1. Bacterial cell structure

Cells are of two types: “eukaryotic” and “prokaryotic”. Sizes of cells are in the range of 1 - 5 µm. Despite their simplicity, bacteria contain a well-developed cell structure which is responsible for many of their unique biological properties. Many structural features are unique to bacteria and are not found among archaea or eukaryotes. Because of the simplicity of bacteria relative to larger organisms and the ease with which they can be manipulated experimentally, the cell structure of bacteria has been studied, revealing many biochemical principles that have been subsequently applied to other organisms. Bacteria appear in a variety of different shapes, viz., spherical (coccus), rod like (bacillus), curved rod (vibros) and spiral (spirill).

*Figure 1.* Different types of bacterial cell

Cell shape is generally characteristic of a given bacterial species, but on the basis of growth condition, it can vary. The most obvious structural characteristic of bacteria is (with some exceptions) their small size. For example, *Escherichia coli* cells, an “average” sized
bacterium, are about 2 µm long and 0.5 µm in diameter, with a cell volume of 0.6-0.7µm [1] Most of the bacterial cells are surrounded by cell wall which is responsible for the structural integrity to the cells. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organism by the presence of peptidoglycan (poly-N-acetylglucosamine and N-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for small substrates. While all bacterial cell walls contain peptidoglycan, not all cell walls have the same overall structures. Since the cell wall is required for bacterial survival, but is absent in eukaryotes, several antibiotics (penicillin and cephalosporin) stop bacterial infections by interfering with cell wall synthesis, while having no effect on human cells.

2. Intracellular Bacterial cell structure

In comparison to eukaryotes, the intracellular features of the bacterial cell are extremely simple. Bacteria do not contain organelles in the same sense as eukaryotes. Instead, the chromosome and perhaps ribosomes are the only easily observable intracellular structures found in all bacteria. Specialized groups of bacteria do exist that contain more complex intracellular structures.

3. Gram positive and gram negative bacteria

On the basis of the response of the bacterial cell wall with gram stain, the bacterial cell walls can be classified as “gram positive” or “gram negative”. For both the gram positive and
gram negative bacteria, particles of approximately 2 nm can pass through the peptidoglycan [2].

![Schematic diagram of bacterial cell.](image)

Figure 2. Schematic diagram of bacterial cell.

Gram positive bacteria react with gram stain to appear purple whereas gram negative bacteria do not react with gram stain. Peptoglycans (mucopeptides, glycopeptides, mureins, etc.) are the structural elements of almost all bacterial cell walls. They constitute ~95% of the cell wall in some gram positive bacteria and as little as 5-10% of the cell wall in gram negative bacteria. Peptoglycans are made up of a polysaccharide backbone consisting of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in equal amounts [3]. The cell wall of some gram positive bacteria is completely dissolved by lysozyme. In addition, the cell wall of gram positive bacteria contains teichoic acid. Teichoic acids [4], in which low molecular weight carbohydrates are joined through phosphoric diester linkages, are another type of polymer present in cell walls and membranes of gram positive bacteria. On the external side of the cell wall, prokaryotes have the glycocalyx, a gelatinous polymer, consisting of polysaccharide chains [5]. Gram negative bacteria have much more complex cell wall. On the cytoplasmic side, the plasma membrane is connected to a single layer of peptidoglycan [6]. This is responsible for the cell wall’s inability to retain the crystal violet stain upon decolourisation with ethanol during gram staining. This peptidoglycan layer
is connected to a lipoprotein unit. The lipoprotein layer connects to an outer membrane [7] which contains a lipopolysaccharide [8,9] that is made up of a lipid portion (two sugar units connected to a hydroxyl fatty acid) and an O-antigen subunit. The lipid portion is also referred as an endotoxin since it is a toxic in a host’s bloodstream or gastrointestinal tract. The O-antigen portion consists of sugar molecules and it is the primary site of gram negative bacteria, recognized by antibodies. The variability of the O-antigen chain can cause problems with the immune response. As the lipopolysaccharides are highly-charged, the gram negative cell walls have overall negative charge. The chemical structure of the outer membrane lipopolysaccharide is often unique to specific bacterial strains (i.e., sub-species) and is responsible for many of the antigenic properties of these strains [10]. As the bacterial cytoplasmic membrane is composed of a phospholipid bilayer, so it has all of the general functions of a cell membrane such as acting as a permeability barrier for most molecules and serving as the location for the transport of molecules into the cell. In addition to these functions, prokaryotic membranes also function in energy conservation as the location about which a proton motive force is generated. As a phospholipid bilayer, the lipid portion of the outer membrane is impermeable to charged molecules. However, channels called ‘porins’ are present in the outer membrane that allow for passive transport of many ions, sugars and amino acids across the outer membrane. These molecules are therefore present in the periplasm, the region between the cytoplasmic and outer membranes [11]. Because of the location between cytoplasmic and outer membranes, signals received and substrates bound are available to be transported across the cytoplasmic membrane using transport and signaling proteins embedded there [12]. Gram positive and gram negative bacteria both can produce extracellular polysaccharides [13,14], which surrounds the bacterium like a capsule. The bacterial capsule increases the overall dimensions of the organism. The capsule is generally composed of hydrophilic polysaccharides carrying negative charges [15]. A large number of
serologically distinct capsule types have been recognized on the basis of their respective antigenic determinants [16]. Capsulated cells are also found in the oral flora [17], with polysaccharides serving as aggregation substances [18] and possibly playing a role in dental plaque and caries. Besides the formation of well defined capsules, it has been reported that a large number of species of the Enterobacteriacea produce an extracellular carbohydrate lining composed largely of colonic acid [19-21]. Enterobacteriacea is a family of gram-negative bacilli that contains more than 100 species of bacteria that normally inhabit the intestines of humans and animals [22]. Enterobacteriaceae, that are commonly part of the normal intestinal tract flora, are referred to as ‘coliforms’. Members of the Enterobacteriacea are relatively small, non-spore forming bacilli. Some are motile, while others are not. Some have capsules, others do not. Members are frequently resistant to common antibiotics. They ferment a variety of different carbohydrates. The patterns of this fermentation are used to differentiate and classify them. Some members are found in soil, water, and decaying matter. Some pathogenic strains also produce exotoxins, while others produce exotoxins that are called "enterotoxins" because they specifically affect the intestinal tract, causing diarrhea and body fluid loss. This is, indeed, a diversified family. The bacterial capsule shows antigenic character. The immunological reactions of these K-antigens are the basis for the serological classification of bacteria [23]. Various species of the Enterobacteriacea are able to cause pneumonia and urinary tract infections [24]. They are also recognized as the major cause of wound infections and other nosocomial (hospital acquired) infections [25]. They may also cause bacteremia and meningitis if conditions are right [26]. These bacteria are estimated to be responsible for about 100,000 deaths each year in the USA, and account for about half of all the clinically significant bacteria isolated by hospital laboratories [22]. Sometimes they succumb relatively low concentrations of common disinfectants, including chlorination; but their susceptibility to antibiotics varies; and they are now becoming frequently resistant [27].
However they cannot be destroyed by freezing the food or water [22]. As these bacteria are found in large numbers in the intestinal tract, they are transmitted most often through foods, ground beef is the most frequent route [28]. Nonfood borne transmission includes person to person which mostly occurs in child day care centers, waterborne transmission occurs due to drinking of contaminated water.
4. The gram negative bacteria *Klebsiella*

The gram negative bacterial genus *Klebsiella* belongs to the tribe *Klebsiellae*, a large member of the family *Enterobacteriaceae*. The organisms are named after Edwin Klebs, a 19th century German microbiologist, *Klebsiellae* is non-motile, rod shaped gram negative bacteria with a prominent polysaccharide capsule [29]. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms.

*Klebsiella* infection is a well recognized problem and causes many diseases [30,31]. The expression of K antigen is an important virulence determinant in *Klebsiella* spp. since it plays a role in resistance to phagocytosis [32-34]. Several workers have demonstrated that the size of the capsule and the rate of its synthesis are important in virulence in pulmonary [35,36], intraperitoneal [37,38] and burn [39] infection models. One consequence of higher levels of K antigen synthesis is the release of larger amounts of polysaccharide from the cell surface [35,40], providing cell-free K antigen which could neutralize circulating anticapsular antibody [41]. In addition, purified K antigens have been shown to exert a number of effects which would have a significant influence on pathogenicity. These effects include induction of immune tolerance [42-44] and impairment of the maturation and function of macrophages [45,46]. Infection with *Klebsiella* organism occurs in the lungs, where they cause destructive changes, necrosis, inflammation and hemorrhage occur within lung tissue, sometimes producing a thick bloody mucoid sputum described as ‘current jelly sputum’. The illness typically affects middle aged and older men with debilitating diseases such as alcoholism, diabetes or chronic broncho-pulmonary disease. *Klebsiella* have also been incriminated in nosocomial infections [47] common sites include the urinary tract, power respiratory tract, biliary tract and surgical wound sites [48]. *Klebsiella aerogenes* infection became epidemic
in neurosurgical intensive care ward. It has been observed that antibiotic therapy has a little impact in the mortality rate for *Klebsiella* infections [49]. The *Klebsiella* capsular polysaccharides are now used as human vaccines [50,51] which are non toxic and immunogenic. As bacterial polysaccharides has potential use in immunological and vaccine preparations, so primary structural studies and conformational analysis as well as studies on various physico-chemical properties of these biopolymers are gaining more and more importance.

Members of the *Klebsiella* genus typically express themselves in two different types of antigens on their cell surface. The first one is a lipopolysaccharide (O-antigen); another one is a capsular polysaccharide (K-antigen). Both of these antigens contribute to pathogenicity. More than 80 K-antigens and about 90 O-antigens exist [52,53]. The variability in structures of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes of appears to be similar. Primary structures of most of the capsular polysaccharides of *Klebsiella* are known [54]. All of them are acidic in nature and are composition of definite repeating units. As they are acidic in nature, they are called saue polysaccharides, SPS (the term ‘saue’ means sour in German). The structure of four SPS K28, K43, K20 and K51 are shown below.

![Structure of Klebsiella K28](image)

*Klebsiella* K28 [ Structure adopted from [55] ]
5. Physiochemical characterization of capsular polysaccharide

As bacterial polysaccharides are quite relevant in terms of vaccine preparation, hence their detailed primary structural analysis and conformational analysis, alongwith detailed elucidation of physico-chemical properties are warranted. There are different ways of characterization of bacterial capsular polysaccharides (SPS). Some specific methods are described below.
5.1. **Compositional analysis.** Compositional analysis of the acidic, neutral and basic monosaccharides obtained from the acid hydrolysis of the bacterial cell wall polysaccharides could be done by using a high-performance liquid chromatography (HPLC) method with pulsed-amperometric detection (PAD) [59]. Hydrolysis of the *Klebsiella* polysaccharides is generally done by aqueous hydrofluoric acid. The bacterial polysaccharides were found to be made up of oligosaccharide repeating units. Most of them are comprised of D-glucose, D-galactose, D-mannose, L-rhamnose, L-fucose, D-glucuronic acid and D-galacturonic acid [60]. The compositions of the four SPS, which will be discussed later, were found to be as follows:

<table>
<thead>
<tr>
<th>SPS</th>
<th>D-Glcp</th>
<th>D-Galp</th>
<th>D-Manp</th>
<th>D-GlcpA</th>
<th>Mass per unit charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>K28</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>980</td>
</tr>
<tr>
<td>K43</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>820</td>
</tr>
<tr>
<td>K20</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>646</td>
</tr>
<tr>
<td>K51</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>594</td>
</tr>
</tbody>
</table>

5.2. **Structural analysis.** Compositional analyses can not predict about the sequence of sugar units in any SPS. For the detailed knowledge of the sequential variation, additional information is required which is obtained by the structural analysis. The process includes glucose analysis, methylation analysis and NMR spectroscopy [56,61]. Additional evidence for the structure of the native polysaccharide is obtained from base-catalysed degradation of the methylated polysaccharide and from the NMR spectroscopic analysis of the lithium-degraded polysaccharide band of the oligosaccharide-alditol derived from the repeating unit oligosaccharide obtained from the bacteriophage degradation. The structure of the four SPS about which present work is concerned is already given in Figure 4.
5.3. *Studies on the interaction of SPS with oppositely charged dye molecules.* The physico-chemical properties of different SPSs could be carried out using dye-polymer interaction and polymer-surfactant interaction studies. From the study of dye-polymer interaction detailed structural aspects of the polysaccharides can be obtained. They are further described as individuals.

5.3.1 *Dye-polymer interaction technique.* Studies of interaction of small dye molecules with the biopolymers are expected to produce useful information regarding the conformation of the SPS, equivalent weight per repeating units, whether it is susceptible to bind with the dye. The studies could also be correlated as the interaction of drug molecules with the SPS, which eventually will help in combating different bacterial derived diseases. One can also determine the thermodynamic parameters of interaction from this simple technique when the spectral measurements are recorded at different temperatures. Specificity in the interaction of different dyes with polysaccharides has also been well studied earlier [62,63]. The dye-polymer interaction technique provides the following informations.

5.3.1.1. *Metachromasy.* Metachromasy is a well known phenomenon in the case of dye-polymer aggregates and has been defined as the blue shift of the main absorption band of a cationic dye in dilute aqueous solution caused by some added polyanion [64,65]. It was generally applied to the aggregations of cationic dye on anionic polymers [66,67]. However, metachromasy has also been observed in same cationic polyelectrolyte and anionic dye systems [68-70].
Figure 5. Absorption spectra of a cationic dye pinacyanol chloride in water (1) and in the presence of anionic polyelectrolyte (2).

Formation of metachromatic compound is characterized by the shift of the absorption maxima to shorter wavelengths (hypsochromic effect) and a decrease in absorbance (hypochromic effect). A polyelectrolyte with relatively high charge density is found to be efficient in inducing metachromasy. Metachromasy also depends on the conformation of the polyanions as well as the dye ions in solution. Extent of metachromasy for a particular dye on different polymers arises from differences in the strength of electronic interaction which depends upon the effective inter-dye system [71].

From the above Figure it is observed that a blue shift of the main absorption band of a cationic dye in aqueous medium was caused by the addition of polyanion [72]. The peak at lower wavelength is called metachromatic band.

5.3.1.2. Reversal of metachromasy. Different techniques for the isolation and stability determination of the metachromatic compound have been reported [71,73]. The stability of the metachromatic compound can be determined by using the concept of reversal of metachromasy. It has been reported that the reversal of metachromasy occurs by the addition
of urea, alcohol, neutral electrolytes, and excess polyanion and also by increasing the temperatures of the systems [74-76].

5.3.1.3. Metachromatic titration. The spectrophotometric (metachromatic) titrations of various classes of acid polysaccharides (polycarboxylates [77], polysulfates [78] and heparinoids [79]) in dilute solution with different metachromatic dyes like acridine orange [78], neutral red [78], methylene blue [80], pinacyanol chloride[81], etc., are also available in the literature. This technique has been found to be used satisfactorily to determine the equivalent weight of the polymer. It can also be used to determine stoichiometry of the polymer/dye in the metachromatic compound [72].

5.3.1.4. Fluorescence studies. Substances containing delocalized electrons present in conjugated double bonds can display fluorescence and are known as fluorophores. The fluorescence spectral data depends upon the chemical nature of the fluorophore and solvent in which it is dissolved. Fluorescence quenching [82] is a process of deactivation of the excited state which competes with fluorescence and results in a decrease in fluorescence intensity. Fluorescence quenching has been chosen as one of the method to study the excited state of a system, specially the dye-polymer complexes [83]. Fluorescence spectra can give a clear idea about the complex formation [84]. The number of binding sites on the polymer molecule can be evaluated from fluorescence quenching technique. Fluorescence spectroscopy can be used to study the interactions of drugs with DNA [85]. The interaction strength of the drug with DNA is reflected by the decreasing sequence of fluorescence intensity and may have much to do with the anticancer activity and toxicity of the drugs. Spectrofluorimetric titration of different fluorescent dyes by the polymers can also be used to determine the equivalent weight of the polymer [84].
5.3.1.5. Thermodynamic studies. Determination of thermodynamic parameters of the interaction can reveal the nature of the metachromatic complex and also the suitable conditions for the interaction between the cationic dye and anionic site of the macromolecule [86,87]. Evaluation of thermodynamic parameters has, therefore, great importance in dye-polymer interaction [84]. By suitably analyzing the spectral data one can determine the binding/interaction constant between the dye molecule and polyanion [88]. Once the binding constant ($K_c$) is known, then the change in the standard free energy ($\Delta G^0$) can easily be calculated using the relation $\Delta G^0 = -RT \ln K_c$. This value could predict the spontaneity of the reaction. From the graphical plot of $\Delta G^0$ vs. T and the value of the enthalpy change ($\Delta H^0$) and entropy change ($\Delta S^0$) could be determined using the relation, $\Delta G^0 = \Delta H^0 - T\Delta S^0$. Changes in the standard enthalpy value can shed light on the nature of the reaction i.e. whether it is exothermic or endothermic while from the changes in the standard entropy value one can predict whether any organized structured states are formed or not [89].

Beside the dye-polymer interaction, polymer surfactant interaction studies also plays an important role in determination of various other molecular properties like hydrodynamic radius, weight average molecular weight, radius of gyration, zeta potential (Z.P.) etc. However, before proceeding to the detailed study on polymer- surfactant interactions, it is worthwhile to first discuss briefly about surfactant.

6. Surfactants

Surfactants are the entities that lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents and dispersants. The term surfactant is a blend of SURface ACTive AgeNT [90]. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their hydrocarbon tails) and
hydrophilic groups (their heads). When a surfactants are added in water the surfactant molecules first get dissolved in water like normal solute, after which they migrate to the air-water interface, where the insoluble hydrophobic group may extend out of the bulk water phase, either into the air or, if water is mixed with an oppositely charged polymer, while the water soluble head group remains in the water phase.

Figure 6. General structure of a surfactant molecule.


6.1.1. Classification based on the source/origin:

a) **Natural surfactants**: The surfactants which are directly or indirectly derived from the natural sources are termed as natural surfactants. Soaps are natural surfactants which are obtained by the process of saponification of fats or triglycerides, e.g., sodium stearate (C<sub>17</sub>H<sub>35</sub>COO"Na"<sup>+</sup>).

b) **Synthetic**: Synthetically derived surfactants are known as detergents, e.g., sodium lauryl sulfate (SLS, SDS).

6.1.2. According to the composition of their tail: The tail of surfactants can be classified according to the different types of hydrocarbon chains as follows:

a) **A hydrocarbon chain**: Aromatic hydrocarbons (arenes), alkanes (alkyl), alkenes, cycloalkanes.

b) **An alkyl ether chain**: Ethoxylated surfactants: polyethylene oxides are inserted to increase the hydrophilic character of a surfactant. Propoxylated surfactants:
polypropylene oxides are inserted to increase the lipophilic character of a surfactant.

c) A fluorocarbon chain: fluorosurfactants.

d) A siloxane chain: siloxane surfactants.

A surfactant can have one or two tails. These are called double-chained.

6.1.3. According to the composition of their head. Surfactants can be classified into different category based on the charge that they carry on their head groups.

Figure 7. Surfactant classification according to the composition of their head: (from top to bottom) nonionic, anionic, cationic and zwitterionic.

A surfactant can be classified by the presence of formally charged groups in its head. A nonionic surfactant carries no charge. The hydrophilic headgroup of an ionic surfactant carries a net charge. If the charge is negative, the surfactant is more specifically called anionic; if the charge is positive, it is called cationic. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic.
Table 2. Classification of surfactants based on the charge of the head groups.

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anionic</strong></td>
<td>Sodium lauryl/dodecyl sulfate (SDS/SLS)</td>
</tr>
<tr>
<td><strong>Cationic</strong></td>
<td>Cetylpyridinium chloride (CPC),</td>
</tr>
<tr>
<td></td>
<td>Cetyltrimethylammonium bromide (CTAB)</td>
</tr>
<tr>
<td><strong>Zwitterionic(amphoteric)</strong></td>
<td>Sodium lauroamphoacetate</td>
</tr>
<tr>
<td><strong>Nonionic</strong></td>
<td>Polyoxyethylenesorbitan monolaurate (Tween 20)</td>
</tr>
</tbody>
</table>

Surfactants assemble in the bulk solution into aggregates. The example of such aggregates is vesicles and micelles. The concentration at which surfactants begin to form micelle is known as the critical micelle concentration (CMC). When micelles form in water, their tails form a core that can encapsulate an oppositely charged site of a polymer and their heads form an outer shell that maintains favourable contact with water. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterinonic (dual charge).

*Figure 8A. Schematic diagram of a micelle in water.*
It has been always found that the mixed surfactant systems exhibit better performance than the individual components [91] and references therein. For this reason, in most of the cases, commercial detergents are comprised of a number of surfactants as mixtures. If nonionic surfactants are added to the ionic surfactants then the CMC value of the mixed surfactant decreases than the value of a pure ionic surfactant [92]. Sometimes the CMC of the mixture at certain composition can have even lower CMC value than the individual components and thus can exhibit better detergency. Besides a decrease in the CMC value leads to a strong binding of the polymer with the surfactant leading to enhanced interaction of the polymer with the mixed surfactant compared with the interaction of the same polymer with the cationic surfactant only. When the oppositely charged polymer-surfactant systems are concerned, there occurs a problem of precipitation, which can be overcome with the aid of a nonionic surfactant.

7. Polymer-surfactant interaction

Polymer-surfactant interaction study is an interesting and a promising field of research due to its wide spread applications in different spheres. Such systems are considered to be an important subject of research for both fundamental and application reasons [93-99]. Polymer-
surfactant mixtures are widely exploited in commonplace formulations to manipulate their performance behaviors. The ternary systems of surfactant, polymer and water have potential for domestic, industrial and technological applications, viz., foods, paints, drug, laundry products, cosmetics, etc. [100,101]. Oppositely charged polymer-micellar aggregates can serve as model for polyion-colloid systems [102]. The coulombic polyion-colloid interaction guides the flocculation of inorganic materials important in water purification [103,104]. Polymer-surfactant interaction could be studied using different techniques viz. viscosity, turbidimetry, dynamic light scattering, zeta potential measurements, etc.

7.1. Viscosity measurement of polymer-surfactant aggregate. Viscosity is an important parameter which can provide information regarding the hydrodynamic radius of biomacromolecule in aqueous solution [105]. Different molecular properties of macromolecule like shape, non electrolytic or polyelectrolytic nature, molecular weight, etc., influence the viscosity of polymer solutions. Several theories in polymer physics literature [106] correlate molecular properties of polymers such as molecular weight overlap concentration, radius of gyration and pore size of concentrated polymer with the intrinsic viscosity. The intrinsic viscosity determination helps in the determination of the solubility parameters of the polymers in different solvents which in turn are applied to drug-excipient interactions [107]. The degree of hydrophobic associations, hydrolysis and size of miceller clusters can be determined from intrinsic viscosity measurement [108,109]. In the viscosity method, which is used for studying polymer-surfactant interaction, it can be assumed that coiling up of the polymer takes place for which reduces the viscosity. Beyond the point of precipitation the viscosity increases due to the formation of macroscopic aggregates.
Figure 9. Variation of different physic-chemical properties, along with the corresponding conformation of a polymer with the addition of oppositely charged cationic surfactant. Parameters: $\eta$, viscosity; $d_h$, hydrodynamic diameter; $\tau$, turbidity and Z.P., zeta potential.

7.2. Turbidimetry. The effect of polymer on CMC values of surfactants, effect of charge density as well as the effect of structure of polymers on the polymer-surfactant binding can be ascertained by the turbidimetric titration [110-112]. As the surfactants get adsorbed on the surface of the polymer, the turbidity increases due to the formation of a larger particle but when the polymer-surfactant aggregate gets resolubilized in excess micelles, the turbidity decreases. The extent of binding and thus the interaction could thus be established.

7.3. Dynamic light scattering studies. Dynamic light scattering measurement is a useful method to determine the hydrodynamic radius of a polymer in solution and also to measure the zeta potential for charged colloids [113-115]. These studies lead to determination of extent of binding of the cationic and cationic-nonionic mixed surfactants with the bacterial polysaccharides (SPS). Size enhancement and extent of zeta potential change depends upon the CMC values of the surfactants and also on the structural variation of the SPS.
Although several works have been reported so far that involve polymer-dye [62-71,73-76,78,86,87,90] and polymer-surfactant [110-115] of different kinds but studies involving bacterial polysaccharides (SPS) and dye/surfactant and are not plenty. So the scopes of studies on the interaction between bacterial polysaccharide and dye/surfactant were plenty.

The present investigation deals with the studies on different physico-chemical properties of the capsular polysaccharides isolated from four different K- serotypes K28, K43, K20 and K51 belonging to the same bacterial genus *Klebsiella* as their properties has not been studied previously in detail. The investigation includes detailed studies on dye-polymer interactions by spectrophotometric and spectrofluorimetric techniques under various environmental conditions for establishing chromotropic character of the SPSs with respect to induction of metachromasy in cationic dyes pinacyanol chloride, pinacyanol bromide and acridine orange. It also includes determination of thermodynamic parameters of interaction, turbidity, viscosity, size measurement and measurement of zeta potential. Cationic surfactants benzylidimethyl-n-hexadecylammonium chloride (BDHAC), cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), dodecylpyridinium chloride (DPC) and nonionic surfactant polyoxyethylenesorbitan monolaurate (Tween 20) were used to study the polymer-surfactant interaction.
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