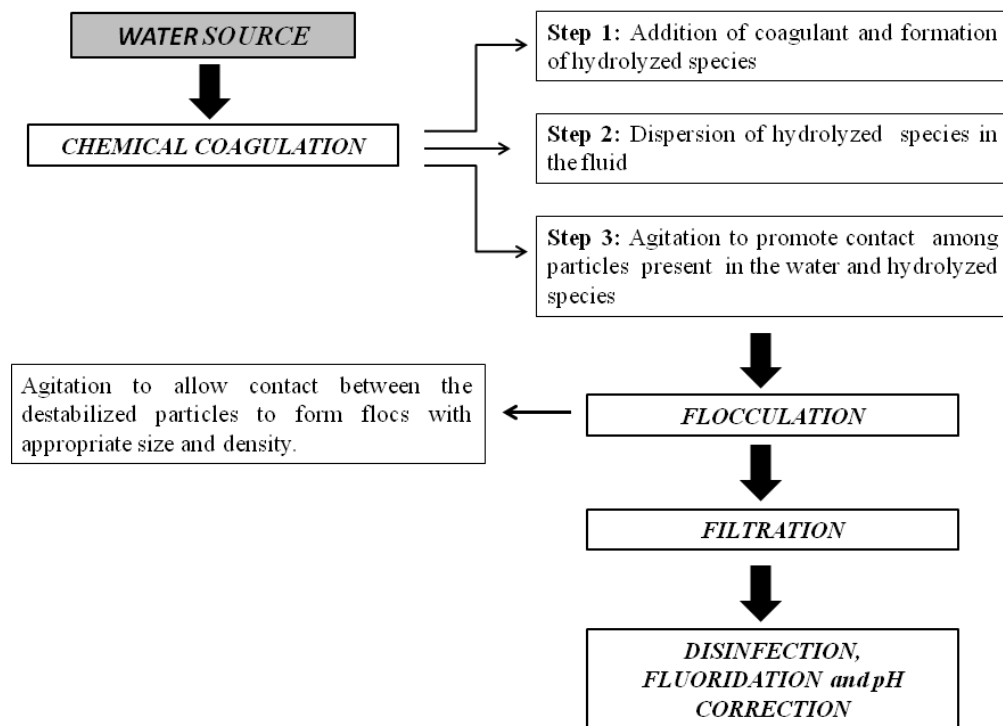


Figure 1. Steps of water treatment.

Coagulation and flocculation constitute delicate steps of water treatment. Conventionally, the water is mechanical or hydraulically stirred, followed by the addition of coagulants, which act by reducing the repulsive forces between particles (impurities) increasing collisions and floc formation (Mcconhachie et al., 1999). The efficiency of the coagulation-flocculation

method depends on the water characteristics, pH and temperature of solution, the type and quantity of coagulants as well as intensity and duration of mixing (Radoiu et al., 2004). These processes are interconnected and must be lucrative and easy to operate (Bromley et al., 2002).

Coagulation, in an electrostatic approach, starts with the reduction of zeta potential, which is a measure of particle stability and represents the potential required to breaking the protective layer of ions surrounding a particle and depends upon the electrostatic forces between charges carried by the colloidal particles (Ndabigengesere et al., 1995). The coagulation process can be achieved by adding cationic electrolytes that promote a compression of the electrical double layer surrounding suspended particles destabilizing them by decreasing the magnitude of the repulsive interactions and allowing their attraction through van der Waals forces (Prabu et al., 2011).

Adsorption-charge neutralization occurs, after diffuse layer compression, when the addition of coagulant releases metal cations which trigger a hydrolysis reaction with production of soluble hydrolysable species (positively charged) promoting aggregation of negatively charged suspended particles (Gassenschmidt et al., 1995). Adsorption and bridge formations occur by addition of synthetic or natural organic materials that have ionizable sites along their chains. Flocculation as a physical phenomenon follows the rapid mixing and coagulation. In this process the size of particles increases as a result of collisions among them. The large particles formed can be easily removed by inexpensive procedures, such as gravity sedimentation and filtration (Metcalf, 2003). The chemical coagulation process can occur in a few seconds, while the aggregation of destabilized particles forming flocs can take hours and is usually held in mechanical or hydraulic units. The speed of flocculation depends on temperature, time and intensity of agitation (Wong et al., 2007).

The flocculation of negatively charged particles occurs due to Coulomb forces among their surfaces and positively charged macromolecules, resulting in a neutral charge. It is possible to connect simultaneously several particles, since only a little moiety of the macromolecule binds to the surface of a single negatively charged particle leading to formation of flocs (Gassenschmidt et al., 1995).

Metal salts such as polyaluminium chloride and aluminium sulphate, and synthetic polymers such as polyacrylamide are frequently used as coagulant agents for water treatment (Duan et al., 2002, Tzoupanos and Zouboulis, 2011). These compounds can promote deleterious effects on the environment and aquatic organisms such as fishes (Thomas and Jurgen, 2002). Particularly, the polyacrylamide residues (acrylamide) are toxic for humans and other animals by affecting the peripheral nervous system (Smith et al., 1996).

Several studies have been performed in order to optimize the coagulant action, such as determination of optimal pH and addition of flocculants, to reduce the environmental impact and health damage (Tatsi et al., 2003). Natural compounds, in this sense, have been considered as substitutes for chemical coagulants due to their abundance, low price, innocuity and biodegradability. More details on natural coagulants are presented in section 1.3.

### 3. SYNTHETIC COAGULANT SUBSTANCES

Chemical coagulation is a complex phenomenon involving several inter-related parameters. Hence, it is very critical to define if a coagulant will operate efficiently under

given conditions. The chemical coagulants can be classified as acidic (aluminum sulfate, ferrous sulfate, ferric chloride, ferric sulfate) and basic (sodium aluminate). Coagulant dosages vary in a wide range aiming maximum removal efficiency of pollutants using minimum doses at optimum pH (Szpak et al., 1996).

The main inorganic coagulants used are salts of aluminum and ferric ions. The latter compounds are often chosen to destabilize colloidal and suspended solids (Tak-Hyun et al., 2004); the most common coagulants used in water and wastewater treatment are the  $\text{Al}_2(\text{SO}_4)_3 \cdot 4\text{H}_2\text{O}$  and the polyaluminum chloride (PAC) due to their effectiveness in treating a wide range of water types at relatively low cost (Hassani et al., 2008).

The aluminum sulfate is probably the most widely used chemical for coagulation of public water supplies, due to excellent floc formation, low cost and relative economy; it is ease to handling, transport, and management. Also, aluminum sulfate is very effective for reduction of color, turbidity, chemical oxygen demand (COD) and biochemical oxygen demand (BOD). However, depending on the dosage, the ingestion of drinking water containing residues of this coagulant can cause renal failure and, when carried to the brain, can lead to dementia, loss of motor coordination, cognitive decline, and Alzheimer's disease (Flaten, 2001, Rondeau et al., 2009).

Synthetic polymeric forms of Al, such as polyaluminium chloride (PAC) and polyaluminium sulphate, have become the most common alternative coagulants (Hassani et al., 2008). The use of PAC has some advantages over aluminum sulphate including reduced acidity, positively charged monomers and polymers, rapid formation of denser flocs, and reduced sludge (Tang et al., 1998). The higher charge density of PAC species often results in a decrease in the coagulant dose and the associated solid production.

Other coagulants such as ferrous sulfate ( $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ ) are very useful to treat waters with pH in the range of 8.5 to 11.0. The ferric sulfate ( $\text{Fe}_2(\text{SO}_4)_3$ ) is convenient for treatment of acidic or highly colored water and is effective in the pH range 5.0 to 11.0. The ferric chloride ( $\text{FeCl}_3$ ) produces good flocs also in the pH range 5.0 to 11.0. In most water treatment systems, synthesized polymers have been used such as polyacrylamide.

The textile industry is one of the most chemically intensive industries and the major polluter of water. Its effluents are discharged as a wastewater which has high color, BOD, COD, pH, temperature, turbidity and toxic chemicals. Pre-hydrolyzed coagulants such as PAC, polyaluminium ferric chloride (PAFCI), polyferrous sulphate (PFS) and polyferric chloride (PFCl) are effective in color removal even at low temperature and produce lower volume of sludge (Verma et al., 2012). It has been reported that pre-hydrolyzed metallic salts are often found to be more effective than hydrolyzing metallic salts which are readily soluble in water (Jiang and Graham, 1998).

#### 4. NATURAL COAGULANT SUBSTANCES

Although the use of natural coagulants of plant and mineral origin for water treatment was very common in the past, the lack of scientific knowledge about their action mechanisms and the modernization of techniques using chemical coagulants led to a decrease in their use (Ndabigengesere and Narasiah, 1998). Recently, the interest in natural coagulants has risen

due to their biodegradability, safety for human health and low cost. Natural coagulants can also be extracted from microorganisms and animal tissues (Šciban et al., 2009).

Seeds of 14 species from *Moringa* genus have been reported to possess coagulating properties in different degrees (Jahn, 1988) and the species *Moringa oleifera* is the most studied. Aqueous extracts from dry *M. oleifera* seeds have been extensively used for removal of water turbidity due to their natural coagulant ability; Gassenschmidt (1991) suggested that a cationic peptide with molecular weight between 6 and 16 kDa with an isoelectric point at pH 10.0 was the active principle. Ndabigengesere et al. (1995) confirmed that the active component was a dimeric protein with coagulant properties better than those of aluminum salts. Also, the authors reported that the use of *M. oleifera* coagulant generated less residues than when aluminum salts were employed and that residual material was innocuous to the environment. Gassenschmidt et al. (1995) isolated two flocculating and basic proteins ( $pI > 10$ ) from *M. oleifera* seeds called MO<sub>2.1</sub> and MO<sub>2.2</sub> with molecular masses of 6.5 and 7.0 kDa, respectively. Amino acid sequencing of MO<sub>2.1</sub> revealed 60 residues in the full sequence (ZGPGRQPDFQRGQQLRNISPPQRCPSLRQAVQLTHQQGQVGPQQVRQMYRVAS NIPST) and high contents of glutamine, arginine and proline. The authors also demonstrated that MO<sub>2.1</sub> showed a flocculant capacity higher than a cationic polymer on polyacrylamide basis.

Okuda et al. (2001) isolated a non-proteinaceous coagulant from saline extracts of *M. oleifera* seeds; this compound corresponded to a polyelectrolyte with molecular mass around 3.0 kDa. Other coagulant proteins from *M. oleifera* seeds were subsequently isolated. Ghebremichael et al. (2005) purified a cationic protein on a cation exchanger column which showed pI greater than 9.6, molecular mass lower than 6.5 kDa and flocculant and antimicrobial properties. Santos et al. (2009) purified a cationic lectin (carbohydrate-binding protein) which showed coagulant property and was named coagulant *M. oleifera* lectin (cMoL). Further, Ferreira et al. (2011) reported that another lectin isolated from *Moringa* seeds called WSMoL (water-soluble *M. oleifera* lectin) was able to reduce turbidity in water.

*M. oleifera* seeds, in addition to coagulant power, have been applied to remove different components in aqueous solutions and suspensions. Beltrán-Heredia (2011) reported that *M. oleifera* seed extract acted as an agent for removal of the anionic surfactant sodium lauryl sulphate in aqueous solutions (removal of 65% was reached). Sharma et al. (2006) and Meneghel et al. (2013) reported the seed powder ability to remove cadmium (Cd) by biosorption and results suggested that the interaction between amino acids of seed proteins and Cd was mainly responsible for the removal of Cd(II) ion. *M. oleifera* seeds were also tested as a sorbent for removing Ag(I) in aqueous solutions and the best results were obtained using 2 g of adsorbent with particle size of 75-500  $\mu\text{m}$ , at pH 6.5 (Araújo et al., 2010). *M. oleifera* seed lectin was effective in promote sedimentation of bacteria present in water (Ferreira et al., 2011).

Other natural coagulants from plants have been searched and studied. In Venezuela, the coagulant potentials of *Cactus latifaria* and seeds of *Prosopis juliflora* were tested using synthetic water formulated to resemble drinking water. When starting from high (100-200 NTU) and low (30-40 NTU) initial turbidities, both materials promoted reduction in turbidity and final results were close to the required standard of 5 NTU. Their optimum dose was 20-40 mg/L which is comparable with that achieved using extracts from *M. oleifera* (50 mg/L) and was about 75% lower than aluminum sulphate (Diaz et al., 1999).

Crude extract from common bean (*Phaseolus vulgaris*) seeds showed the ability to act as a natural coagulant for water treatment with a few advantages over *M. oleifera* seeds, such as, no oil presence. In this study the authors reached partial purification of the coagulant components through anion exchange chromatography. The fraction having the highest coagulation activity (72.3%) was eluted with 0.875 mol/L NaCl and the optimal coagulation dosage was 0.081 mg/L. Coagulation activity of partially purified common bean coagulant was almost 22 times higher than that of crude extract (Antov et al., 2010).

Seed extracts from Horse chestnut (*Aesculus hyppocastanum*), and Common oak (*Quercus robur*), Turkey oak (*Quercus cerris*), Northern red oak (*Quercus rubra*) and European chestnut (*Castanea sativa*) were also investigated for potential use in water treatment. The natural coagulants were extracted with water or NaCl solutions and all these plant materials showed coagulant properties, although extracts from seeds of *C. sativa* and *Q. robur* were the most efficient expressing coagulant activities of 80% and 70%, respectively (Šciban et al., 2009).

Chitosan is a polysaccharide prepared by de-N-acetylation of chitin, which is the main constituent of crustacean shells (Chen et al., 2007). A number of studies have assessed its use as a coagulant or flocculant for the removal of mineral colloids (Huang et al., 2000, Roussy et al., 2004) and it has been used to treat inorganic solid suspensions in wastewater treatment systems (Roussy et al., 2005). Chitosan is a polymer with a moderate to high molecular weight and cationic charge; the coagulation process using chitosan seems to be charge neutralization (Huang et al., 2000). Chen and Chung (2011) compared the coagulation performance of acid-soluble chitosan, water-soluble chitosan, a coagulant mixture of chitosan with alum, and a coagulant mixture of chitosan with PAC. The results showed that when chitosan was mixed with alum or PAC in a mass ratio of 1:1, the coagulation efficiency of the mixtures was better than those of chitosan, alum, or PAC alone in terms of a wider dosage range and high settling velocity. These findings suggest that alum or PAC can be partially replaced by chitosan as a simple and cost-effective alternative.

Tannins are mostly water-soluble plant polyphenolic compounds with molecular weight ranged between 500 and some thousand daltons. These compounds contain enough hydroxyl groups for effective cross linking of other compounds and are actually a natural and feasible source for coagulant synthesis. The production process of these kinds of coagulant is well-known and possible even to optimize it in terms of efficiency (Beltrán-Heredia et al., 2010). The tree *Acacia mearnsii* (Black wattle) is a well-known tannin source and was revealed as an efficient product in anionic surfactant removal (Sánchez-Martín et al., 2009).

The studies regarding the application of plant-based coagulant can be considered a promising alternative to remove contaminants from water destined for public supply. The use of natural coagulants can avoid solid residues that are generated in conventional water treatment processes.

## 5. AGGLUTINATION AND HEMAGGLUTINATION

The agglutination phenomena can be defined as the linkage of particles or cells suspended in a liquid, resulting in formation of clumps. In detail, this aggregation is the outcome of connections established by agglutinating agents among different components of

the particulate material. Agglutination is a term broadly used in medical-clinical area to designate the formation of visible aggregates as a result of interaction among specific antibodies and insoluble particles containing antigenic determinants. The agglutination can occur with particles having natural antigenic determinants on their surface (erythrocytes, bacteria, protozoa, etc.) and with inert particles (latex, polystyrene, bentonite, etc.), or even with antigenically unrelated cells (blood cells) which adsorb or attach to soluble antigens. When agglutination interactions use erythrocytes can be called hemagglutination.

Agglutination interactions may occur through a direct or indirect form (Figure 2). In both agglutination reactions, the biological fluid is tested for the presence of antibodies that will bind the antigens (Stanley, 2002). Insoluble antigenic particles in direct agglutination are used at their entire or fragmented forms. Bacteria, fungi, protozoa and erythrocytes can be directly agglutinated by antibody.

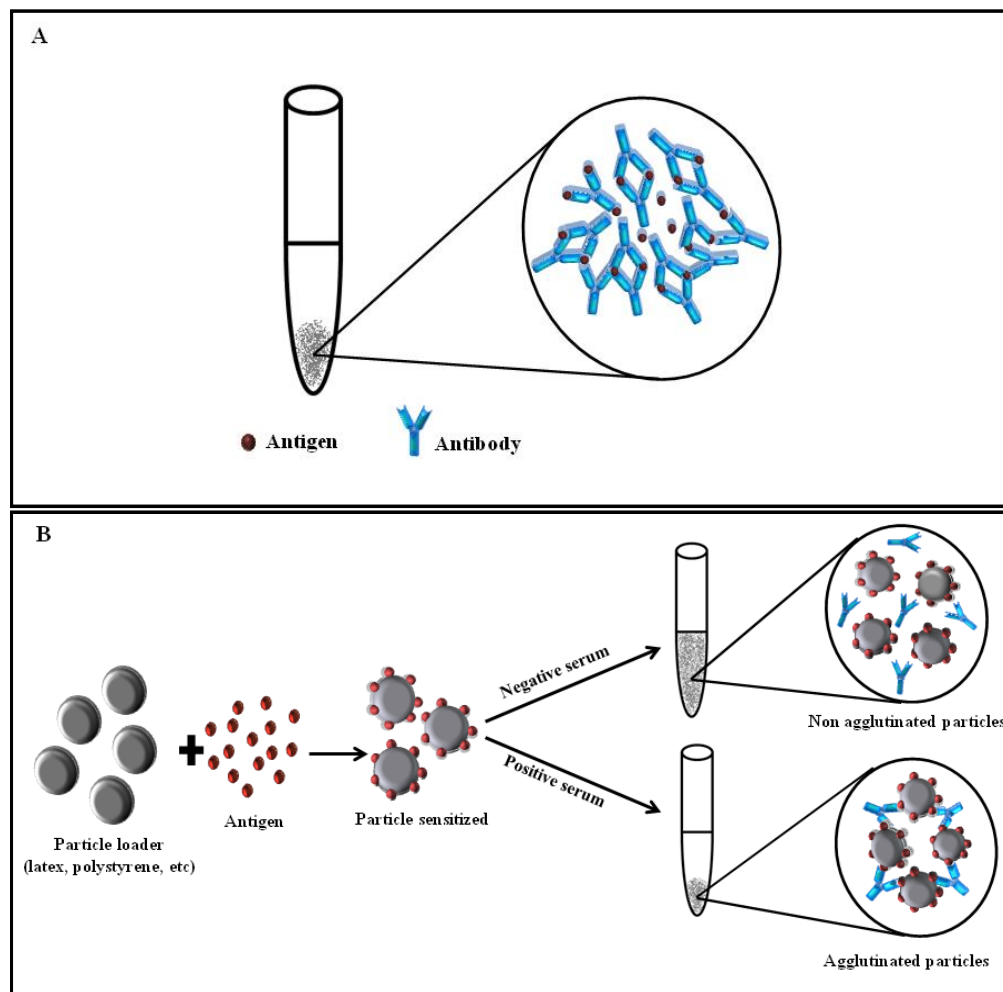


Figure 2. Schematic representation of agglutination interactions. A: Direct agglutination reaction where antibodies recognize and establish links with antigens forming aggregates. B: Indirect agglutination using particles. These reactions occur when antigens or parts of antigens are first bound (adsorbed) to a carrier particle to become resistant and more easily recognized by specific antibodies. Sensitized carrier particles amplify the recognition by antibodies and clump.



On the other hand, in the indirect agglutination the erythrocytes and inert particles (latex, bentonite, yeast, etc.) can be sensitized by passive adsorption via chemical agents such as tannic acid and chromium chloride, and by conjugation of the antigen by means of covalent chemical bonds by providing stable reagents. The agglutination assays can be performed in tubes or plates.

## 6. DIAGNOSTIC APPLICATIONS

A diagnostic assay for an infectious agent can be used to demonstrate the presence or absence of infection, or to detect evidences of a previous infection (for example, the presence of antibodies). Agglutination reactions are much employed for the laboratory diagnosis of diseases caused by viruses, bacteria, protozoa, fungi, and autoimmune diseases (Stanley, 2002). In Microbiology, agglutination is an important technique for diagnosis commonly used as a method of identifying bacteria and its specific antigens (Gaidamashvili et al., 2002).

Direct or indirect agglutination and hemagglutination assays have been increasingly applied in various research fields and clinical diagnosis of several diseases such as visceral leishmaniasis (Srivastava et al., 2011) and typhoid fever (Abdoel et al., 2007). This technique has been mainly performed as a qualitative analysis but demonstrate versatility, reliability and speed of execution.

Leptospirosis, a zoonosis with worldwide distribution, is an acute febrile illness caused by spirochaetes of the pathogenic *Leptospira interrogans* group. Latex beads sensitized with recombinant LipL32 (a highly conserved leptospiral antigen) were used to detect specific antileptospiral antibodies from human and dog sera. Recombinant antigen-based latex agglutination assay is a suitable technique for the examination of a large number of sera which involves the LipL32 immunodominant antigen. The test was found to be sensitive, specific and accurate as compared to the standard microscopic agglutination assay, mainly in the acute phase of the illness (Dey et al., 2007). Hemagglutination assays are also widely used in clinical laboratories for the diagnosis of leptospirosis (Levett and Whittington, 1998).

Brucellosis is a zoonotic disease that, despite being long recognized, continues to afflict humans and domestic animals in many countries around the world (Araj, 2010). A rapid latex agglutination test was developed and evaluated for the serodiagnosis of human brucellosis (Abdoel and Smits, 2007). Latex particle agglutination test (LPA test) is also used to detect bacterial antigen in cerebrospinal fluid to diagnose bacterial meningitis. The LPA test was positive in 30 of the 36 cases studied, with a sensitivity and specificity of 83% and 100%, respectively (Das et al., 2003).

Chagas' disease is a complex zoonosis caused by the parasite *Trypanosoma cruzi*. Serological assays are widely used for the diagnosis, particularly regarding the diagnostic of disease chronic stage. The indirect hemagglutination is included among these techniques as the most commonly used approaches and is also used in epidemiological surveys, in medical care tasks and in scientific research (Souza and Neto, 2012).

The determination of C-reactive protein (CRP) is an assay that measures general levels of inflammation in human body. In recent years it has been given particular interest to the measurement of serum CRP as a marker of inflammation associated with cardiovascular diseases. In clinical procedures/protocols, lipid agglutination and latex agglutination are

among the most used techniques to analyse CRP. The basic biochemical reaction that constitutes blood CRP detection is the selective association of the protein with a specific analyte adsorbed onto particles of cholesterol or latex (Algarra et al., 2012).

Ye et al. (2011) developed a latex agglutination inhibition reaction test (LAIRT) to detect aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in agricultural commodities, foods and feeds. Aflatoxin B<sub>1</sub> is a toxic metabolite produced mainly by *Aspergillus flavus* and *A. parasiticus* and humans would be exposed to AFB<sub>1</sub> directly by eating contaminated products (Tan et al., 2009). The method developed was simple, easy to perform and interpret, and the process could be completed within 10 min using minimal equipment.

## 7. LECTINS AS COAGULANT AND AGGLUTINATING AGENTS

Lectins are proteins or glycoproteins able to bind reversibly to carbohydrates without altering the covalent structure of any of the recognized ligands (Sharon, 2007). These proteins are broadly distributed in nature (Santos et al., 2013). Lectins can precipitate soluble glycoconjugates and bind to carbohydrate of membrane glycoproteins and glycolipids thus inducing agglutination of various cell types (Vazquez et al., 1996). These proteins play an important role in immunological defense systems since they can sequester viruses, bacteria and other cellular-micro invaders, as well as substances that they secrete. Also, these proteins are involved in other cellular events besides agglutination process; they act in proliferation, opsonization, signal transduction, metastasis and apoptosis (Dutta et al., 2005, Nunes et al., 2012, Coriolano et al., 2012a, Coriolano et al., 2012b).

The presence of a lectin – carbohydrate recognizing protein – is detected using a hemagglutination assay. These molecules have the ability to induce cell agglutination phenomenon (Kennedy et al., 1995). The hemagglutination assay is performed by serial dilution of a sample containing lectin and incubation with human or animal red blood cells; in this process, the carbohydrate-binding sites interact with carbohydrate or glycoprotein present in the erythrocyte surfaces forming a network of agglutination among the cells (Correia et al., 2008). This process is shown in Figure 3A.

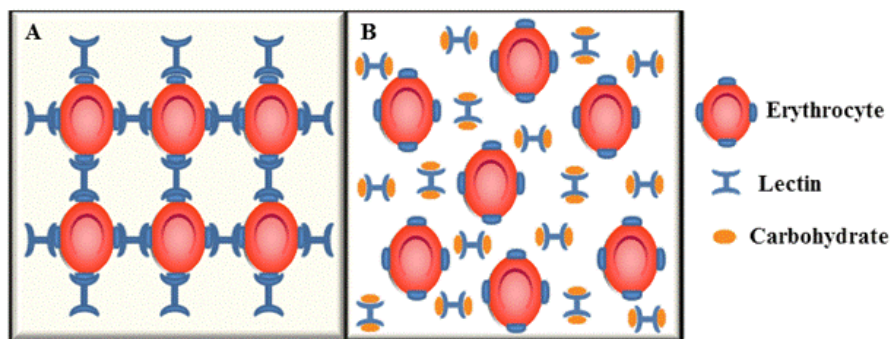


Figure 3. Schematic representation of lectin hemagglutination (A) and carbohydrate inhibition assays (B). Carbohydrates present on the surface of erythrocytes are recognized by the binding sites of the protein forming the network (A). Lectins with more than one binding site are capable of promoting the agglutination phenomenon. The lectin binding sites also recognize carbohydrates free in solution (B) and interaction is inhibited; free erythrocytes precipitate.

The assurance that the agglutinating agent is a lectin is provided by inhibition assay of hemagglutinating activity using a solution of a specific carbohydrate or glycoproteins (Correia et al., 2008). If the lectin binding sites are occupied by free carbohydrate, the lectin binding to erythrocyte surface is blocked and the network agglutination will not be formed (Figure 3B).

Lectin hemagglutination is distinct from tannin induced pseudo-hemagglutination; lectins bind cell polysaccharides differently from tannins (Figure 4, A and B). Thus the connection between tannins and polysaccharides present in the membrane of erythrocytes promote a wrap that induces repulsion between cells; this phenomenon is seen macroscopically as an apparent agglutination, but at the microscopic level it is, in fact, a pseudo-hemagglutination. Beside tannins compounds also able to cause pseudo-hemagglutination are lipids or bivalent cations at high concentrations (Rüdiger, 1998).

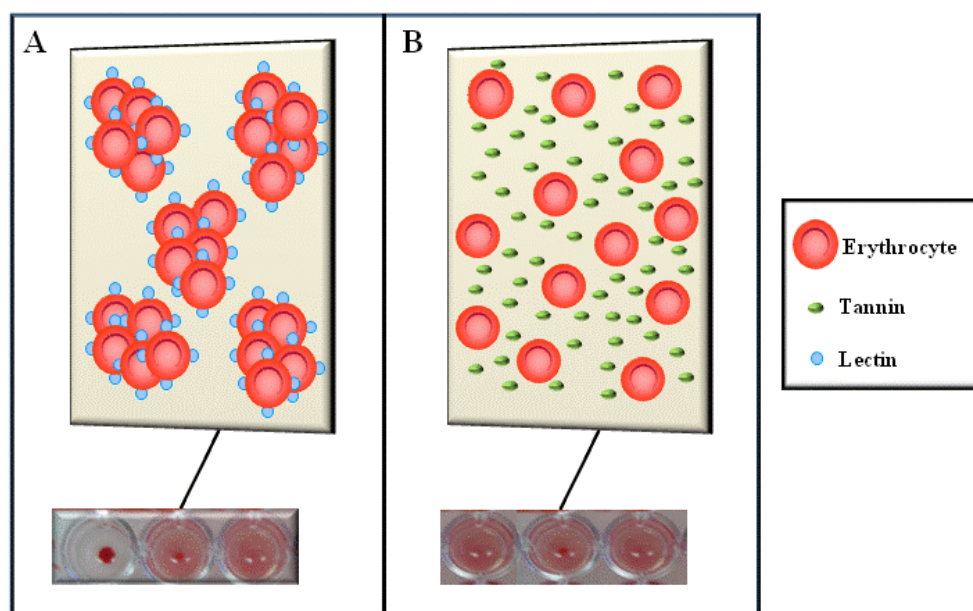


Figure 4. Schematic representation of hemagglutination and pseudo-hemagglutination assays as revealed by optical microscopy. A: Hemagglutination with formation of red blood cell aggregates due to the presence of lectin. B: Pseudo-hemagglutination with dispersion of red blood cells induced by substances such as tannin.

### 7.1. Bacterial Agglutination by Lectins

The ability of lectins to interact with bacteria has already been reported for different purposes. Commercial lectins of *Canavalia ensiformis*, *Ulex europaeus*, *Phaseolus vulgaris*, *Triticum vulgare*, and *Swartzia pickellii* of undefined specificity interacted with *Yersinia pestis* strains isolated from rodent fleas and human biological fluids. Most of the *Y. pestis* strains did not agglutinate with *U. europaeus* or *C. ensiformis* lectin; *P. vulgaris* lectin agglutinated suspensions of all the bacillus strains used. Fifteen of the 19 strains tested positive for assays using *S. pickellii* lectin. A similar agglutination pattern was obtained for lectins with specificity for oligosaccharides containing *N*-acetylglucosamine and *S. pickellii*

lectin, which did bind to the affinity matrix chitin, a polysaccharide of *N*-acetylglucosamine. The use of bacterial strains and commercial lectins of defined specificity may be an approach to provide evidence about lectin binding sites of undefined monosaccharide specificity (Cavalcanti et al., 1990).

## 7.2. Lectins with Antibacterial Activity

Some lectins have antibacterial activity through cell agglutination and variable effects against different microorganisms (Oliveira et al., 2008; Nunes et al., 2011). Glycoconjugates such as peptidoglycans, lipopolysaccharides and teichoic acids are present on bacterial cell surfaces and constitute potential lectin targets (Nunes et al., 2011).

A lectin from *Bothrops leucurus* snake venom (BIL) exhibited antibacterial effects against human pathogenic Gram positive bacteria and was not effective against Gram negative bacteria. A possible reason for the difference in susceptibility is the difficulty that BIL encounters in crossing the outer cell wall of Gram-negative bacteria to reach the periplasmic space. This lectin may interact with the peptidoglycan present in the Gram-positive bacteria cell wall while may not be able to bind peptidoglycans of Gram-negative bacteria, whether it does not enter in the periplasmic space. In the presence of 200 mM galactose this lectin loses its antibacterial effects and agglutination properties; so, the carbohydrate-binding property of BIL is linked with its antibacterial activity (Nunes et al., 2011).

A lectin from *Phthirusa pyrifolia* leaf exhibited antibacterial activity and was more effective for Gram-positive than for Gram-negative species. This greater interaction observed with Gram-positive bacteria may be explained by the high levels of peptidoglycan on the wrapper. Probably, this protein was able to agglutinate the bacteria, promoted their immobilization, and inhibited their growth or even destroyed the bacteria (Costa et al., 2010). This kind of interaction (lectin-bacteria cells) may exist by covalent/or noncovalent aggregation, depending on the molecular weight of the oligomers and its subunits (Rittidach et al., 2007). A lectin from *Eugenia uniflora* seeds demonstrated a remarkable nonselective antibacterial activity. This lectin strongly inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella*; it moderately inhibited the growth of *Bacillus subtilis*, *Streptococcus* sp. and *Escherichia coli* (Oliveira et al., 2008).

## 7.3. Lectins with Coagulant Properties

Some proteins have coagulant properties and can be used in water treatment. *M. oleifera* is a plant whose seeds contain natural coagulant proteins (Okuda et al., 2001; Ghebremichael et al., 2005; Bhuptawat et al., 2007, Santos et al., 2009, Ferreira et al., 2011). It is widely known that the plant have numerous uses (Santos et al., 2011). Antibacterial activity has been attributed to different parts of the plant, such as leaves, roots, seeds, flowers, fruit peel and unripe pods (Anwar et al., 2007).

Coelho et al. (2009) purified by chitin chromatography a lectin from *M. oleifera* seeds called WSMoL (water-soluble *M. oleifera* lectin). Ferreira et al. (2011) demonstrated that this lectin has coagulant activity, reducing turbidity and bacteria contamination. The study

showed that WSMoL aggregated and coagulated these microorganisms from environmental water as well as interfered in the growth of *S. aureus* and *Escherichia coli*.

The coagulant *M. oleifera* lectin (cMoL) is a native basic protein that was isolated after saline extraction and guar gel column chromatography. This lectin was active at pH range 4.0-9.0 and its hemagglutinating activity was inhibited by carbohydrate and abolished by azocasein as well as asialofetuin. Polyacrylamide gel electrophoresis under reduced conditions revealed a main polypeptide band of 26.5 kDa; cMoL showed coagulant activity in turbid water, similar to aluminium sulphate, the coagulant most widely used in water treatment (Santos et al., 2009). Santos et al. (2012) showed that a saline extract from *M. oleifera* seeds with lectin activity removed humic acid from water; this preparation acted better than aluminum sulphate and can be an interesting natural alternative to remove humic acid.

cMoL coagulant property is showed in a simple assay. First, a tap water sample was treated with kaolin clay, stirred for 30 min and allowed to settle for 24 h to complete hydration (Figure 5, left). cMoL (200  $\mu$ L, 1 mg/mL) was then incubated with kaolin suspension (1 mL), pH 6.0, for 30 min (Figure 5, right). Kaolin was used to give the desired turbidity to water sample which was clarified with cMoL. Water remained turbid in the control tube.



Figure 5. Aspect of coagulation assay using kaolin clay 10 g/L (a model of turbid water) and cMoL (1mg/mL) as coagulant. Left tube represents the control and right tube water treated with cMoL evidencing clarification.

#### 7.4. Coagulation Mechanism Proposal of Coagulant *M. oleifera* Lectin (cMoL)

The understanding about the mechanisms involved in the coagulation process by proteins from *M. oleifera* seeds has always been a challenge for researchers, usually because this activity is reported only for crude extracts. cMoL is thermostable, pH resistant and have a molecular weight of 26.5 kDa (Santos et al., 2009). Okuda et al. (2001) proposed a model where coagulation by purified coagulant solution (MOC-SC-pc) from *M. oleifera* seeds occurs due to interaction of MOC-SC-pc with bivalent cations, forming *net-like* structures. This model can not be applied to cMoL since the presence of bivalent ions ( $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$ ) did not improve the efficiency of coagulation (Santos et al., 2009).

Molecules vary in their charge properties; all molecules with ionizable groups can be titrated and their net surface charge is highly pH dependent. Proteins are built up with many different amino acids containing weak acidic and basic groups; their net surface charge will change gradually as the pH of the environment changes, so the proteins are amphoteric components.

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Figure 6 shows a proposal for the interaction mechanism involved in cMoL coagulation process. cMoL, a basic positively charged protein (Santos et al., 2009), interacts with colloidal particles like kaolin, which zeta potential is negative (Table 1).

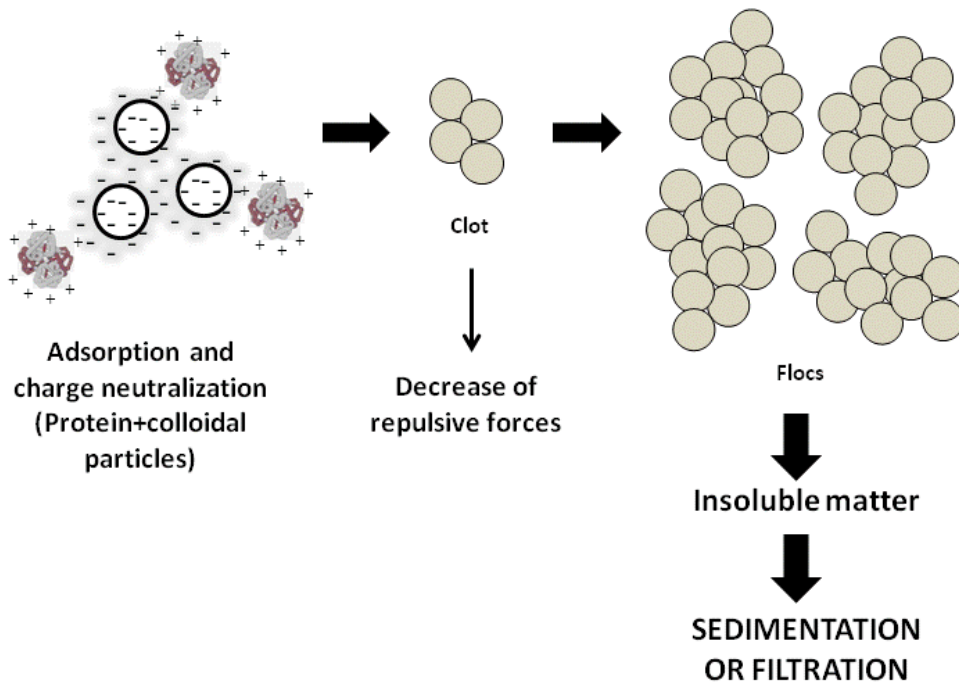


Figure 6. Coagulation mechanism proposed to coagulant *Moringa oleifera* lectin (cMoL).

The decrease of repulsive forces leads to formation of aggregates, which become increasingly larger and denser forming an insoluble material subsequently removed by sedimentation or filtration. Zeta potentials of kaolin (0.5 g/L) in different pH values were determined using a Malvern Zetasizer instrument equipped with the zeta potential cell DTS1060 at 20 °C. Values were derived from the electrophoretic mobility using the Smoluchowski approximation (Hunter, 1981).

**Table 1. Zeta potential of kaolin clay in different pH values**

Kaolin clay 0.5 g/L	Zeta potential (mV)
pH 5	-4.66 +/- 0.32
pH 6	-7.75 +/- 0.04
pH 7	-2.51 +/- 0.15
pH 8	-4.66 +/- 0.25

Coagulant activity of high-molecular cationic polyacrylamide derivatives has been explained by the bridge formation model. Coagulation of negatively charged particles is a result of binding by Coulomb forces of positively charged particles and neutralization of part of the surface charge. Reduced electrostatic repulsion leads to the agglomeration and formation of flocs by interaction between negatively charged particles (Gassenschmidt et al., 1995); cMoL may acts in a similar way.

## CONCLUSION

The purpose of this chapter was to review the differences among coagulation, flocculation, and agglutination processes and the applications of molecules and substances able to exert these effects. Coagulation, flocculation, and agglutination differ in the manner how linkage occurs among aggregate components and whether aggregates remain in suspension or not. The agglutination reaction by antibodies or lectins can be employed for different purposes such as identification of bacterial isolates or diagnosis of infection diseases. Coagulant and agglutinating properties of lectins from *M. oleifera* seeds confer to these proteins a potential as water treatment agents by removing particulate materials and microorganisms.

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