CHAPTER 3

COMPARATIVE STUDY OF SDS POLYACRYLAMIDE GEL ELECTROPHORESIS OF PROTEIN EXTRACTED FROM CESTODES *MONIEZIA* SP., *STILESIA* SP. AND *AVITELLINA* SP. A PARASITE OF THE DOMESTIC GOAT *CAPRA HIRCUS* (L.) IN JALNA DISTRICT (M.S.), INDIA.

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ABSTRACT

SDS-PAGE is a commonly used method for analyzing protein samples that can give the information on purity, subunit composition, molecular weight, and relative abundance. A large number of mammals are the hosts of Anoplocephalidean cestodes. The cestode *Moniezia* sp., *Stilesia* sp. and *Avitellina* sp., were the worms isolated from the infected host i.e. *Capra hircus* at Jalna. Present study was used to examine the electrophoretic patterns of extracted proteins of *Moniezia*, *Stilesia* and *Avitellina* isolated from the infected goats i.e. *Capra hircus* by using SDS-PAGE technique. The SDS-PAGE of the extracted proteins resulted evident molecular weights of different polypeptides of cestodes, that ranged from below 3 kda to above 205 kda, but most of bands were observed between the range of molecular marker and very few bands observed in above high molecular protein bands 205 kda.

Keywords: Protein *Moniezia*, *Stilesia* and *Avitellina*.

INTRODUCTION

The biochemical methods used for the identification of organisms emphasized the application of electrophoresis as an analytical tool for fixing the taxonomic problems (Sibley 1960). On this background thereafter, a number of researches reported the usage of electrophoresis of protein having been done on a huge sort of vertebrates and invertebrates. Some of the trematode parasites, *Schistosoma mansoni* and *Schistosoma japonieun* had been used to study their protein polymorphism by use of disc electrophoresis (Sodemen, 1967; Yoshimura, 1968).

It was observed that the saline extract of *Schistosome* species produced characteristic species specific electrophoretic patterns which can also useful tool in the identification of these parasites (Yoshimura, 1968). Ruff *et al.* (1973) also used disc electrophoresis for the identification of different races of *S. japonicum* on the basis of their geological distribution. Diverse species of the genus *Paragonimus* were identified on the basis in their characteristic protein patterns, mainly *P. westermani*, *P. ohirai* and *P. miyazaki*. The morphological characteristic of the adult worm of these species are difficult to distinguish due to the fact that the taxonomic characteristic are not recognized (Yoshimura, 1969). The maximum species-specific differences in the polypeptide profile as well as glycoprotein profile have been found in the Schistosomes (Aronstein and Strand, 1983).

Kumartilake and Thompson (1979) obtained reliable variation within the protein profile of various strains of *E. granulosus* as well as generic differences in *Taenia crassiceps*, and *Mesocestiodes corti*. Bursey *et al.* (1980) have also used polyacrylamide gel electrophoresis for the separation of various species of *Taenia*.

The soluble protein in *Taenia*, by means of polyacrylamide gel electrophoresis, was studied by Barrett (1982), who confirmed that the band patterns in different parts of strobilae were basically similar regardless of the state of development. Wright (1974) talked about the use of electrophoresis technique as a valid taxonomic device, however, particular research is required on different organisms. The species particular protein profiles would not only help in the identification but also serve as an excellent source for specific diagnosis of the helminthes worms. The intrinsic property of protein being the charged molecules has furnished an advantage that they can be resolved into different feature fractions according to their electrophoretic mobility. In the study of pouched amphistomes, the seasonal biochemical changes are related to the scale and shape of gonads. Such morphometric differences can be influenced by the reproductive and metabolic status of the parasite. Therefore morphological features alone cannot be used as standard method for the characterization of closely related worms.

In current study an attempt has been made for unique identification of a large quantity of closely associated helminthes infecting the gastrointestinal tract of the ruminants. SDS PAGE is a commonly used method for the identification of parasite proteins. The method depends upon the migration of solubilized proteins in an electric field and depending upon the conditions in size, charge, or both. The rate of migration of protein within an electric field is determined by the intrinsic characteristics of the protein (size, mass, shape and charge) and by the physical characteristics of the medium (ionic strength, viscosity, pH, temperature). Many different methods of electrophoresis have been described but the most commonly used technique in the field of parasitology is SDS-PAGE, i.e. polyacrylamide gel electrophoresis (PAGE), carried out in the presence of the detergent sodium dodecyl sulphate (SDS) (Eileen Devaney, 1997).

In the present work, comparative study of protein profile has been carried out by SDS- polyacrylamide gel electrophoresis technique as described by Laemmli (1970).

SDS-PAGE ELECTROPHORESIS

MATERIALS AND METHODS

In present study of the protein polymorphism in cestode parasites, a number of species inhabiting same or different host (sheep and goat) were used. The cestodes, i.e. *Moniezia, Stilesia* and *Avitellina,* were collected from intestine of *Capra hircus.*

All of the worms have been processed individually for the extraction of proteins. The worms, washed with saline and maintained at 37±2 °C, have been homogenized in 0.1 M Phosphate buffer, pH 7.4, containing 0.25 M sucrose, in a glass teflon tissue homogenizer with a motor driven pestle. The homogenate was then centrifuged for 10 min. to dispose of debris and unbroken cells. The clear homogenate was treated with ammonium sulphate for extraction and partial purification of total proteins. Following gradual addition of ammonium sulphate (65% w/v) to the homogenate, constant stirring was done and any change in the pH was checked so that pH remained between 7 and 7.4. At 65% ammonium sulphate concentration, majority of the proteins are precipitated out. The precipitated proteins were centrifuged and the pellet was suspended in 0.1 M Tris-HCl, pH 7.4, washed twice in the same buffer. Finally the pellet was air dried and subsequently the dried proteins were solubilized in sample buffer containing 20% sodium dodecyl sulphate (SDS), 5% alpha-mercaptoethanol, 10% aqueous bromophenol blue in 0.12 M Tris-HCl, pH 6.75 (Laemmli, 1970) and stored in liquid nitrogen for subsequent use.

Protein concentrations of the samples have been determined by the method of Bradford (1976) as modified by Spector (1978).

The electrophoresis of proteins in presence of SDS was essentially carried out as described by Laemmli (1970) with some minor modifications. The separating gel were prepared by a linear gradient of 7-15% polyacrylamide which was allowed to polymerize at room temperature for about 45 min., and following polymerization it was overlaid with a stacking gel consisting of 4% polyacrylamide. Since the stacking gel shrinks on storage, it was always prepared fresh just before the start of electrophoresis.

Always freshly prepared ammonium sulphate was added in separating gel solutions and mixed thoroughly before pouring into the chambers of gradient gel marker. The gel solution was poured into the glass plate's mould using 1.5mm thick spacers so that 1.5 mm thick slab gel of linear gradient of acrylamide (7% - 15 %) is formed. Once the solution is pureed, it was carefully overlaid with few drops of distilled water and left for polymerization at room temperature for 45 min. After the polymerization, distilled water was aspirated and then 4% stacking gel was prepared.

Before carrying out electrophoresis all the protein samples in Laemmlis sample buffer were boiled for 5 min. in water bath, pre-maintained at 1000 c. The gels were pre-run at 5 mA for 30 min. after which the power was disconnected. The tank buffer was changed prior to the application of sample containing 80-100μgm protein of each parasite on the stacking gel. The gel slab thickness was 1.5 mm, with 130 mm total gel length consisting of 10mm stacking gel and 120 mm gradient separating gel. The electrophoresis was initially carried out at 5 mA for 8-10 hour's in a refrigerator to minimize the heating effect. The standard molecular weight markers were simultaneously run in one lane of the same gel slab. The medium molecular weight standards contained the following highly purified proteins (3.5 kda to 205 kda).

After electrophoresis the side spacers were removed and the gels were fixed in methanol: acetic acid: water mixed in the ratio of 45:10: 45, for 1 hour at room temperature. The gels were then stained for 2 hours in 0.25 percent coomassie brilliant blue R-250 prepared in the fixative. The over stained gels were initially de-stained in the same fixative without dye and finally de-stained in methanol: acetic acid: double distilled water (5:7:88) until the background was clear and protein bands became distinct. The gels photographs were taken with the help of camera.

SDS-PAGE of cestode parasites i.e. *Moniezia*, *Stilesia* and *Avitellina*
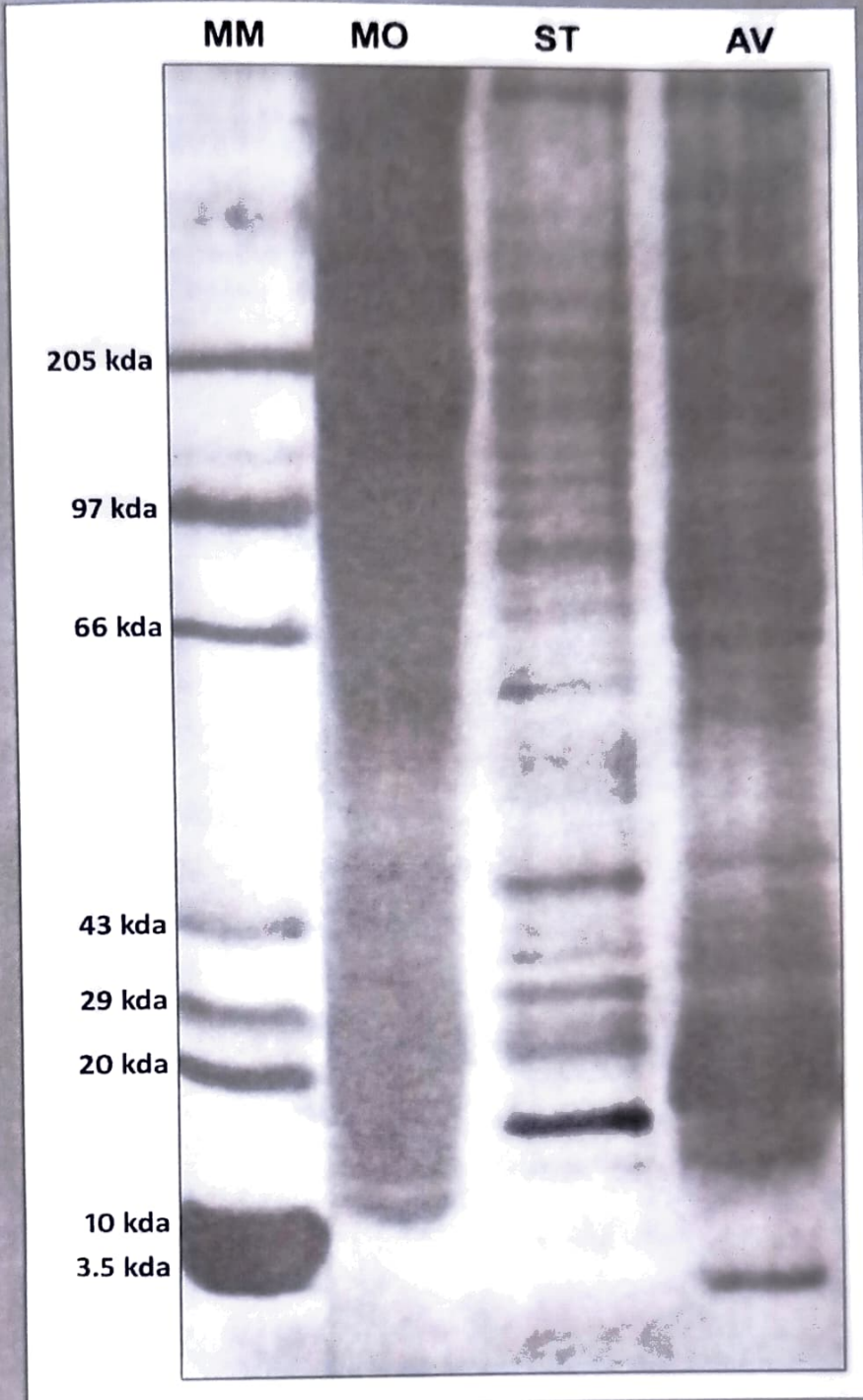
Lane-1: Standard Molecular weight Marker (MM)
Lane-2: MO (*Moniezia*)
Lane-3: ST (*Stilesia*)
Lane-4: AV (*Avitellina*)

(See coloured view on Plate-I)

RESULTS AND DISCUSSION

In the present study, for investigation into the molecular heterogeneity in three cestode parasites, their proteins were subjected to SDS-PAGE and result exposed that the parasite protein molecules were separated into different fractions according to their molecular weights. The variation in the protein profile of different parasites is shown in the figure below.

It was found that the most molecular weight of different polypeptides of cestodes ranged from below 3 kda to above 205 kda, but the majority of bands have been observed among the range of molecular marker and very few bands observed in above high molecular protein bands 205 kda.

In the present study the cestode parasites i.e. *Moniezia* sp., *Stilesia* sp. and *Avitellina* sp. were studied using SDS-PAGE and the pattern of the parasites' proteins is shown in the photoplate. The *Moniezia* sp. contents showed broad range of proteins between 10 kda to 205 kda and beyond. *Stilesia* sp. showed protein bands from 10 kda to 205 kda and beyond the 205 kda. In *Avitellina* sp. the banding pattern in the gel was obtained between 3 kda to 205 kda and beyond. These parasites showed different molecular weight of proteins because all the parasites were of different genus. Similar results were obtained by Ashour *et al.,* (1995) in their comparative study by SDS PAGE of four nematode species *T. vitulorum, T. leonina, T. canis,* and *P. equorum* in which most of the bands were common among the four species, but certain unique bands were also found.

The results of the present study clearly show the prominent variation in the protein profile of different cestode parasites under study. Analyses of data at the same time also reflects their differences and shows an overall similarity among the different cestodes. The cestodes i.e. *Moniezia, Stilesia* and *Avitellina* represent the same order Anoplocephalidea but two different families. The comparative qualitative analysis showed that the cestode parasites *Stilesia* sp. had low concentration of proteins than the other cestodes of *Moniezia* sp. and *Avitellina* sp. These results shows that habitat may not be the only factor which influence the polypeptide profile of these intestinal parasites but there must be some genetic factors which regulate the similarities and diversities in the closely related taxonomic genera.

Similar result were obtained by Osikovski, *et al.* (1978) who differentiated three species of the genus *Paramphistomum* viz., *P. microbothrium, P. cervi* and *P. microbothrides* with the help of their protein profile and proposed that SDH electrophoresis can serve as a most powerful tool for biochemical taxonomy. Yoshimura *et. al.* (1970) also used protein profile to correctly identify the

morphologically similar parasite species and also to synonymize several controversial species with weak taxonomic descriptions.

Recently Sulima *et al.* (2018) found out in comparative SDS Page electrophoresis study, the protein similarities and differences between the cysticercoid larva and adult *Hymenolepis diminuta* stages.

According to Bien *et al.* (2012), specific antibodies against *T. britovi* were found while the specific antibodies against *T. spiralis* from infected mice.

Kumartilake and Thompson (1979) have also proposed that the genetic constitution of parasites is reflected in their characteristic protein profiles. According to Ferguson (1980) the genetic composition of a population will change gradually over the generations due to mutation, over production of offsprings, and natural selection; and only those are selected which are best adapted to the conditions of the macro or micro habitats.

The present study (SDS-PAGE) concluded that the cestode parasites are not only quantitatively different but they also show some qualitative differences. On the basis of earlier studies (Yoshimura, 1968, 1969; Bylund and Djupsund, 1977; Osikovski, *et al.*, 1978 and Kumartilake and Thompson, 1979) as well as the results of the present investigation, it can be concluded that protein polymorphism can be used as a valid parameter for generic, specific and intra-specific characterization of helminth parasites.

ACKNOWLEDGMENTS

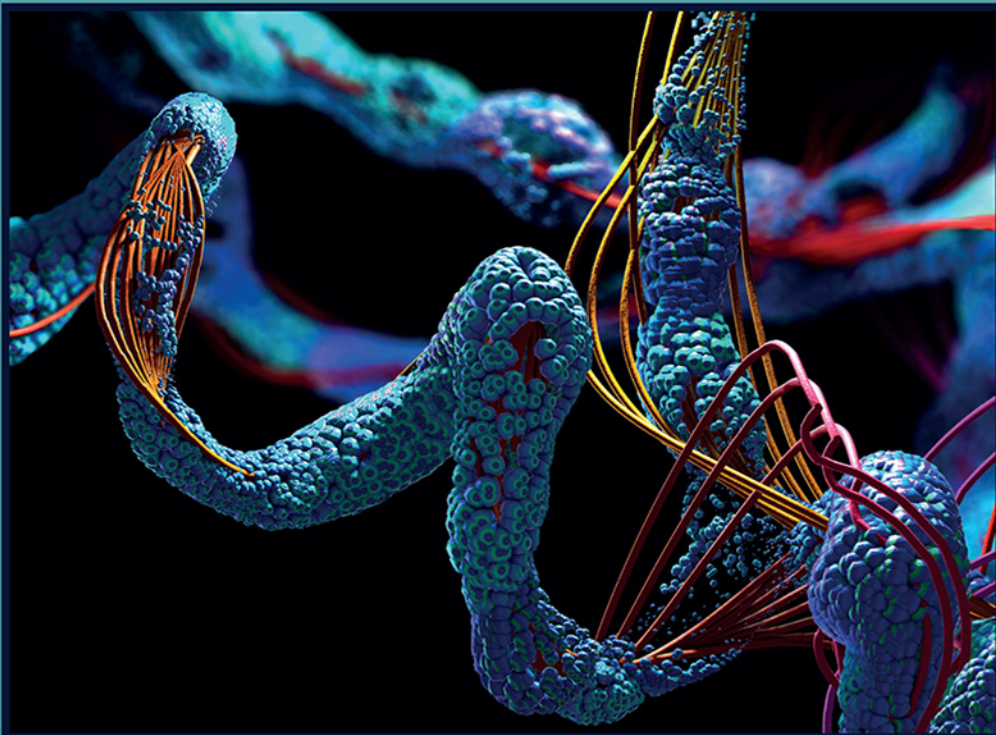## REFERENCES

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PROTEIN-BASED BIOPOLYMERS

FROM SOURCE TO BIOMEDICAL APPLICATIONS

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CHAPTER 1

An introduction to protein-based biopolymers

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1.1 Introduction

Since the inception of time, polymers are a vital and unavoidable part of our daily life. Polymers are bulky molecules encompassing distinct monomers linked together to form elongated chains. Monomers are simple building blocks whereas complex building blocks are considered as "repeat units" (Fig. 1.1). In general, polymerization is a process where monomers are treated either chemically or biologically to form polymers. Homopolymer is obtained from only one type of monomer whereas copolymer results from distinct monomers. They can be either linear or branched.

Broadly, there are three ways to get biopolymers: from (1) biomass, (2) microorganisms, and (3) synthesizing bioderived monomers (Choi et al., 2018a, 2018b). Moreover, starting materials such as sugars, amino acids, natural fats or oils can be considered as monomers of biopolymers like polysaccharides, proteins, and lipids, respectively (Choi et al., 2018a, 2018b). With the advent of modern technology in chemistry and material science, the properties of biopolymers can be tuned by undergoing reinforcement to encounter specific requirements.

Some biopolymers like proteins and nucleic acids are considered a carrier of bio-information whereas polysaccharides are the energy source for cell activity and are commonly known as sugar family biopolymers. Biopolymers are biodegradable and have numerous applications in soil strengthening, drug delivery, food packaging, tissue engineering, composites, and many other structural materials. Although some biopolymers are

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† Author deceased

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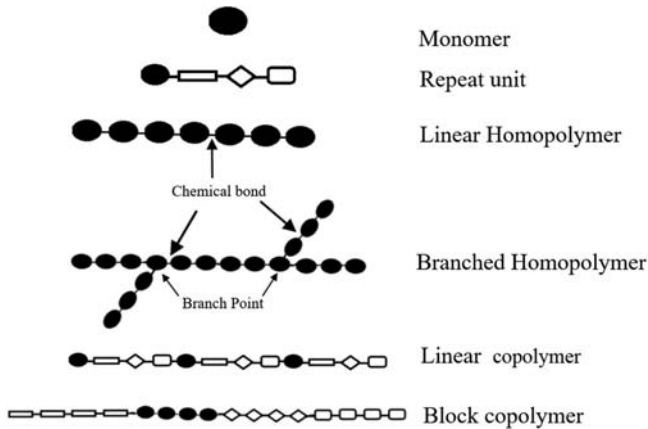
Figure 1.1 Fundamental structural features of polymers..

in the developing phase, nearly few find replacements with synthetic materials whereas others can have unique properties which open up commercial breaks. Mostly, well-recognized firms such as agriculture, chemical, and also biotechnology ventures are constantly searching for new novel biopolymer compounds. Furthermore, some biopolymers are preferable to conventional polymers mainly due to environmental features. Also, much work needs to be done to overcome commercialization hurdles, as many biopolymers are in the developmental stage.

Among these biopolymers, proteins are the most challenging and versatile to have numerous possibilities in many fields of applications. PBB can be obtained from plants and animals, and also derived from microorganisms as shown in Fig. 1.2 (Choi et al., 2018a, 2018b). Proteins, commonly known as polypeptides, are complex copolymers made up of amino acids (20 different amino acids as shown in Fig. 1.3 as starting material units or building blocks (Belitz et al., 2009). Protein or polypeptide is considered as protein-based biopolymer (PBB) has amino acid as a monomeric unit, which is connected by amide bonds ($-NHCO-$) obtained by the reaction of carboxylic acid ($-COOH$) group of one amino acid and of amine ($-NH_2$) group of another amino acid. Based on their side groups amino acids can be cationic, anionic, aromatic, polar, and nonpolar [(Silva et al., 2014)]. Proteins or PBB can account for nearly 50% of the dry weight of cells, and on average has 12%−19% nitrogen, 20%−23% oxygen, 6%−7% hydrogen, 50%−55% carbon, 0.2%−3% sulfur, and traces of phosphorus on an elemental basis (Silva et al., 2014). Recently, it is reported that numerous polypeptides which are similar to
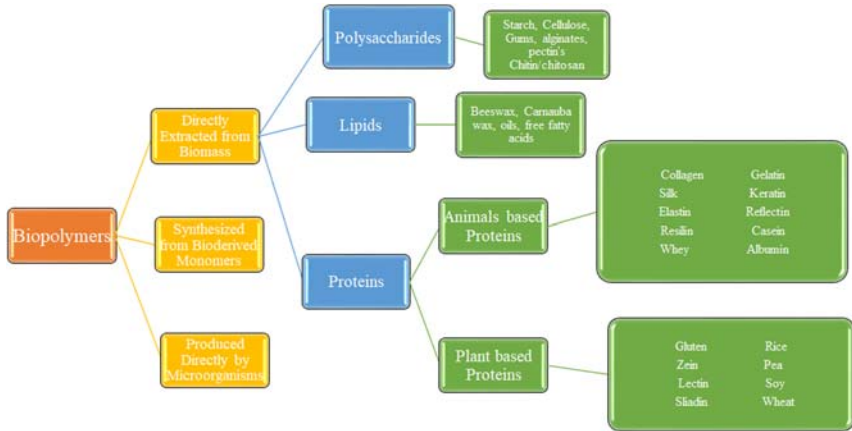
Figure 1.2 Types and origins of the biopolymers.
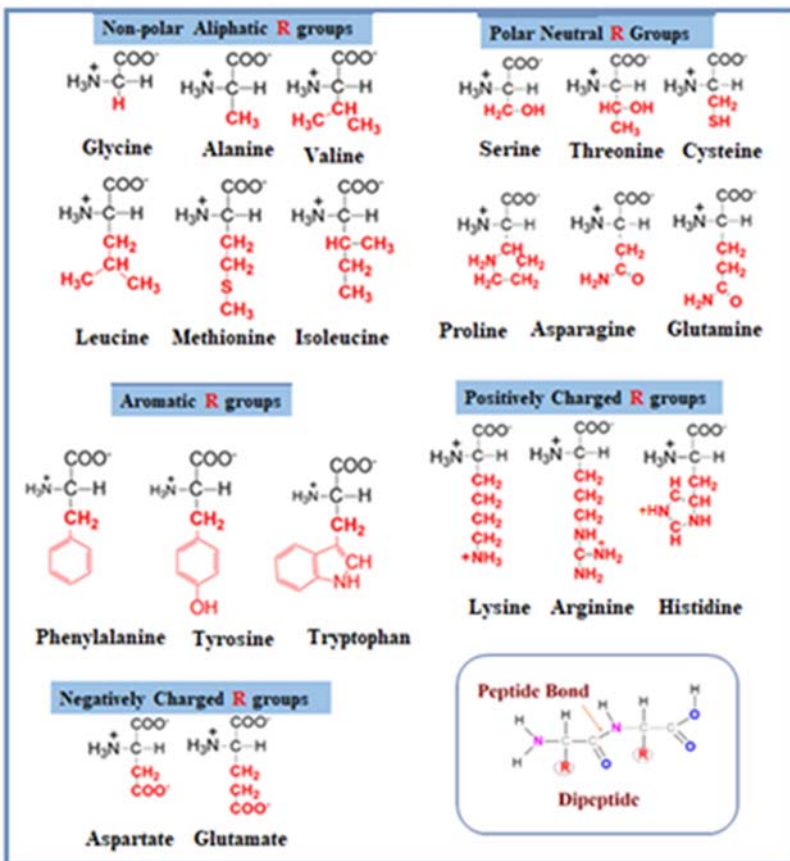
Figure 1.3 Peptide bond, dipeptide and types of amino acids based on their R groups.

natural proteins found in shells or bones have been synthesized. Generally, aspartic acid is used as the amino acid precursor for the synthesis of polypeptides. An amino acid is starting material of proteins or PBB that can be fabricated into composites, films, sponges, hydrogels, microspheres, tubes, and fibers by various solvent processing methods which can have a wide range of applications.

1.2 Protein and its biopolymers

1.2.1 Structure and properties of proteins

Currently, the most active and challenging area of research is to understand the relationship between polymer structure and its physical properties. Protein has four levels of structural forms (Hardy et al., 2008; Silva et al., 2014; Zhang & Zeng, 2008) such as primary (1°), secondary (2°), tertiary (3°), and quaternary structures (4°). The primary structure is considered as amino acid sequence (AAS), whereas the secondary structure is the conformational relationship of amino acids with each other. There are two common and stable conformational arrangements of secondary structure, either α–helix, and the β-sheet, which are confirmed with the help of the XRD analysis study. The tertiary structure is obtained by folding protein chains and the physical properties, as well as biological functions, depending on the way protein chains, get folded. There are two classes of tertiary protein structures such as fibrous and globular. Globular proteins are nearly spherical, soluble, or form colloidal dispersions in water whereas fibrous proteins are elongated filaments or chains and are insoluble in water. Well, a known example of fibrous protein is keratin found in hair and wool, which is composed of coiled alpha–helical protein chains with other coil analogs. On the other hand, globular proteins may clump into a shape of a globe which have the same arrangement of types and structures. Commonly known examples of globular proteins include insulin, hemoglobin, and most enzymes. Moreover, the quaternary structure of the protein is obtained by the style of organization of assemblies of two or more protein chains (Hardy et al., 2008; Silva et al., 2014). These complex structures of proteins via noncovalent bond interactions can get affected by some constraints like solvents, pH, and temperature. These physicochemical parameters can make changes in quaternary, tertiary, and secondary protein structures and result in protein denaturation. In addition, the next property of protein structure is an isoelectric point, which is related to the basic and acidic groups present in their constituent amino

acids. At an isoelectric point, there is no net charge on the protein, which means the negative charge and positive charge due to residues gets nullified(Hardy et al., 2008; Zhang & Zeng, 2008). Basically, at the isoelectric point, proteins get aggregation either above or below the pH of the isoelectric point. At such conditions, the protein has either a net positive or negative charge which enables the denaturation, commonly known as the opening of the proteins (Silva et al., 2014). Here, some of these PBBs are discussed individually by considering their origin and class.

1.2.2 Origin and types

1.2.2.1 Collagen

Collagen PBB (C-PBB) is fibrous PBB and present in ample amounts in mammals. Structurally, it is the main constituent of extracellular matrices (ECM) and has Glycine (Gly) which is present in often repeating three long chains of peptides. In addition, glycine-proline-hydroxyproline (Gly-Pro-Hyp) is a common tripeptide unit of collagen. This tripeptide unit forms one helical turn which resulted due to Gly and Hyp intramolecular hydrogen bonds (Lee et al., 2001; Zeugolis et al., 2008). Nearly, ~ 1400 amino acid residues are present in that Gly as a repeating sequence. It can promote cell proliferation and differentiation and has good biocompatibility, cell adhesion as being a structural protein (SP). The connective tissue of mammals is mostly made up of collagen which is the most abundant protein found in mammals. In total body protein, collagen constitutes 25%—35% share, which inside the body forms collagen fibrils resulted in the fiber of collagen (Di Lullo et al., 2002). It can offer support to tissues and is considered the major component of ECM. It also possesses high tensile strength and presents abundantly in fascia, tendons, cartilage, ligaments, bone, and skin.

Collagen, a PBB for biomedical application which has excellent biocompatibility, good bio–absorbability, and immunogenicity is also negligible. It is also a nontoxic biomaterial (Thumann et al., 2009). C-PBB chemically converts into gelatin by partial hydrolysis in mild reaction parameters which resulted in the separation of globular, three strands, and random coils. In comparison to C-PBB, gelatin displays low antigenicity.

For the last many years, researchers have assessed C-PBB for many applications like in drug delivery, in bone and cartilage constructs, wound healing, vascular diseases, neural, urogenital, skin, and ocular. Hydroxyapatite(Ciardelli et al., 2010; Dubey & Tomar, 2009; Susan et al., 2009) or brushite (Jayaraman & Subramanian, 2002; Tamimi et al., 2008)

is used as a cross-linking agent in conjunction with collagen-based porous scaffolds in an osteochondral defect. However, some researchers display that few numbers of autologous chondrocytes grow on type I and type II collagen without any important difference (Glattauer et al., 2010; Tebb et al., 2006). Also, C–PBB has been confirmed propitious protein–based biopolymer material (PBBM), as a replacement scaffold for a very complex structure like the meniscus in optimal condition (Glattauer et al., 2010; Tebb et al., 2006).

C–PBB can be used as a biomaterial in vascular diseases. There are some irregularities and improper functioning of the heart and atherosclerosis observed in vascular diseases. Currently, due to the complex structure of heart tissue engineering application limited up to colonization of acellular matrix and implantation of some specific part (Eitan et al., 2010; Tedder et al., 2009).

Commonly, xenogeneic heart valves are used frequently, but due to their tendency of getting calcified and immunogenic nature, it is not considered concrete treatment(Van Nooten et al., 2009). In the field of regenerative medicine, heart decellularization (Ott et al., 2008), production of heart valves and veins (Teebken et al., 2009) would lead the advancement based on C–PBB for cardiovascular disease. It can be used in the treatment of skin and ocular type problems and also used in wound and ulcer treatment triggered due to burning.

Many C–PBB substitutes like Alloderm, Integra are commercially offered and also developed tissue-engineered skin by using collagen with melanocytes models (Régnier et al., 1997; Regnier, 1983). C–PBB with stem cell is the best combination that can treat cornea defects. Moreover, collagen scaffolds were widely inspected in the last decade for damaged cornea treatment by delivering limbal epithelial cells (Dravida et al., 2008; Grueterich et al., 2003; Levis & Daniels, 2009; Schwab, 1999; Shortt et al., 2009; Zakaria et al., 2010). For the treatment of urogenital complications, collagen scaffolds can be used.

C–PBB composite with the Patient's urothelial cells is a promising way for augmentation bladder (Atala et al., 2006; Bouhout et al., 2010; Magnan et al., 2006). A collagen–based biomaterial is a very good nerve guide as well as auspicious in the field of delivery systems. Also, C–PBB and collagen composite can be used in the reconstruction of the abdominal wall (Ansaloni et al., 2007; Bellows et al., 2008; Liyanage et al., 2006), in ulcer treatment (Sun et al., 2010) and have shown a great delivery potential.

1.2.2.2 Gelatin

Gelatin is a PBB that originated from animals and can be used in osteo-porosis, osteoarthritis, brittle nails, obesity, skin aging, and many other conditions and uses. Moreover, gelatin PBB (G–PBB) is a common ingredient in sauces, marshmallows, gummy candies, soups, broths, cosmetics, and medications. G–PBB can be produced from the partial degradation of collagen. Basically, due to different degrees of hydrolysis of collagen gives gelatin of different molecular mass nearly 65,000 & 300,000 g/mol. In general, G–PBB includes ingredients like proline, 4–hydroxyproline, and glycine which also varied from source to source. The contents available in gelatin obtained from pigskin are proline (13%), 4-hydroxyproline (9%), and glycine (33%) (Ta, 2007a, 2007b). G–PBB can be considered as notable starting material for the preparation of bio–decomposable films because of its low cost, wide existence, biodegradability, biocompatibility, and good film–forming properties, (Jongjareonrak et al., 2006).

G–PBB can be used for the preparation of films by casting technique, either by cold-casting and by hot-casting. The cold-casting films prepared in less than or at room temperature, have a spiral structure whereas hot-casting films prepared at more than 35°C, have a statistical coil structure. In addition, the coil structured hot–casting films are more brittle than the former ones (Fraga & Williams, 1985; Menegalli et al., 1999) G–PBB films obtained from these three different methods are different in thickness, ranging from 357 to 55 m, and also very thinner films obtained by casting. The films prepared by casting have high tensile strength whereas the films obtained by the extrusion method are extendable and stronger (Andreuccetti et al., 2012). The obtained G–PBB films are oxygen impermeable, transparent, and more reversible and are used for the edible film's production (Ta, 2007a, 2007b) because it has melting point very close to body temperature. However, G–PBB edible films may possess antioxidant, antimicrobial properties and have low oxygen permeability by adding some agents like carvacrol, citrus essential oils and so on (Ahmad et al., 2012; Kavoosi et al., 2013; Tongnuanchan et al., 2012).

1.2.2.3 Keratin

Keratin PBB (K–PBB) is a fibrous as well as a SP, which is tough and insoluble. Architecturally, it can form structures like hooves, nails, hair, feathers, and horn of many animals. The formation of K–PBB is started from fibrous monomers, which are initiated by keratinocytes, as special

cells. An intermediate filament is obtained from these fibrous monomers by the action of twisting and wrapping around each other. It is found after keratin analysis that it has an extraordinarily high percentage of cysteine, which is ranging from 7%−20% of total amino acids. Cysteine is a sulfur-containing amino acid that is known to form inter and intramolecular disulfide bonds which allow flexibility and persistent amino acid property to the tissue (Dowling, Crewther, Inglis, et al., 1986; Dowling, Crewther, Parry, et al., 1986; Katoh, Tanabe, et al., 2004). Structurally, α-keratin is helical in structure which upon stretching to form β-keratin. This structural transformation leads to change in the thermal, mechanochemical properties of the material (Pauling & Corey, 1953). K-PBB can be classified into hard and soft based on its regulations and functions. Tough structure, hard keratin obtained by compact and ordered arrays of intermediate filaments (IFs) which are embedded in a matrix of proteins where cysteine is in abundance. On the other hand, soft keratin formed by cytoplasmic IFs are loosely packed and offered mechanical flexibility to the epithelial cells (Coulombe et al., 2000; Fraser et al., 1986; Moll et al., 1982).

Although, keratin obtained from a natural source such as wool is nonsoluble but which on reductive treatment or stretching that can get soluble keratin. The chemical extraction of K-PBB is possible with mercaptan, sodium dodecyl sulfate, and aqueous urea and can make biodegradable scaffolds and films (Katoh, Shibayama, et al., 2004; Katoh, Tanabe, et al., 2004; Yamauchi et al., 1996). K-PBB is used to make three-dimensional scaffolds and films but the scaffolds are very fragile and cannot be used singly for a biomedical application like tissue engineering.

Till very less research done on K-PBB but it is found very propitious PBBM for biomedical applications and can be used in drug delivery, regeneration or nerve guiding wound healing, and ocular implants. K-PBB film developed with a softening agent such as 1%−3% glycerol, used for the reconstruction of the ocular surface. It is then confirmed by testing it in vitro and has a promising result with good bioactivity and mechanostability (Reichl et al., 2011). It has been found that keratin-PBB can also be used in nerve repairing. In addition, Hill et al. reported the repairing of rabbit's peripheral nerves by the use of hydrogel scaffolds of keratin. Even though these scaffolds were not that useful to repair the nerve channel, but briefly it shows better results in terms of conduction delay compared to empty nerve channel and autograft (Hill et al., 2011). Moreover, it has also been verified the competence in enhancing the nerve guiding

and neuro-inductivity in peripheral nerve injury model of mouse (Apel et al., 2008; Sierpinski et al., 2008). K-PBB is bioactive, biocompatible, and biodegradable and has sequences for cell adhesion such as Arg-Gly-Asp and Leu-Asp-Val (Tachibana et al., 2002, 2006).

1.2.2.4 Fibrin

Fibrin is a fibrillar PBB, which is insoluble and produced to stop bleeding for blood clotting. It is obtained from a soluble fibrinogen protein which is found in blood plasma, secreted by the liver. Fibrin PBB (F-PBB) is fibrous can be used for drug delivery and tissue engineering due to its cross-linking properties (Bootle-Wilbraham et al., 2001; Jegoux et al., 2005; Le Guehennec et al., 2005; Yamada et al., 2003). The activated factor III is responsible for cross-linking in fibrin, which catalyzes the formation of Υ-Υ dimers by catalyzing the lysyl-Glu covalent bond formation (Ehrbar et al., 2007; Ju et al., 2007). F-PBB and its degraded product can promote cell attachment and cell proliferation. Along with all these qualities, fibrin has certain drawbacks like instability, low mechanics, and fibrinolysis.

F-PBB is very important for many biomedical applications such as cell adhesion, differentiation, and proliferation and is also used in repairing neural injuries and cartilages (Ju et al., 2007; Willerth et al., 2006). F-PBB and its fiber-based scaffolds are used as a cell carrier for cardiovascular tissue engineering and also tested its effects on ECM by seeding the cell (Mol et al., 2005). F-PBB is used to design artificial kidneys, precisely to fabricate renal proximal tubule, which was also verified in vitro and found efficient toward the regular functioning of the normal kidney such as correct polarization for the absorption of desired solutes from the glomerular filtrate (Ng et al., 2013). Moreover, composite scaffolds (CSF) of fibrin and thrombin prepared to apply in bone tissue engineering, CSF found suitable and efficient which has a high concentration of fibrin and low thrombin (Karp et al., 2004). Later on, CSF of gelatin, fibronectin, and growth factors with F-PBB was prepared and experienced steady cell proliferation as well as good cell proliferation. Also found that these CSF in vitro does not allow the de-differentiation of endothelial cells (Wang et al., 2010).

1.2.2.5 Silk fibroin

Silk Fibroin PBB (SF-PBB) is obtained from insects like silkworm Bombyx mori (Vepari & Kaplan, 2007). This fibrous PBB is similar to

keratin and collagen and is also produced by spiders and scorpions. Here, in different species beta-sheet structures are similar but variations are observed in physical properties and AAS. SF-PBB has a structural role in the formation of traps, cocoons, safety lines, and the protection of eggs due to its periodic protein sequences. Also, SF-PBB has some good properties like stability, biocompatibility, mechanical strength, ease in processing, biodegradability, flexibility in morphology, and low toxicity (Numata et al., 2010, 2011) which can be helpful in biomedical applications like drug delivery, tissue engineering, and regenerative medicine (Holland et al., 2019; Numata & Kaplan, 2010). In addition, SF-PBB fibers can be used as sutures. Also, some silks are with an arginine-glycine-aspartic acid sequence, which helps in cell adhesion and allows them as useful biomaterials (Gupta et al., 2016; Mandal et al., 2010; Numata et al., 2015). The alanine/glycine copolymer composition (Ageitos et al., 2016) is responsible for the crystal region and pyrolysis behavior of the silk, which resulted due to major secondary structures (Malay et al., 2016). From this correlation, it has been very clear that the physical properties of the material may change with the partial modifications in the polypeptide sequences of silk proteins. Silk Fibroin scaffold (SFS) is highly porous and their connectivity is also quite good. The observed dimension of the pore is between $100-1000$ μm in size and has more than 90% porosity. SFS has characteristically better biocompatibility, biodegradability, and mechanical properties (Hofmann et al., 2007; Kim, Kim, et al., 2005; Kim, Park, et al., 2005; Wang et al., 2008) and the nanosized SFS possesses very close similarity to ECM.

Moreover, it can up-regulate the integrin-β1 expression for cell adhesion, which has been resulted in the low processing conditions that can help in drug delivery (Bondar et al., 2008). In addition, Y. Gotoh et al. reported that the scaffold made from silk fibroin and lactose is used for the hepatocyte cells adhesion and is found similar to the collagen-based scaffolds (CBS) for the same. Morphologically, it was found different from collagen (Gotoh et al., 2004). A combined knitted SFS and microporous silk sponge prepared by H. Liu et al. for tissue engineering. They reported better cell adherence, cellular and growing functions in comparison to knitted SFS Liu(Liu et al., 2008).

1.2.2.6 Elastin

Elastin mainly presents in vascular, connective, and load-bearing tissues and consists of proline, valine, glycine, and alanine residues with ~ 66 kDa molecular mass. Elastin has good mechano-elastic properties

and can observe it in rat skin at small stress and deformations(Oxlund et al., 1988). However, it has resilience, elastic modulus, extensibility, strength and toughness are 90%, 0.0011 GPa, 150%, 0.002 GPa, and 1.6 MJm-3 respectively (Aaron & Gosline, 1981). Therefore, elastin PBB is considered a significant SP as well as material of scaffolds (MSF). Moreover, a general problem with this SP is that it is difficult to process because of its poor solubility in water. Elastin PBB is neither easy to synthesize, obtain and produce in large quantities nor having many reports on biomaterials composed of elastin, compared with other SPs. It has been reported that MSF of elastin obtained from bovine ligament used to study its mechanical properties, focusing on stretchy modulus (Kirkpatrick et al., 2003). It is reported that elastin is considered a useful SP and an effective additive in hybrid composites which offers unique properties (Berglund et al., 2004).

1.2.2.7 Resilin

Resilin is a less soluble SP found in biological structures which require long-range elasticity and energy storage. Resilin PBB (R–PBB) is an elastic material like rubber, thermally stable up to ∼140°C, and possesses 300%−400% extendibility (Tatham & Shewry, 2002). Resilin is composed of tyrosine residues which form covalent cross-linking and achieve ∼92% resilience (Gosline et al., 2002). Although many properties of resilin and elastin resemble each other, resilin has a lower modulus and higher resilience. It is reported that resilin can be obtained from vein joints of dragonfly wings (Yazawa et al., 2018).

It has been stated that natural resilin is not commonly accessible for biological and physical analysis. Genetic engineering or recombinant DNA technology (RDT) or Gene cloning provides new routes to the un-imagined modifications in many areas such as Bt-cotton, other genetically modified crops. Moreover, the Drosophila gene is partially cloned and expressed to identify and encode resilin (Elvin et al., 2005). R–PBB could be obtained by photochemical cross-linking. Peroxidase is used to achieve this cross-linking, which catalyzes di-tyrosine formation. The RDT resilin proteins were expressed and assessed as biomaterials (Qin et al., 2009, 2011, 2012). Moreover, it is also inspected that whether the combinations of resilin will work or not, though it has attractive physical properties (Cao & Li, 2007; Lv et al., 2010). Elastomer, a composed hybrid obtained from resilin by photochemical or chemical cross-linking and can be considered as muscle-mimetic

biomaterials. Apart from this, work on resilin is found limited as compared to elastin which is useful as an elastomer in many reports.

1.2.2.8 Reflectin

Reflectin PBB (Rf-PBB) mostly constitutes aromatic and sulfur-containing amino acids. It is a recently identified protein family, originated in certain cephalopods (squid) including Euprymna scolopes and Doryteuthis opalescens (Crookes et al., 2004). Although the basic structure of reflectin is deduced, the exact molecular structure is up till now not known. It is presumed that the reflectin is created from a type of transposon or jumping genes, which is a DNA sequence. This jumping gene can change positions within genetic material by encoding an enzyme. Squid is a type of cephalopod that can change body color to disguise itself. It is going to perform to mimic its surrounding, by the use of reflectin. Researchers are keen to know the exact molecular mechanism and have very limited research work on Rf-PBB. Although, the structure of reflectin is not fully elucidated it is anticipated to have beta casks and random coil (Kramer et al., 2007).

It is recently reported that the camouflage function can be contributed by proteins and their families which comprises pigment cells either cephalopod or rainbow cells and white pigment vesicles. Mechanistically, Rf-PBB can refract incident light in certain areas of cephalopods due to aromatic and sulfur-containing structural amino acids (DeMartini et al., 2015; Naughton et al., 2016). A horizontal gene transmission was observed in Aliivibrio fischeri, which is a marine luminescent bacterium that possesses the reflectin gene(Guan et al., 2017). Currently, many material researchers have been interested in Rf-PBB considering it as a new functional PBB, and also reported recombinant reflectin as novel optical biomaterials(Qin et al., 2013).

1.2.2.9 Casein

Milk is the main source of Casein, constitutes nearly 80% of overall milk proteins. In this casein-PBB (C-PBB), which is commonly known as phosphoprotein, has a huge amount of proline but no cysteine. The absence of disulfide bonds for cross-linking resulted in less conformational secondary as well as foldable tertiary structures. It is sparingly soluble in water and forms a colloidal suspension in the milk due to its hydrophobic property. However, it is used in the manufacturing of food additives, protective coatings, adhesives, binders, and fabrics

(Abu Diak et al., 2007; Somanathan et al., 2000). It is considered the most desirable PBB for tissue engineering purposes due to stability, ease of availability, and inexpensiveness. Also, it is used as a drug delivery vehicle. Apart from this, it is used as a biomaterial for biomedical applications in a very limited or specific area in comparison to collagen, silk fibroin, keratin, and fibrin.

Moreover, very few researchers have done work on bone or cartilage defects. Among them, Ritzoulis et al. verified the use of caseinate composite scaffolds and hydroxyapatite in tissue engineering (Ritzoulis et al., 2005). In addition, for controlled delivery of drugs, F. Song et al. demonstrated the use of casein hydrogel in which genipin is used as a cross-linking agent. It is observed that both gelling time and the strength of hydrogel increases with increasing genipin concentration. It is seen that variable temperature also made an effect on gelling. Moreover, it is reported that in gastrointestinal conditions genipin can stimulate protein or drug release (Song et al., 2009).

1.2.2.10 Whey

Whey is a kind of milk protein, obtained from milk as a byproduct during cheese manufacturing. Also, whey PBB (W-PBB) is a globular obtained after the curdling of milk as a liquid residue. In cow's 20% of proteins are W-PBB and 80% are casein PBB (C-PBB) whereas in human milk nearly 70% and 30% are W-PBB and C-PBB present, respectively (Hoffman & Falvo, 2004; Luhovyy et al., 2007). Moreover, W-PBB is composed of immunoglobulins, α–lactalbumin, protease peptones, β–lactoglobulin, and serum albumin and all their natural forms are soluble, irrespective of pH (Farrell et al., 2004). In addition, the amino acid cysteine present in W-PBB is used as a starting material for the synthesis of glutathione in the body which is universally known as a cellular antioxidant. Also, W-PBB and its components proposed to decrease the risk of cancer in animals and considered a new route for the forthcoming medical research (Parodi, 2007).

Here, W-PBB films can be prepared from whey protein isolates (WPI) and their concentrates (WPC). These two different materials, WPC and WPI are abundant in methionine, cysteine, and sulfur-containing amino acids and constitute at least 90% and 50%—80% protein contents, respectively (Jauregi & Welderufael, 2010). In the film formation process, W-PBB in aqueous solution undergoes thermal denaturation and the 3-D structure of W-PBB can be modified by heating, and also upon drying, which has promoted the

hydrophobic and intermolecular S-S bonding (McHugh & Krochta, 1994; Shimada & Cheftel, 1989). Generally, the W-PBB solution with a concentration of 8%–12% is heated at 75°C–100°C to obtain plasticized WPI films (Mchugh et al., 1994). Moreover, the WPI solution with a lower concentration such as 5% (w/w) can be used for whey-based film preparation (Pérez-Gago & Krochta, 1999). W-PBB undergo irreversible thermal denaturation and obtained films have a consistent structure (Lent et al., 1998). The film-forming conditions of WPC can be optimized by altering the denaturing temperature and pH of the film-forming solution. For example, for the uniform film formation, the pH of the solution used for film-forming was set at 6.6 by using 2M NaOH aqueous solution and also heating temperature, time were controlled at 75°C and 30 min, respectively (Banerjee & Chen, 1995).

In addition, some other methods such as ultrasounds (US), ultraviolet (UV) radiation, and alkalization can also improve the properties of the whey-based films (WBF). Upon UV treatment, the WBF undergoes significant improvements in its mechanical properties and color properties (Díaz et al., 2016). Also, pH acts a very important role in influencing the properties of WBF. Mostly, alkaline pH promotes protein unfolding, denaturation, and solubilization (Bourtoom et al., 2006). It is reported that the WPI films obtained in the strong alkaline conditions are more useful in food packing as compared to the WPI films obtained through heating (Quinn et al., 2003). It is also observed that the films obtained after UV radiation and 7 or 9 pH are strong with improved puncture resistance compare to the films obtained at higher 11 pH because of aggregation and denaturation (Díaz et al., 2017). In addition, the US can also make stronger WBF with more exposure time (Banerjee et al., 1996). The WBF made with plasticizers can act as an excellent barrier for oxygen, aroma, and oil, also they are flexible, transparent, and bland. They could work as poor moisture barriers due to their hydrophilic nature which can be enhanced by the addition of lipid materials (fats & oils). Commonly, lipid materials used for improved moisture barrier were waxes (Janjarasskul et al., 2014; Pérez-Gago & Krochta, 2001; Soazo et al., 2013), plant oils (Javanmard & Golestan, 2008; Kokoszka et al., 2010; Shaw et al., 2002), fatty acids (Fernández et al., 2007), and acetylated monoglycerides (Anker et al., 2002).

1.2.2.11 Albumin

Albumin is a globular protein and undergoes coagulation when exposed to heat. It is water-soluble and available majorly in egg white, blood serum, milk, also it can be found in several other plant and animal tissues. It is the most abundant serum protein present in blood plasma, which constitutes about 55%

of total blood plasma protein. Albumin PBB (A-PBB) used their microspheres in pharmaceuticals which vary from nanometer to micrometers. Rhodes et al. prepared albumin microspheres (AMS) with 5−15 μm size for the first time. These AMS have ϒ radiation sources for the determination of irregularities in the pulmonary circulation (Rhodes et al., 1969; Zolle et al., 1970). Although, these AMS have been used mostly for cell culture and drug delivery to various organs and tissues (Gan et al., 2009; Iemma et al., 2006) but not that much used for cartilage and bone tissue engineering like casein. In addition, S. R. Lyu et al. verified the use of an albumin-based scaffold (ABS) for the promotion of neocartilage formation. Here, they have used a matrix of chitosan, polyethylene oxide, and chitin with ABS and undergone seeding and cultivation of the porcine knee chondrocytes. The amount of ABS improves cell adherence but does not show an effect on porcine cell viability (Lyu et al., 2012).

Albumin and its nano-size micelles are used as the carrier for drug delivery to treat several diseases. For the treatment of cancer, Y. Wu et al. established AMS as a drug delivery vehicle by loading it with the cancerous drug. They loaded doxorubicin (DOX) an anticancerous drug on micelles made up from polycationic albumin precursor protein cBSA-147. These micelles are stable in a wide range of pH and different physiological buffers. It has been reported that their uptake into A549 cells was very well after 1 h period of incubation. It is also observed that there is five times more cytotoxicity in comparison to free DOX. Also found that the intracellular drug release was very efficient (Wu et al., 2012). Initially, it is assumed that the bone defect can be overcome by mineralized scaffold, as they will grow mesenchymal stem cells (MSC). Later on, it is confirmed that was not a sure treatment. Moreover, Weszl et al. used human MSC which are isolated from dental pulp or bone marrow with preseeded scaffolds of freeze-dried human or bovine graft or hydroxyapatite. Further, it is observed that the cells are less productive under standard culture conditions whereas it improves the efficiency after coating it with collagen and fibronectin but they are still not sufficient. Here, they used human albumin coating which has provided potency to both seeding and proliferation. And, it is seen that human albumin coating does not affect the mechanical property of the scaffolds (Weszl et al., 2012).

1.2.2.12 Zein

Zein is a PBB of prolamin plant storage protein (PSP), found in corn which has nearly 40%−50% of the total endospermic proteins. It is soluble

in the alcohol–water mixture (60%−95%) and this property resulted due to its composition of amino acids. Zein PBB (Z-PBB) comprises an enormous amount of hydrophobic charge-free or neutral amino acids, mostly constitutes amino acids such as alanine (10%), leucine (20%), and proline (10%). It is a heterogeneous combination of amino acids, has a molecular mass of 44 KDa, which are connected by disulfide bonds. It incorporates nearly 15% β-sheets 50%−60% α-helix, and residual nonperiodic molecules (Lai et al., 1999).

It is not only used as inks, adhesive, chewing gum, and biodegradable plastic but also used as a carrier of DNA for gene delivery, drug delivery vehicle, and as scaffolds for tissue engineering (Lai et al., 1999; Shukla & Cheryan, 2001). Moreover, J. Tu et al. in 2009 demonstrated bone formation by using zein-based scaffold with rabbit mesenchymal stem cells (Tu et al., 2009). Karthikeyan et al. in 2011 verified that zein can be used as a drug carrier. Here, they loaded zein based microsphere, prepared by emulsification and solvent evaporation method with Aceclofenac. In vitro, these microspheres were found stable in gastric pH. This gastric pH condition is suitable for the slow release of drugs which allows the gastric injury risk reduction. Also, it is found that there will be neither inflammation nor side effects of the drug. After 72 h, it has been confirmed by checking drug release at intestinal pH in vitro condition (Karthikeyan et al., 2012). M.C. Regier et al. have successfully demonstrated DNA delivery by using fabricated zein-based nanospheres as a vehicle where the DNA can be entrapped and can be delivered at a specific location. Moreover, the particles used are in the range between 154−410 nm in size (Regier et al., 2012), and also, they maintain the integrity of DNA by avoiding the use of any harsh solvent and temperature.

1.2.2.13 Gluten

Gluten is the main storage Gu–PBB found in cereal grains such as wheat, barley, oats, and rye. Gluten contains 75%−85% of the entire protein in wheat bread (Shewry et al., 2002) which constitutes mainly gliadin and glutenin protein, they can be classified into high molecular and low molecular glutenin and α/β, γ, and Ω gliadins (Payne, 1986). Broadly, the classification of Gu–PBB is based on its solubility in aqueous alcohols, gliadin is soluble whereas glutenin is insoluble (Wieser, 2007). However, their homologous storage proteins in barley, oats, and rye are referred to as hordeins, avenins, and secalins, respectively (Rosentrater, 2018). Gu-PBB is a complex mixture of many related proteins, collectively they are

referred to as gluten (Biesiekierski, 2017). In addition, other grains such as maize and rice have storage proteins, commonly referred to as gluten, but they are not harmful and cannot cause diseases like celiac labeling (Food and Drug, 2007).

The networks of Gu–PBB differ because of different sizes and components and also variability caused by growing conditions, genotypes, and technological processes. Gu–PBB can be used as an additive in processed foods to improve flavor, texture, and moisture retention which could also act as an agent either binder or extender. High resistance is observed to the gliadin peptide sequences which causes pancreatic, gastric, and intestinal proteolytic digestion problems. Wheat, oats, rye, and barley grains are important main foods that cause several disorders due to the intake of gluten which is about 5–20 g/day in western countries.

There are two methods to obtain gluten films, of which the one is drying followed by casting, and the other is thermos pressing after boiling the protein solutions (Cuq et al., 1998; Gällstedt et al., 2004; Mangavel et al., 2004). However, the films obtained by these two methods are characteristically different, the casted films have higher elongation properties whereas the thermos pressed have stronger rupture resistance. It is reported that these films have a different stress-strain relationship which clarifies the effect of production methods on the network structure of proteins (Zuo et al., 2009).

The film-forming solution (FFS) is most commonly a mixture of water and ethanol and also found that the uniform films can be obtained by regulating the pH of FFS. It is observed that the films produced from alkaline FFS are with significantly higher tensile strength than that of films produced from acidic FFS (Gennadios, Brandenburg, et al., 1993). Other than this, the films produced from ethanol solution have good properties compared to the films made from alkaline solutions(Krishna et al., 2012). In addition, the characteristics of the films can be enhanced by heat and mechanical mixing which helps to disperse the gluten (Arnon-Rips & Poverenov, 2018).

Also, the properties of the films such as oxygen isolation, resistance to water vapor, and mechanical properties can be improved by taking various measures (Krochta & Mulder-Johnston, 1997). It is reported that water vapor permeability can be reduced by 25% compared to the control group by the addition of the hydrophobic nonpolar substance into the FFS dispersion(Gennadios, Weller, et al., 1993). The mechanical properties of the gluten-based films can be enhanced by casting which was possible through

covalent cross-linking of gliadin polypeptide chains (Gao et al., 2006). These films can be used as edible films and also thyme essential oil (TO) was added to gluten-based edible films to improve in vitro antioxidant and antimicrobial properties of samples (Ansorena et al., 2016).

1.2.3 Synthetic protein material products in the industry

Several research groups studied the properties of protein-based biopolymer materials (PBBM) (Abascal & Regan, 2018; Hu et al., 2012; van Hest & Tirrell, 2001). It is expected that the mechano-physical properties of SPs in nature should be in the recreated PBBM. Although there are several methods for the synthesis of SPs it seems difficult to retain the innate biophysical functions of a SP using regenerated or recombinant proteins. Not only do artificial synthetic methods need to be optimized to achieve the desired biophysical properties of the structural PBBM but also, it is difficult to reproduce the biological ordered structure. Nowadays, there are several commercial products of SPs available in the market.

Recently, Bolt Threads firm based in CA, USA has industrialized artificial silk fiber Microsilk using recombinant protein and made ties as well as caps from it. These artificially made ties and caps sold out immediately. These prepared ties are made from 100% Microsilk whereas caps are composite of American Rambouillet wool and Microsilk. Also, a Dress was prepared from 100% Microsilk. In 2019, artificial silk fibers, T-shirts, textiles have made and commercialized by Moon Parka. Also, prepared films, silk resin, and other silk materials by this firm and soon have commercialized these products.

In Germany, in 1898 casein plastic or lactic casein have been invented and is used commercially for clothing buttons, impressions, as biodegradable materials. Similarly, in the 1970s Toyobo Co., Ltd, have developed Promix, which is a fiber made up from polymerization of casein with acrylonitrile. In such a way, proteins extracted from plants such as soya beans, peanuts, corn, and textiles have been developed. Commercially, it was found difficult to fibrillate these proteins, hence a composite fiber was made of 60% polyester, 20% soybean protein, and 20% nylon.

1.2.4 Reinforcement and modification techniques

PBB are strong candidates for polymer engineering which offers advanced polymer composites or blends with state-of-the-art properties for their anticipated applications (Gandini, 2011; Graupner et al., 2009;

Thomas et al., 2013). Here, the AAS present along the polypeptide chain is considered as the deciding factor for variable physicochemical features of these proteins. The core chain of the peptide is constant but it has side chain "R" and it has variable functional groups. The side chain "R" is playing a vital role in verification and is responsible for the nature, functions, sequence, and shapes of the protein (Biro, 2007). The interaction of the synthetic and natural polymer with "R" groups of the peptide provides different reinforcement approaches such as physical and chemical treatment (Kuzuhara, 2003), preparation of blends (Averous, 2009; Barone & Schmidt, 2005; Liu et al., 2013; Zhang et al., 2011), and chemical block copolymerization (Morell, 2013; Reynhout et al., 2013; Schlaad & Antonietti, 2003) for the development of protein-based polymer. Several scientists have reported composites or blends of protein with synthetic, natural, and nonprotein molecules such as keratin–polyethylene (Barone & Schmidt, 2005), keratin–chitosan (Tanabe et al., 2002), keratin–polypropylene, keratin–cellulose polypropylene (Bullions et al., 2003, 2004; Schuster, 2003), and glutenmethyl-cellulose (Zuo et al., 2009). K-PBB is used to prepare nanofiber (Aluigi et al., 2008), film (Barone, Schmidt, & Liebner, 2005), and composites (Bertini et al., 2013) while edible films can be prepared by using gluten (Mojumdar et al., 2011), milk protein (Bahram et al., 2013; Ramos et al., 2012), and soy proteins (Su et al., 2010). These natural polymers could be extensively used as biomaterials (Maskarinec & Tirrell, 2005; Xing et al., 2011), packaging material, and in coating industries (Scheller & Conrad, 2005; Wittaya, 2012).

1.3 Applications

1.3.1 Soil Strengthening

Since, population growth and urbanization have increased enormously, which is a great concern for geotechnical engineers regarding soil or sand stabilization. Although, normal soil or sand stabilization materials (SSM) such as cement have a harmful environmental impact (Chang et al., 2016), because of this, researchers are always in search of alternative eco-friendly materials like PBB. Despite advantages such as adequate strength, availability, and low cost of cement, which is also a global concern due to CO_2 emission (Chang et al., 2016). Cement production and use are one of the main reasons for global warming (Dale et al., 2001), which is due to constant increment in greenhouse gases (Lashof & Ahuja, 1990) resulted in

glacier melting and sea level rising (Meehl et al., 2005), drought and intense storm and tornadoes (Dale et al., 2001). In addition, other environmental drawbacks are changes in soil pH, inhibition of surface vegetation growth, urbanization, and also concrete dust (Chang et al., 2016).

This leads to the use of benign, sustainable, and eco-friendly materials and methods. It is reported that the microbial soil treatment is time-consuming compared to other methods (Ivanov & Chu, 2008). Many biopolymers such as lignin (Zhang et al., 2015), gellan gum and gar gum (Chang, Prasidhi, et al., 2015), xanthan gum (Chang & Cho, 2012; Chang, Im, et al., 2015), Guar gum (Gupta et al., 2009), Alginate (Galán-Marín et al., 2010), starch, and tannin (Keita et al., 2014) tried and found effective in soil strengthening. In addition, Hadi Fatehi et al. stated the use of PBB such as casein and sodium caseinate as new soil additives to improve the dune sand strengthening. They also reported that the uniaxial strength, compressive strength, California bearing ratio test (CBR) values increased with the application of these milk proteins with its sodium salt on dune sand.

1.3.2 Food packaging: films and coatings

Protein-based biopolymers has become the most attractive and leading food packaging materials due to its biodegradability, processability, combination possibilities, and eco-friendliness. Also, the raw materials for PBB films and coatings are of low cost, easily available, and can be classified into two categories: PBB of plant origin and PBB of animal origin. The sources of plant PBB for packaging materials are soybean Zhang et al. (2010), peanut (Jangchud & Chinnan, 1999), corn (Aydt et al., 1991), and sunflower seed whereas animal PBB are gelatin, collagen (Gomez-Guillen et al., 2011), and casein, whey (Tien et al., 2001). These PBB are mostly used for the preparation of edible films because of their protecting potential of food products, nutritional values, and sensory features. Moreover, many globular proteins such as gluten (Zhong & Yuan, 2012), corn (Aydt et al., 1991), soy (Tian et al., 2011), whey (Kinsella & Whitehead, 1989) have been established as good films and coating materials. These obtained products such as films and coatings from PBBs have incomparable gas barrier properties and also reasonable mechanical properties. In addition, poor water barrier property exists due to hydrophilicity of proteins, which can be overcome, and also other properties can be improved by the application of plasticizers and post-treatments. Also, microbial viability or antimicrobial activity and lipid oxidation can be inhibited or delayed by the addition of some active compounds in these PBB films and coatings.

1.3.3 Protein purification

Recombinant proteins are vital for numerous biomedical applications, also significant for high-throughput screening, lead identification & validation, and reagents for drug discovery. However, protein expression and purification are the major issues in biopharmaceutical applications. Protein expression can be improved by selecting different hosts like bacteria, mammalian cells, and insect cells based on properties helping in protein expression, and also the expression conditions can be optimized for maximum yield (Baneyx, 1999; Makrides, 1996). Commonly known methods for protein purification are precipitation, electrophoresis, and chromatography. Among these methods, chromatography is most widely used, which involves solid stationary phase as columns to undergo protein separation based on physicochemical properties such as hydrophobicity (Regnier, 1983), size (Lathe & Ruthven, 1956) or shape (Bailon et al., 2000) and charge (Weiss & Weiss, 2004).

Apart from the conventional chromatography practices, recombinant proteins (RCP) can be purified by the use of gene-engineering and affinity techniques combinedly. An affinity tag, which is a small peptide aid as a binding partner to incarcerate molecules attached on chromatography beads, is labeled on target protein at the C- or N-terminus using gene-engineering methods. Though many tags have been developed, affinity chromatography is being limited to RCP with some exceptions. It is also reported that the affinity labels can have adverse effects on solubilization (Makrides, 1996), crystallization (Makrides, 1996), and also on proteolytic cleavage (Greenwood et al., 1992).

To overcome many challenges associated with affinity chromatography, a new method is needed for protein purification, which can be temperature-sensitive elastin-like polypeptides (ELP). These ELP can circumvent difficulties associated with chromatographic approaches analogous to an affinity tag, where an ELP tag works. ELP tags are available in different sizes and compositions, which in fusion with protein can be used in drug delivery and tissue engineering other than protein purification (Meyer & Chilkoti, 1999).

1.3.4 PBBM in healthcare: tissue engineering, drug delivery, surface engineering

PBBM has been used to advance scaffolds for innumerable biomedical applications like tissue engineering (TE), drug delivery (DD), emulsifiers, filters, detectors, and transducers in biosensors and wound dressings. Despite advantages like biocompatibility (Cho et al., 2008) and degradability by enzymes (Cho et al., 2008), PBB has disadvantages such as

immunogenicity, antigenicity, and solubility issues. PBB such as silk & silk fibroin (Bhardwaj & Kundu, 2011), gelatin (Prasong & Pak, 2011), and elastin (Nettles et al., 2010), collagen, fibrin, keratin, Zein, casein, and albumin shows good biocompatibility and used as a scaffold in tissue engineering constructs, active pharma ingredients drug delivery carriers with considerable success. PBB composite films formed by blending and block copolymerization offer new avenues of properties such as controllable drug delivery and tissue engineering by providing good cell support and also with physical and biochemical support (Haghpanah et al., 2009; Hu et al., 2010; Jaklenec et al., 2008).

Researchers have been carried out continuous developments in the PBBM which substantially impact medical exercise in surgery and regenerative medicine. Also, PBBM has an impact on controlled drug delivery systems which brings awareness about the difficulties with old and new novel drugs (Qiu & Park, 2012). Advancement in the PBBM discipline has directed the improvement of innovative drug delivery systems by considering surface and bulk properties with considered structural and chemical features. For drug delivery, smart hydrogels which carry drug formulations found promising for effective drug release (Loh et al., 2010) and are also cost-effective for a variety of applications in therapeutics.

Moreover, Almany and Seliktar (2005) demonstrated promising fibrinogen-based hybrid hydrogel scaffolds which are crosslinked with bifunctional polyethylene glycol side chains and provide more advantages than other hydrogel scaffold materials. Likewise, Koutsopoulos et al. reported a gel that is a successful carrier and can release drugs of variable size, potentially for insulin and Herceptin (Koutsopoulos et al., 2009; Loh et al., 2010; Luo et al., 2011). This gel which is commonly known as "nanofiber hydrogel scaffold" can regulate the degree of release by varying density of the gel that enables over a definite period (Koutsopoulos et al., 2009). Due to purity, ease of design and use, nontoxicity, nonimmuno-genicity, bio-adsorbable, and also have local applicability to a particular tissue, these PBBS are ideally suitable for drug delivery.

1.3.5 Recombinant protein polymers

Recombinant protein polymers (RPP) are long polypeptides made up of amino acid units that mimic normal structural PBB. Genetic engineering offers modifications in the selected area of structural units of PBB, which resulted in RPP and permits the formation of tunable protein polymer

with advanced functionality (Wang et al., 2019). Moreover, the genetically modified polymers are polypeptides obtained from natural amino acids and followed biochemical pathways. Since, last 3 decades, the heterogeneous microbial structural PBB and RPP have been produced a characteristic class of polypeptide polymers (Qian et al., 2020).

1.4 Protein-based biopolymers nanoparticles

Protein-based biopolymers nanoparticles have been extensively used for many biomedical applications, mostly for the delivery of materials, such as anticancer drugs, growth factors, genetic materials or DNA, and RNA and peptide hormones. These nanoparticles are a good alternative to synthetic polymers due to their biocompatibility, biodegradability, and non-immunogenicity, which are generally used for nanoparticle formulations. PBB nanoparticles can be prepared from proteins, such as fibroin, albumin, gelatin, gliadin, legumin, lipoprotein, 30Kc19, and ferritin. Methods used for PBB nanoparticle synthesis are emulsification, desolvation or coacervation, self-assembly, electro-spraying, and salt precipitation as shown in Fig. 1.4 (Jain et al., 2018). Among them, desolvation is the most
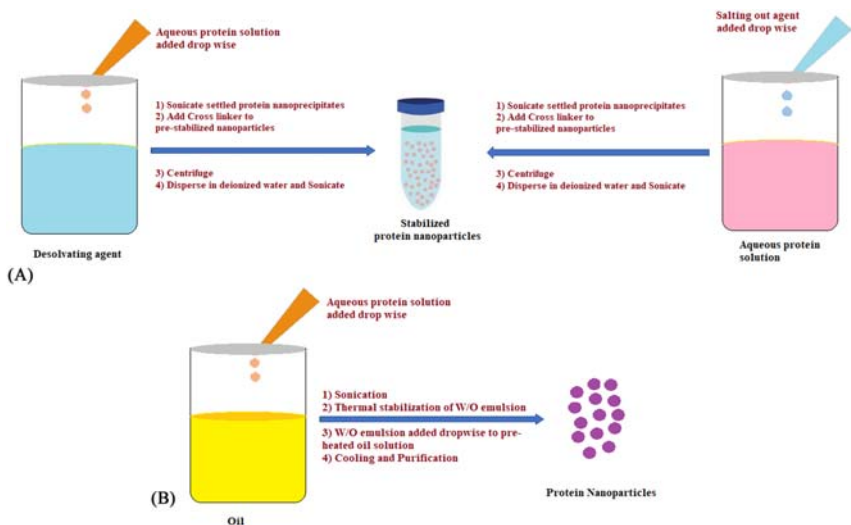

Figure 1.4 (A) Desolvation method for protein nanoparticle synthesis and preparation of protein nanoparticles by salt precipitation. (B) Emulsion technique for preparing protein nanoparticles: aqueous protein phase and non-aqueous (oil) phase are combined and emulsified to yield protein nanoparticles.

commonly used method, where cross-linking takes place using chemical cross-linkers such as polyphenols, or glutaraldehyde with aggregation of protein followed by dehydration by desolvating agents such as acetone or alcohol.

Due to their small particle size, quick desolvation in the bloodstream, easy transmission through cells via endocytosis (Jacob et al., 2018), and target specificity, PBB nanoparticles are considered the most promising agents for drug delivery systems. These PBB nanoparticles have many advantages as drug delivery systems, such as safety, hydrophilicity, ease of particle size control, efficacy, stability, surface modification, biocompatibility, biodegradability, and nonimmunogenicity, which helps to improve pharmacokinetic and pharmacodynamic actions of the therapeutics (Langer et al., 2003). Like drug delivery, these PBB nanoparticles are specifically used in a variety of targeted therapies such as vaccines (Sahoo et al., 2015), lung delivery (Mottaghitalab et al., 2017), tumor therapy (Sabra et al., 2018), and cancer therapy (Saleh et al., 2019), due to their nonantigenic property (Verma & Garg, 2001). Currently, many scientists use PBB or PBB nanoparticles for the preparation of vaccines to protect from the COVID-19 international crisis.

1.5 Challenges and future prospects

This introductory chapter, discussed PBB origin, types, properties and also emphasized their synthesis, blends or composites, purification techniques, and applications in various fields. In the production of films for food packaging and many other purposes, PBB dispersions are made into either water or ethanol or water-ethanol mixtures. This solvent limitation is a challenge, where other solvents need to use for the production of good quality films. Various researchers have been used tunable features of these PBB for edible food packaging (Khwaldia et al., 2010; Oussalah et al., 2004; Tien et al., 2001; Zhang et al., 2010). Unlikely, in nonfood packaging, there are many challenges in the properties of films such as strength, toughness, tensile modulus, and elasticity. As the interaction of peptide chains is through hydrophobic interactions, ionic bonding, hydrogen bonding, and covalent bonding. So, it is challenging to have control over the bond formation in the peptide chain to chain interaction, which could be controlled by the deviation in a degree of bond formation. Blending, copolymerization, or cross-linking are the processes of improvement of the adhesive or cohesive properties of PBB to face future

challenges (Aluigi et al., 2008; Nandagiri et al., 2011; Prasong & Pak, 2011; Zhong & Xia, 2008; Zoccola et al., 2008). Chromatographic techniques have many challenges in protein purification, mostly column cost, and affinity. There are many faceted challenges of using PBB which include their synthesis, purification, material design, and processing. There is a hope that more researchers will take part in this domain of research and will have innovative techniques to achieve SPs with tunable biophysical properties. At this time, PBB nanoparticle's research work is restricted to their physicochemical properties and toxicity including their pharmacokinetics and pharmacodynamics. It is assumed that more advanced PBB nanoparticle drug delivery systems will be available shortly.

Acknowledgments

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Details the latest developments in the synthesis, characterization, and biomedical applications of protein biopolymers.

- Covers a range of protein-based biopolymers, including gluten, collagen, keratin, soy, and more
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- Explores the biomedical potential of protein biopolymers, covering applications such as cancer therapy, tissue engineering, and drug delivery

Protein polymers have garnered increasing focus in the development of biomedical materials, devices, and therapeutics, due to their intrinsic bioactivity, biocompatibility, and biodegradability. This book comprehensively reviews the latest advances in the synthesis, characterization, properties, and applications of protein-based biopolymers; each chapter is dedicated to a single protein class, covering a broad range of proteins, including, silk, collagen, keratin, gluten, and more. The book explores the biomedical potential of these polymers, from tissue engineering to drug delivery and wound healing.

Protein-based Biopolymers: From Source to Biomedical Applications offers a valuable resource for academics and researchers in the fields of materials science, biomedical engineering, and R&D groups working in pharmaceutical and biomedical industries.

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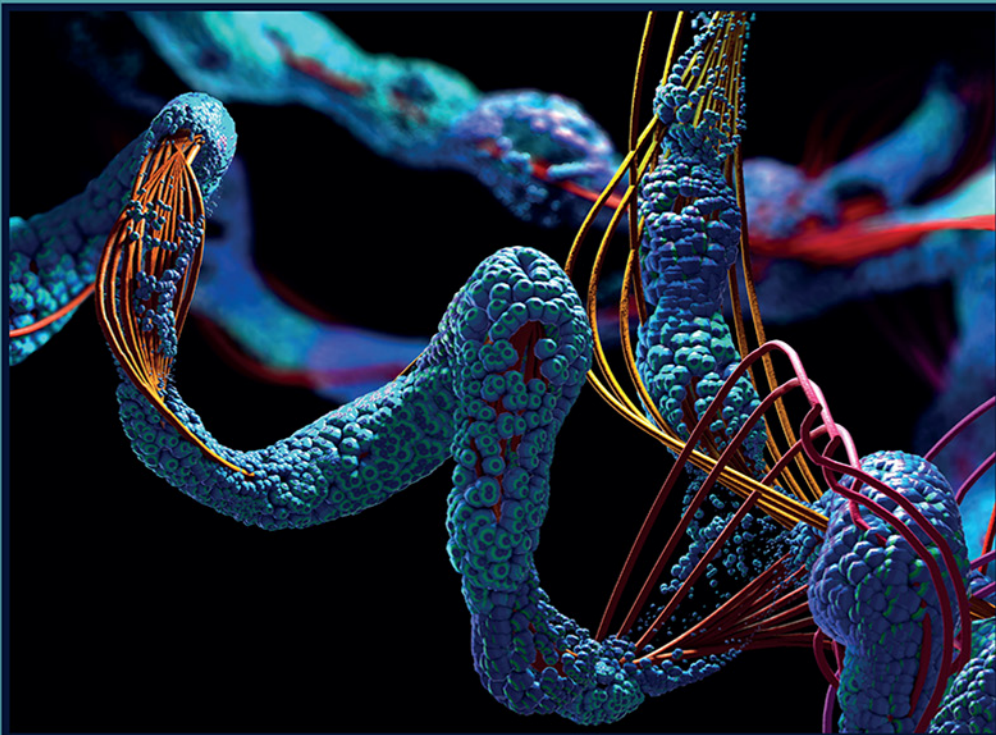
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CHAPTER 4

Fabrication, properties, and biomedical applications of soy protein-based materials

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4.1 Introduction

Biopolymers play an important role in the biological milieu aiding for several functions like transport of ions, drugs, small molecules, etc. Among these, natural polymers tend to have immense demand in the field of biomedical as compared to synthetic due to their impeccable biocompatibility, and biodegradability without immunogenicity. Natural polymers include carbohydrates (cellulose, starch, pectin, etc.), proteins (albumins, collagen, soy protein, zein, and silk), and nucleic acids (DNA, RNA) have been used for food, therapeutic, and tissue engineering applications (Ebhodaghe, 2020; Jacob et al., 2018; Shankar et al., 2016). Among these, protein or protein-based biomaterials are good in correspondence with biological tissues (extracellular matrix) due to their exclusive structural and functional characteristics (Khan et al., 2021; Yao et al., 2021). With respect to structure, proteins provide an opportunity for surface modification or functionalization owing to their free carboxyl and amino groups present on the exterior of the proteins. In addition, the unfolded proteins provide the scope to interact with hydrophobic groups. The functionality of proteins offers cell adhesion and proliferation properties facilitating biomedical applications (Ju et al., 2020). Soy protein (SP) is considered as the blend of albumins and globulins, mostly the globular structure. Among the proteins, SP is a good functional food constituent derived from soybean (legume) with a varying protein content of 36%—56% depending

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on the geographical origin (Chen & Zhang, 2005; Cuq et al., 1998; Zhang & Kang, 2017). This low molecular weight protein is produced from the by-product of soy oil and is predominantly has been used due to its abundance, ease of use, low cost, stable storage, significant water resistance, nonimmunogenic, biodegradability, and biocompatibility. In prospect of nutritional value, this plant-derived protein is loaded with nine essential amino acids (AAs) and contains low methionine/glycine and lysine/arginine ratios. This tends better proteolytic cleavage that eases the digestion progress (Singh et al., 2008) as analogous to animal-derived proteins (Young, 1991). The SP illustrates not only the use of agriculture-based products but also prevails over safe from zoonotic infection, biodegradability (enzymatic), immunogenic and environmental problems in comparison to animal proteins (Xu et al., 2015). The SP is prepared by grinding the dehulled and defatted soybeans into powder form, subjected to decarbohydration (by solubilizing in water), and further purified. SP is commercialized as three products namely soy flour, SP concentrate, and SP isolate holding 50%, 70%, and 90% protein, respectively. Based on the sedimentation coefficients, SP is fractionated into 2S, 7S (β-conglycinin–SG), 11S (glycinin–SC), and 15S globulins contributing to the total of 8%, 35%, 52%, and 5% respectively. Among the subunits, 7S and 11S contribute to about 60% of the storage protein with their corresponding molecular weights are 360, and 175 kDa (Petruccelli & Anon, 1995). The former subunit accounts for more than half of the total protein component. Similarly, the pI's of the subunits are 6.4 and 4.8, respectively. In general, the pI of SP is considered as 4.8 due to the presence of highly negatively charged AAs. The functionality of SP is based on the dissociation, denaturation, and aggregation of the subunits (Hermansson, 1978; Petruccelli & Añón, 1994; Sorgentini et al., 1995). The 7S subunit has greater α–helix and random coils accounting for flexibility in the protein structure as compared to the 11S (Ali et al., 2010). Structurally, SP is packed compactly with hydrophobic globular core and hydrophilic side chains toward the exterior as of general protein structure. Thus SP is considered as an amphiphilic which provides an opportunity to interact with both polar and nonpolar groups, acting as an emulsifier (Kumar et al., 2002). Chemically the SP has 18 AAs, where the side chains mostly contribute to hydroxyl ($-OH$), carboxyl ($-COOH$), an amino ($-NH_2$) groups tending toward hydrophilic as well as facilitating easy reactivity or surface functionalization to amplify its properties. For instance, phosphorylation of the protein facilitates hydrophilic nature ultimately leading to enhance water holding and emulsifying properties. SP has been structurally modified by physiochemical and enzyme-mediated methods. The latter is

exemplified with *peptidoglutaminase* for deamination, required for the solubility in an acidic environment. In prospect of biomedical and food applications, SP contains isoflavones, Phytic acid (a rich source of phosphorus) (Meikle et al., 2012). The phytic acid is well known as a free radical scavenger, anticancer and antiinflammatory agent thereby SP acting as a potential biomaterial (Kumar et al., 2004; Vucenik & Shamsuddin, 2003; Yoon et al., 1983).

4.2 Soy protein properties

These are essentials for biomedical applications. The exclusive features of any protein are reliant on the chemical structure and its response to subjected environments like pH, temperature, and ionic strength. The properties were explained below.

4.2.1 Surface properties

The surface charge or zeta potential of the protein, hydrophobicity, charge density, and solubility are responsible for their fabrication of SP-based biomaterials. The zeta potential is dependent on the pI of the SP and the pH of the suspended medium (Akio et al., 1983). In general, hydrophobic amino acids are present in the core of all the proteins. So, the value is relatively low in comparison with other proteins. The value increases upon subjecting the protein to pH far from its pI. Then the number of hydrophobic amino acids present on the surface of the SP exposed or interacted with the medium decides the hydrophobicity (Zhang et al., 2012). The SP solubility is higher at acidic and alkaline pH as compared to its pI. This is due to the ionic strength that is developed by the salt bride formation between the SP and cations in the medium (Teng et al., 2009).

4.2.2 Mechanical properties

The SP solution at its pI lacks intermolecular electrostatic repulsion hence exhibits low viscosity. While in gels the intermolecular attractions, covalent (disulfide) bonds, Vander Waal forces, hydrophobic interactions, are improved that are favorable for gelation. This could be achieved by altering pH, ionic strength, and temperature that triggers the SP to unfold (Malhotra & Coupland, 2004). The 7S and 11S subunits behave independently and exhibit different viscoelastic behavior. The latter has increased

elasticity due to the presence of a high proportion of disulfide bonds and the rigid β–pleated sheets structure (Tang et al., 2006a).

4.2.3 Biodegradability

In the context of biomedical, the degradation of the complex material into less complex or simple entities either by hydrolysis (chemical/enzymatic) or by the body's metabolism. The end products of the SP biopolymer will be removed from the body by means of bioabsorption or bioerosion (Kamath & Park, 1993). The SP is intrinsically nontoxic and biodegradable. The polymers or cross–linkers (synthetic/natural) are used along with the SP to form the biomaterial that determines biodegradability. For instance, the SP-Polyurethane composite implants promoted biodegradation in vitro and in vivo. The in vivo studies for 9 months clearly justified the disappearance of the implants due to improved cell adhesion and proliferation (Li et al., 2020).

4.3 Fabrication of soy protein-based biomaterials

There is a wide range of materials that have been developed and explained in detail and represented in Fig. 4.1.
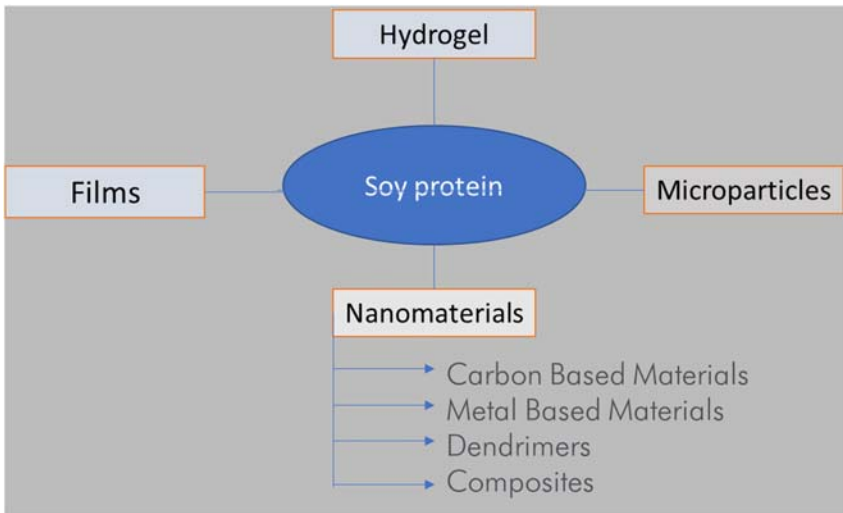
Figure 4.1 The biomaterials were fabricated using soy protein in various dimensions. Watermark from PDB ID. 1OD5.

4.3.1 Soy protein films

The films are two-dimensional structures with significant flexibility, mechanical stability, and good gas barrier property as compared to the other biomolecules such as sugars and fats (Cho & Rhee, 2004; Gennadios et al., 1993; Kunte et al., 1997; Silva et al., 2014a). Higher protein content facilitates the better film–forming property so, SPI is preferred as compared to SF and SPC (Guerrero et al., 2011; Mo & Sun, 2002; Sothornvit & Krochta, 2001). In the film fabrication, the stable intermolecular interactions or bonds are reduced, rearrangement and spatial reorientations happen within the SP chains that eventually form the stable three-dimensional porous networks with micro-architecture due to newly formed physicochemical interactions. Various physical and chemical modifications have been used for the preparation of the films. In the former, hydrothermal, high rpm, elevated temperatures are used to create the sulfhydryl functional groups from disulfide bonds. This reasons the unfolding and structural reorientations. The protein structure favors emulsification and solubility. The films generated by subjecting to high temperature exhibit enhanced elongation at break and minimize their water vapor permeability as compared to films generated by other methods. Chemical modifications such as phosphorylation, thiolation, acetoxylation and use of cross-linkers (Chemicals and enzymes), etc. have been used to augment the foaming and emulsification properties of SP. The use of a cross-linker enhances the mechanical stability, water barrier characteristics by minimizing its swelling behavior, solubility, and water vapor permeability. Several conventional chemicals such as glutaraldehyde (toxic above 0.3 ppm) (Vaz et al., 2003a), formaldehyde, carbodiimide 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, glyoxal, epoxy, phenolics, and synthetic and natural polymers poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), polyvinyl alcohols, gelatin, pectin, Chitin, Alginate, etc. have been used as cross-linkers (Barkay-Olami & Zilberman, 2016; John et al., 1998; Akkasit et al., 2006; Zhang et al., 2006; Zheng et al., 2003; Zhong & Sun, 2001). Hydrolysis of SP with enzymes such as papain, transglutaminase, horseradish peroxidase (HRP) is used to enhance the functionality (Kinsella, 1979). For example, The HRP was cross-linked covalently with SP to form the films with enhanced mechanical property and protein solubility (Stuchell & Krochta, 1994). SP films were fabricated by two methods solution casting and film extrusion which are explained in detail.

4.3.1.1 Solution casting

In this method, proteins are suspended or solubilized in the solution with alkaline pH ($>$ pI of protein) or solvent constituents (polymers or nano-material) that are further subjected to solvent evaporation. Mostly, water is used as the solvent medium in this method. Blending the protein solution with water-soluble polymers like poly(ethylene oxide), agar, poly-vinyl alcohol (Ji et al., 2012; Su et al., 2010; Tian et al., 2011). Also, hydrophilic polymers like rubber, polystyrene, polyurethane, etc. could be used (Liu et al., 2008; Tian et al., 2010a; 2010b). However, water-insoluble polymers like PCL and PLA were also used with uncommon solvents. This method makes the changes in solubility of protein leading to protein unfolding which is dependent on the AA composition, AA sequence, and degree of denaturation. In this context, the protein unfold-ing refers to the opening of the peptide chains from the three-dimensional protein structures (Cho et al., 2007). The basic pH and heat treatment is applied which allows the peptide chains to get exposed to the solvent environment before casting on the flat surface (Mauri & Añón, 2006; Wang et al., 2013). The pH, temperature, flow rate, drying period, and substrate surface property are the factors responsible to tailor the films using this wet process. The drying step can be performed by infrared, microwave energy, and hot air (Denavi et al., 2009). As compared to the dry process, solution casting is considered as slow processing time with a complicated protocol that involves huge amounts of solvents and energy. After drying, the fabricated films are difficult to be removed from the substrate so, there is a need for the spraying of certain agents is required to aid the film removal. Also, the yield efficiency of the product is less, and pollutants used in the protocol may be harmful thereby narrowing down its applica-tion toward commerciality (Shi & Dumont, 2014).

4.3.1.2 Film extrusion

In this dry process, the bulk SP is subjected to a thermomechanical extru-sion setup for the film fabrication where the protein is passed through a screw and melted. Under high temperature, pressure, and shear stress the proteins undergo the unfolding, recombination of the functional groups, and cross-linked among the polymers by orienting themselves to form networks at the molecular scale that led to the film formation. Hence, the temperature, pressure, mechanical shear, length to diameter ratio, number of screw extruders (single or dual), screw speed, screw rotation type, and use of plasticizers are influential parameters for film fabrication. In virtue

of films fabrication, the protein decreases its solubility, denatures, and increases viscosity thereby restricting the protein chain movement by involving in the network formation. During the process initially, the intermolecular and intramolecular interactions forms the stable native protein structure were dissociated. Further, reconstructing the interactions like disulfide (−SS) and amide (−CONH) are responsible for forming the porous networks. So, the hydrophobic disulfide and the hydrophilic free side chains (−CO or −NH) flanking on the exterior play a key role in the film fabrication through this method. In addition, noncovalent interactions like hydrogen bonding are involved in reassembling the protein subunits into stable structure formation. In recent times, blends of the polymers have gained researcher's attention to develop SP-based biomaterial either with synthetic or natural polymers such as SP/polyethyleneimine-polydopamine or SP/starch blends respectively (Chang et al., 2021; Ferreira et al., 2021). For instance, the polyethyleneimine-polydopamine blended with SPs for generating the films with injectable, fluorescence, low cytotoxic, biodegradable self-healing properties depicted in Fig. 4.2. The SP is used for biocompatibility and other polymers were used for incorporating microcavities ultimately exhibiting significant mechanical strength. Synergistically, the blended films generated reversible molecular networks with excellent self-healing properties.

In comparison to casting, this method has enhanced mechanical properties (Guerrero et al., 2010). This process is mostly used in the plastic or food packing industry for packaging as this method employs SP isolates for fabrication benefitting the cost-effectiveness. This method is also used in bioelectronics (Guerrero et al., 2021) and controlled drug delivery applications which will be explained in detail. There are certain drawbacks in this process which include processing temperatures during melting and intrinsic brittleness. In the former, the temperature of the melting is close to SP's decomposition that is around 200°C. Secondly, the pure SP has a brittleness that could be overcome by the use of plasticizers. The use of plasticizers like amides, hydroxylamines, and polyols not only generates significant flowability but also flexibility (Chen & Zhang, 2005; Chen et al., 2008a; Kumar et al., 2009; Liu & Zhang, 2006; Liu et al., 2007; Tian et al., 2009). Intrinsically the plasticizers and their interactions involved with the SP being polar facilitate the hydrophilicity and flexibility with varied mechanical properties. In this regard, Water is also considered as a plasticizer as well as an enhancer of flowability. But the SP films generated using water are unstable and fragile. This is due to the
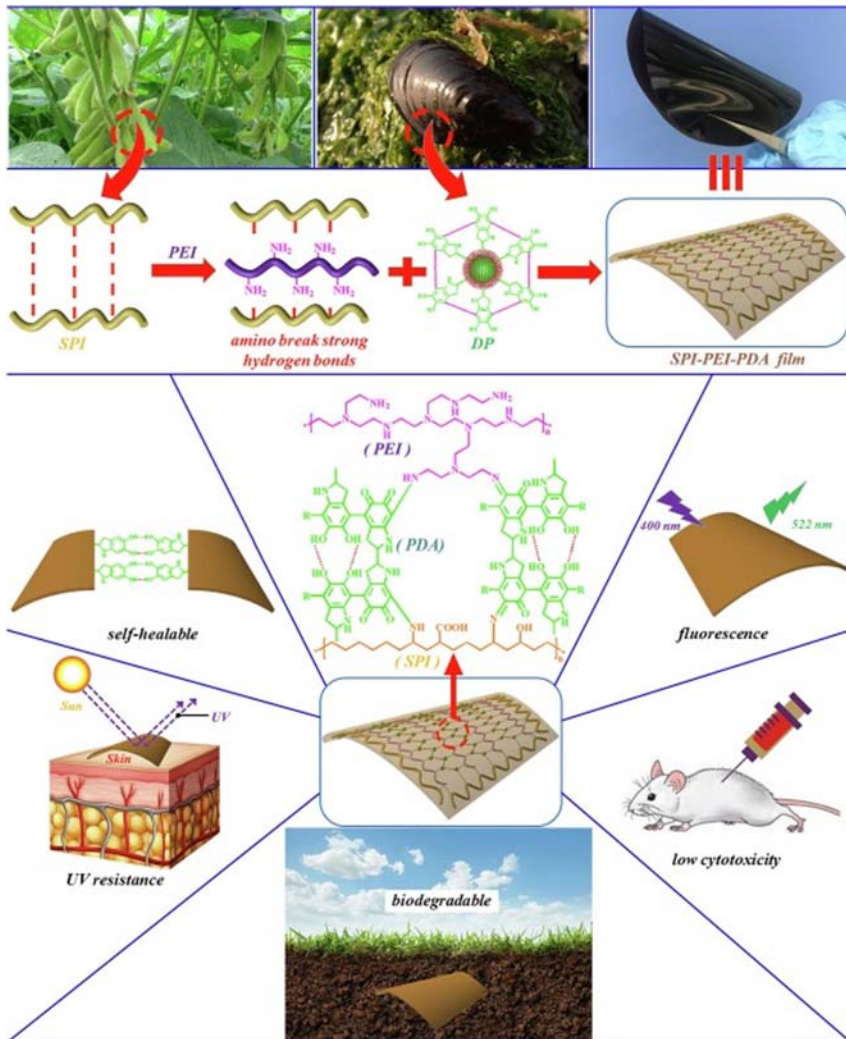
Figure 4.2 The soy protein blended with polyethyleneimine-polydopamine is used for a self-healable film depicting its exclusive characteristics. *Adapted with permission from Chang, Z., Zhang, S., Li, F., Wang, Z., Li, J., Xia, C., Yu, Y., Cai, L., & Huang, Z. (2021). Self-healable and biodegradable soy protein-based protective functional film with low cytotoxicity and high mechanical strength.* Chemical Engineering Journal, *404, 126505. Copyright Elsevier.*

evaporation of water during the process. Therefore the use of the plasti–cizer at the appropriate proportion yields a stable film (Chen et al., 2005; Guo et al., 2015).

4.3.2 Soy protein hydrogels

The cross-linked polymeric material can hold huge amounts of water and bioactive compounds or liquids in their three-dimensional porous networks. The porous networks are open and interconnected that offer a significant surface area to volume ratio eventually playing a role in the entrapment of a good payload of bioactive compounds thereby acting as a noble cargo (Kang et al., 2005). In addition, SP hydrogels have excellent biocompatibility, low toxicity, and biodegradability properties (Caillard et al., 2009). So, the SP-based hydrogels have immense potential in controlled drug or nutrient delivery (Maltais et al., 2009; Meikle et al., 2012) and tissue engineering (implants) applications (Santin & Ambrosio, 2008). The cross-linkers can be either physically or chemically cross-linked. The mode of cross-linking has an effect on the microstructure formation, release behavior of the entrapped bioactive compounds. In general, SP hydrogels were fabricated by self-assembly, blending, and chemical grafting (Li et al., 2008; Xie et al., 2017; Xu et al., 2012). The SP hydrogels are influenced by several factors such as temperature, pH, ionic strength, ultrasound-mediated, the concentration of cross-linkers (chemicals and enzymes), protein, and solvent (Caillard et al., 2009, 2010; Chien et al., 2014). In this context, several physicochemical interactions like noncovalent bonds, electrostatic, disulfide, hydrogen bonds, and covalent bonds contribute to the gelation (Ni & Dumont, 2017). Therefore understanding the factors responsible and their associated interaction among the polymeric subunits is important to fabricate a gel depending on the application. The SP hydrogels are mostly fabricated by heat treatment, cold-induced gelation, chemical or enzyme-induced gelation. The SPI hydrogel is prepared by treatment at high temperatures, the native protein structure is denatured exposing the hydrophobic moieties in the core. Further, the polymeric subunits interact and aggregate among themselves to form a stable hydrogel (Caillard et al., 2008). However, the temperature-based hydrogels are having limitations like being mechanically unstable (stiffness) and low water-holding properties (Tang et al., 2006b). An alternative temperature-based method is a cold gelation method where the protein is denatured by subjecting to high temperatures below the critical concentration for gelation in the first step. Further in the second step, at ambient temperature gel formation is facilitated by the addition of $Ca^{+2}(CaSO_4)$ or glucono-δ-lactone is added which lowers the pH near the isoelectric point. The first step the heat treatment involves protein unfolding and

converting into soluble aggregates. In the later step, the coagulant weakens the electrostatic forces by lowering the pH, promoting the noncovalent interactions, and disulfide bonds that enable aggregation followed by gel formation (Alting et al., 2000; Nicolai et al., 2011). SP hydrogels are subjected to microbial transglutaminase (MTGase) forms hydrogel through N-ε-(γ-glutamyl)lysine cross-links. The mechanical strength of the gel can be well-tuned by varying the time and MTGase/SPI mass ratio. Later Hu et al. fabricated the transglutaminase-SPI hydrogel by mediating ultrasound treatment for enhancing hydrophobicity and mechanical strength.

The ultrasonic waves improve reaction rate and the product yield on the account of reduction of the particle size of the suspensions eventually the size and uniformity of pore size as depicted in Fig. 4.3 using the scanning electroscope microscope analysis. Such changes are due to protein structural modifications that are observed by the micrographs with uniform porous hydrogels (Hu et al., 2015). The ultrasonic treatment accelerates the high degree of glycation between the sugars and SPI by milliard reaction forming acid-induced gelation with good mechanical strength as compared to the nonultrasonic treated hydrogels (Hu et al., 2013; Zhang et al., 2014; Zhao et al., 2016). Recently, pretreatment of ultrasonic waves with the SPI and carbohydrate (maltodextrin) has described an increase in the pI, hydrophobicity, and free sulfhydryl groups that are responsible factors to enhance glycation subsequently gelation with good mechanical strength and water holding capacity (Zhao et al., 2021).
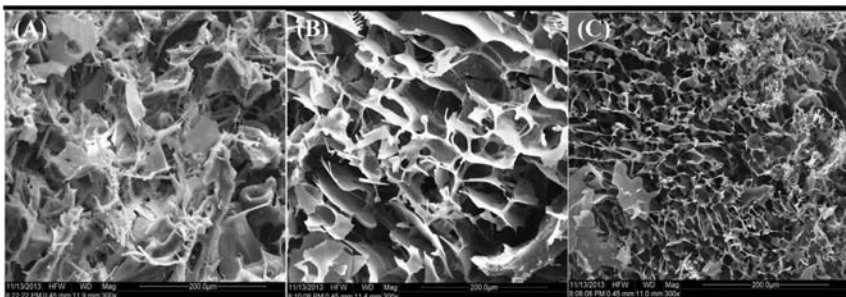
Figure 4.3 Scanning electroscope microscope images of MTGase-induced hydrogels containing riboflavin formed from without ultrasound treated (A), 20-min ultrasound-treated (B), and 40-min ultrasound treated (C). *Reprinted with permission from Hu, H., Zhu, X., Hu, T., Cheung, I. W. Y., Pan, S., & Li-Chan, E. C. Y. (2015). Effect of ultrasound pre-treatment on formation of transglutaminase-catalyzed soy protein hydrogel as a riboflavin vehicle for functional foods.* Journal of Functional Foods*, 19, 182−193. Copyright Elsevier.*

Caillard et al., used a conventional cross-linker, glutaraldehyde to demonstrate the degree of cross-linking and salt concentration with SP hydrogels cross-linked are inversely proportional to the swelling ratio affecting the release behavior of the loaded ionic compounds (Caillard et al., 2009). SP hydrogels are cross-linked with oxidized dextran, a natural polysaccharide derivative for exhibiting excellent flexibility to withstand high compression by controlling the gelation period and pH (Liu et al., 2018). Some polymers like polyethylene glycol (PEG), poly$N$–isopropyl acrylamide-co-sodium acrylate and poly(N–isopropyl acrylamide) were used to cross-link with protein subunits to form SP hydrogels (Liu & Cui, 2011a; Liu et al., 2014; Snyders et al., 2007). Interestingly, the PEG-SP soft hydrogel has a high water-holding capacity of about 96% the average molecular weight was intensified by three times as compared to its theoretical networks, as well as the elastic moduli, can be tuned from 1−17 kPa (Shingel et al., 2006). SP forms composite hydrogels with natural polymers like alginate, bioactive glass, etc. Further advancement includes subjecting to sono-chemical reaction assists the homogenous gelation (Silva et al., 2014b). The blending of poly (acrylic acid) with SPI involves the free radical copolymerization forms an interpenetrating network hydrogel (Liu et al., 2009). The SP is complexed with dextran was allowed to self-assemble with the drug to form small particle-sized nanogels which were induced by ultrasonic waves. The particle size can be tuned from 66−138 nm (Jin et al., 2016) The soy protein is used in blends with zein (Chen & Subirade, 2009), and collagen (Brännvall et al., 2007) to form hydrogels for increasing the mechanical stability.

4.3.3 Soy protein microparticles

The particles with spherical size and having a diameter from 1−1000 μm attributed with the good surface area/volume ratio. These particles contribute to the significant encapsulation of bioactive compounds like vitamins, drugs proteins, etc. Microencapsulation is a process of shielding the core or active materials (bioactive compounds for delivery) with the aid of protein as a membrane or wall material. The microparticles developed from microencapsulation benefit the dispersibility in an aqueous medium, sustained release pattern of active material, protection of active material from degradation from external environments such as pH, temperature, moisture, and oxidation. In general, microparticles exist in four different structural forms that is microcapsule, microsphere, multilayer

microcapsule, multishell–multicore microsphere. Among these, SP mostly exists in microcapsule and microsphere forms. The microcapsule is the active material framed by protein wall material. Microspheres are the active material dispersed in a protein matrix network. The multilayer microcapsule is active material surrounded by more frames of wall material. The multishell–multicore microsphere holds more active materials and more composite frames (Nesterenko et al., 2013). Microparticles are synthesized by coacervation, spray drying, cold gelation, and supercritical CO_2-assisted impregnation methods. The choice of the method depends on the factors like size, physicochemical parameters of active and wall material, and biodegradability of the particles. Lazko et al. were the first to synthesize soy glycinin microcapsules using the coacervation method, a simple and bottom–up approach (Lazko et al., 2004a, 2004b). These microcapsules hold liquid hexadecane inside and solid soy glycinin wall material outside. This method involves the formation of protein stabilized oil emulsion initially by tuning the protein concentration, temperature, and acidic pH. Then protein coacervation involves the precipitating of microparticles by the salting-out method through altering the pH. For stabilization, the cross-linking of the microparticles was done using a cross-linker (glutaraldehyde) to reinforce the wall material which was finally separated by quick decantation and freeze-dried for storage. In addition, stirring speed, pH, time, the concentration of cross-linker, and core have their influence on morphology, metrology, and encapsulation efficiency (Mendanha et al., 2009). For example the increase in the solute or core concentration decreases the encapsulation efficiency. The wall: core ratio was varied from 1:1 to 1:3 which showed a decline in encapsulation efficiency from 98% to 79% (Mendanha et al., 2009). In the same manner, glycinin–SDS complexed microcapsules encapsulated with oil were fabricated. Xiong and co-workers fabricated SP microspheres for the theophylline delivery with the combination of alginate. The combination of the biopolymers offers the synergistic effect of the different functional properties. They had blended both the biopolymers and cross-linked them with Ca^{+2} for stability (Zheng et al., 2007a). Recently, the Inulin: SP ratio is inversely proportional to the encapsulation efficiency of oil (Rios–Mera et al., 2019). One of the limitations of this method is the larger particle size greater than $100\,\mu m$ as compared to the spray drying method (Nesterenko et al., 2013). The SPI and zein complex spheres were synthesized by the cold gelation method yielding the particle size of $15-25\,\mu m$. This technique involves heat treatment and alkaline pH for

SPI and zein denatured solutions respectively. Then, Ca^{+2} ions were added to solubilize the aggregates to form gel networks. Further, this blend was dispersed in the soybean oil to form a water-oil emulsion. At this droplet phase, the Ca^{+2} ions were removed by acid addition thereby forming microspheres under controlled parameters. The mixing of SP with zein protein supplements stability, density, and hydrophobicity. The drug was dispersed along with protein for drug delivery (Chen & Subirade, 2009; Chen et al., 2010). Among various methods of microparticle fabrication, spray drying is the most commonly used method due to the hydrosolubility of the SPI. Typically, the procedure involves the solutions of SPI or wall material, and bioactive compounds were well homogenized under high-pressure resulting in low droplet size of emulsion and low viscosity (Gharsallaoui et al., 2007; Rusli et al., 2006). With an increase in pressure there is an increase in encapsulation (Lazko et al., 2004a). This causes unfolding that allows the exposure of hydrophobic and hydrophilic regions to interact well with the environment and make the unfolded protein surface more active. The highly active SP in the emulsions wraps freshly formed oil droplets at the interface. This surface holds stable from re-coalescence. These solutions were subjected to a spray dryer to form the solid powder collected from the collector using a stream of hot air. This step restricts the core mobility in the cell material induced by the solid content (Charve & Reineccius, 2009). An appropriate temperature is a key factor at this point, higher temperatures make the wall material rigid that do not allow the release of the core and evaporation of the core (Rascón et al., 2011). The SP was blended with polysaccharides for better wall material to protect flavors, oxidative stability, and significant drying properties (Augustin et al., 2006). Nesterenko et al. studied the impact of acylation (grafting of fatty acid chains) with SPI has improved the microencapsulation efficiency of hydrophobic (α-tocopherol) moieties as compared to the native protein (Nesterenko et al., 2014). This was attributed to the increase in the amphiphilicity of the protein. Similarly ascorbic acid, a hydrophilic vitamin was also studied (Nesterenko et al., 2014). As analogous succinylated SP has improved the encapsulation of hydrophobic drugs and thermal stability as compared to the native SP (Castro et al., 2019). Saavedra et al., SP forms microparticles with blends of gum Arabic (AG) and maltodextrin. They have demonstrated the concentration of wall material influenced the wetting time, bulk density, and hygroscopic nature of the particles formed (Porras-Saavedra et al., 2015). Using SP microparticles from spray drying, the novel supercritical

CO_2-assisted impregnation method was developed to protect the nutritional oils or solutes from oxidation. The supercritical CO_2 fluid employed makes this approach green. As the fluid operates the method at a lower temperature (31°C) prevents the degradation of temperature-sensitive compounds, and the inert environment shields from the reactivity toward oxidation. In addition, the ability of the fluid to behave as both gas and liquids allowed to dissolve solutes and polymers supports the impregnation process. The apparatus consists of a cell with a magnetic stirrer, heat jacket for temperature, CO_2 storage cylinder, and pressure generator. The system holds a cell loaded with solute or Chia oil and a cylindrical metal cartridge filled with SP microparticles. Both the compartments are separated by metal support to avoid mixing. Upon stirring, the cell is supplied with CO_2, temperature, and pressure until stabilization. Then, the cell is depressurized to avoid the solid CO_2 microparticles formation due to expansion. Further, the cartridge is removed to impregnate the microparticles with oil. The encapsulated particles were recovered and stored. This method is an alternative approach for shielding Chia oil from oxidation, light, and temperature (Gañan et al., 2020). SP is also used as a stabilizer in microparticle formulations. For instance, solid lipid microparticles encapsulated with vitamins were stabilized with hydrolyzed SP yielded particles with lower diameters that are highly stable (Brito-Oliveira et al., 2017).

4.3.4 Advent of nanoscience

The state of art, Nanoscience has been boon and revolutionized in the biomedical field. The branch of science that deals with materials with nanometer (nm) scale and its corresponding dimension has exhibited unique properties as compared to their counter bulks (Buzea et al., 2007). In prospect of SP, there are various nanomaterials explained below in detail.

4.3.4.1 Soy protein nanoparticles

The particles with all three dimensions in nm scale irrespective of their morphology are referred to as nanoparticles (NPs). The NPs have higher encapsulation efficiency for enhanced bioactivities, and stability for the long-term storage that is useful for biomedical application. With respect to SP fabrication methods, mostly a common step include dissolving the SP bulk powder into an alkali medium and again changing the pH near to neutral followed by centrifugation to remove the larger particles or aggregates.

Ionic gelation method

This method is also referred to as the cold gelation method discussed above. The NPs were synthesized using by increasing calcium concentration and lowering the pH. The SP peptide chains were interacted with one another by salt bridges and the Ca^{+2} favored the beta-sheet formation tending them for aggregation through hydrogen bonding. Further, these aggregates populate themselves by hydrophobic interactions to form the nanonetworks, thus forming SP nanoparticles with spherical morphology (Zhang et al., 2012). Using carboxymethyl chitosan and SPI, complex nanoparticles were fabricated loaded with vitamin D3 where there is an increase of 6% in the loading efficiency and also exhibited better-sustained release of nutraceutical (Teng et al., 2013a).

Desolvation

Wang co-workers had first fabricated SPI nanoparticles by desolvation method. In the typical procedure, the protein was dissolved in alkaline pH ($>$pI) solution followed by the addition of desolvating agents like ethanol ($>$80% to water added), acetone, etc. Further, the cross-linker such as glutaraldehyde ($>$75% to protein concentration used) was added to cross-link with the ε-amino groups of lysine present on the exterior (Teng et al., 2012; Weber et al., 2000). The initial addition of desolvating agent favors the exposure and allows interaction among the protein units by the hydrophobic domains. Further addition involves either the disruption of larger aggregates or ample formation of tiny aggregates. Therefore greater ethanol is required for nanoparticle formation. The cross-linker addition attributes to the hardening and stability of the nanoparticles depicted in Fig. 4.4. Though there is an increase in the size of the nanoparticle as compared to the immediately formed nanoparticle due to the fact of stability which is required for long-term storage that is a prerequisite parameter for a drug carrier (Teng et al., 2012).
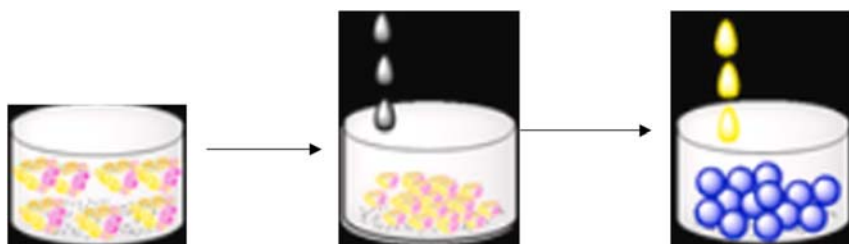
Figure 4.4 Schematic representation of nanoparticulation using desolvation technique.

Microfluidics

The SP nanoparticles were synthesized using the microfluidics technique, a top–down approach. The microfluidizer instrument was operated with temperature control by circulating water and a pressure of about 159 MPa. The alkaline pH favors nanoparticle formation owing to the better dispersion that allows the SP to coagulate and precipitate. The microfluidized NPs blended with polymer matrix yielded film with less cracking and enhanced elastic modulus as compared to larger NPs (Jong, 2013).

Ultrasonication

This top–down approach was assisted by alkaline hydrolysis or by homogenization for conversion of insoluble into soluble protein nanoaggregates (Tang et al., 2009). The alkaline treatment is meant for the dissociation of subunits. The ultrasonication supports the reconstitution of building blocks into ordered nanostructures. The alkaline SP solution was incubated at a high temperature ($>55°C$) for about 2 h followed by suspending to neutral pH and acidic pH to remove the larger aggregates by unfolding the protein bulk aggregates to their individual units. Further, the SP solution is ultrasonicated at 40 Hz for a few mins. Then to attain the stability of the freshly prepared SP NPs were cross–linked using a conventional cross–linker, glutaraldehyde for about 16 h and centrifuged to remove the larger particles as a pellet. To achieve the purity, the SP NPs were subjected to membrane filtration of 3 kDa to remove the excess salts and stored at 4°C for long-term usage. The protein was subjected to alkaline pH to increase the inter repulsive forces further pH is shifted to neutral along with ultrasonication led to the fabrication of soluble nano-aggregates (Lee et al., 2016). The high hydrostatic pressure treatment along with ultrasonication facilitated the nanoaggregation due to favorable rearrangement among the unfolded protein subunits (Wang et al., 2011).

Electrospraying

This technique is similar to spray drying used for NPs, and nanocapsules fabrication. The electric field is applied to the capillary tube filled with SP solution, creating the electrically charged thin jet from the nozzle. The solvent in the formulation is evaporated and the solid nanoparticles were collected from the collector plates. The viscoelastic properties of the solution, the distance between the capillary tube and collector plates play an important role in the NP preparation. The SP solution at lower concentrations (<20 wt.%) exhibited low electrosprayability due to rigid 3D

structure where the obstruction of the chain folding or entanglements between the neighboring molecules (Pérez-Masiá et al., 2014). However, this can be controlled either by heat treatment or the addition of surfactants, gums, etc. This enhanced the electrosprayability favoring the nanocapsule formation. This method has also been used in the microencapsulation of thermosensitive molecules like β-carotene thereby increasing bioaccessibility (Gómez-Mascaraque & López-Rubio, 2016).

Self-assembly

Several proteins are intrinsically considered as self-assembled nanomaterials. In the case of SP, the oligomeric subunits such as 11S or 7S are also considered as self-assembled nanostructures (Tang, 2019). In this bottom-up process, the balance of attractive and repulsive forces between the building blocks forms an ordered nanostructure (Sanguansri & Augustin, 2006). This process is influenced by the temperature, pH, solvent, protein concentration, agitation speed (mechanical or magnetic stirring) in the formation of protein-based nanostructures. In general, proteins are subjected to heat or hydrolysis which denature initially and further the subunits tend to aggregate by means of self-assembly. The denaturation or disintegration of protein and reassembly of the subunits involves noncovalent (hydrogen bonding, hydrophobic) interactions and covalent (disulfide) bonds. Initially, Thanh and Shibaski demonstrated urea treatment at high concentrations induced the dissociation of conglycinin subunits and reassembled β subunit as core and α, α' subunits as shell forming core-shell nanostructures (Thanh & Shibasaki, 1976). Similarly, the heat treatment was initially applied on conglycinin to dissociate and reconstituted by providing favorable ionic strength with alteration in the tertiary structure (Wang et al., 2014). Recently, the disulfide bonds were cleaved by the reducing agents and reassembled into nanoparticles due to hydrophobic interactions. The strong hydrophobic interactions responsible for hydrophobic clusters favor the protein's structural integrity that accounts for the spherical morphology (Wang et al., 2014).

4.3.4.2 Soy protein nanoemulsions

The kinetically stable colloidal dispersion at nanoscale formed of two immiscible liquids (oil and water) using an emulsifier. Nanoemulsions (NE) are mostly in the range of $20-500$ nm. But, there is an argument in the scientific community about the metrology range (Jin et al., 2018; McClements & Rao, 2011; Singh et al., 2017). NEs possess exclusive characteristics such as stability, optical clarity as compared to the emulsion. Mostly proteins are used as an emulsifier. SP nanoemulsions are fabricated

by low energy methods and high energy methods. The latter method includes high–pressure homogenization, ultrasonic homogenization and microfluidics, mostly used for the SP NEs.

Microfluidics

The most commonly used method, one among the two immiscible liquids ruptures the other having the microscale thereby forming nano-scale dispersions. The protein is used as an emulsifier which is a tough task in the breakdown. So, high-pressure air is applied to break the coarse pre-mix to flow through the stainless steel microfluidizer ($\approx 10\,\mu M$) to form the nanoscale droplets. Such a high energy device is necessary to provide the homogenous premix flow and shortest time to produce nanoscale droplet preparation. In this regard, a high concentration of protein, and low concentrations of oil are required to ease the nanoemulsion forma-tion. This method is advantageous for high formulation volume, and uni-form size (de Oca-Ávalos et al., 2017; Tan et al., 2016).

Ultrahigh pressure homogenization

The SP variants (7S and 11S) are used by Xu et al., for the NE formation using this method. Similar to high-pressure microfluidics, high protein con-centrations and low oil concentrations were used to homogenize the disper-sions through a single pass of oil–water coarse emulsion. The NE droplet sizes are in the range of 100−500 nm (Xu et al., 2018). Alike, the NEs pre-pared from SP isolate with low concentrations of phosphotidylcholine had generated droplet size <433 nm (Li et al., 2007). The advantages of NE's fabricated using this procedure include good stability against creaming, agglomeration, and are used for long-term storage (Bhushani et al., 2016).

Ultrasonic homogenization

This method involves homogenization and sonication. The microdroplet coarse emulsion was prepared by homogenization with the agitation speed of 2000 rpm. Further, the ultrasonication is applied for 30 min resulting in the droplet size in the range of 230−350 nm (Jin et al., 2018; Teng et al., 2020). The homogenization facilitates better agitation and emulsification and the sonication involves the shear and cavitations respec-tively that disrupt the microscale to the nanoscale droplet. Ultrasonication is advantageous over microfluidization due to easy aseptic production, experimental setup, and economical. This method minimizes the turbidity subsequently, there is an increase in the zeta potential that in turn accounts for good stability (Teng et al., 2020).

4.3.4.3 Soy protein nanofibers

These are one-dimensional nanostructures with a good diameter and high aspect ratio. These are fabricated using a versatile electrospinning technique shown in Fig. 4.5. In this method, SP alone (mostly SPI) or in combination with both natural polymers (zein) (Phiriyawirut et al., 2008) or synthetic (polyethylene oxide) (Thirugnanaselvam et al., 2013) and polyvinyl alcohol (Cho et al., 2012) were used. In the typical procedure, the SP solution is pumped through a nozzle by applying an electric field. The fibers were collected on the metallic fiber collector that acts as a counter electrode. Considering the low cost mostly blends with the polymers have been used in the fabrication of SP nanofibers. The physicochemical conditions such as solubility, pH, and the right choice of the polymers, solvent for the blended solutions are responsible for the nanofiber fabrication. For instance, the PEO used increases the viscosity and minimized the thermal conductivity of raw materials favorable for forming the uniform fibers (Vega–Lugo & Lim, 2008). In some cases of blended SP/PVA fibers, the greater pH has a
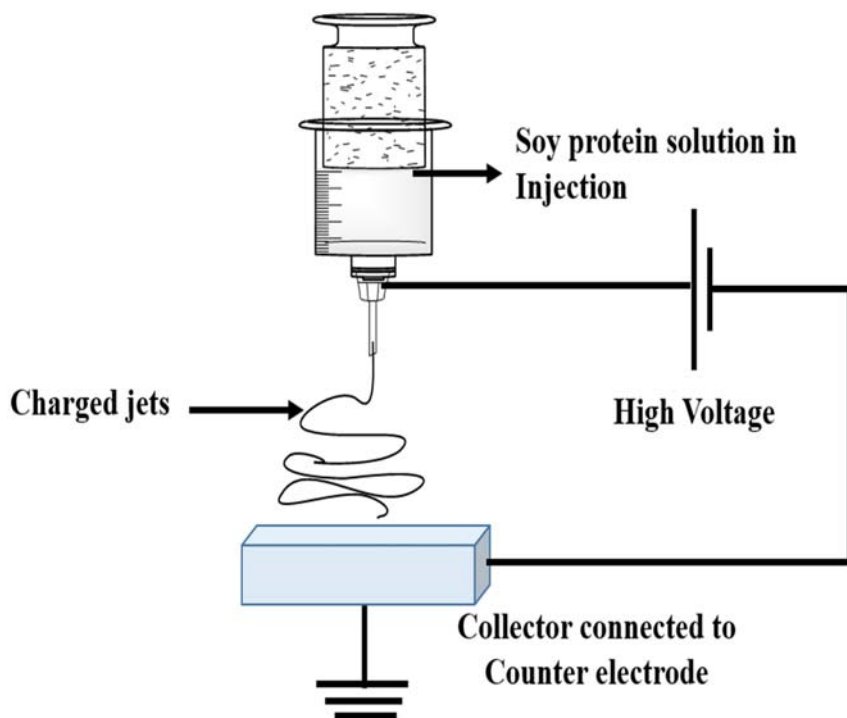

Figure 4.5 Schematic representation of experimental setup for electrospinning.

greater influence on the protein denaturation that corresponds to thinner electrospun fibers. Thus the thinner morphology has significantly reduced the mechanical property (Cho et al., 2012).

4.3.4.4 Soy protein nanocomposites

Soy protein nanocomposites (NCs) are those with two constituents that is SP or its variants and the filler that can be either organic or inorganic with at least one dimension in nanoscale (Xia et al., 2016). The nano-material can be nanotubes, nanofibers, NPs, nanowhiskers, nanocrystals, etc. The NP size, surface area to volume ratio, high aspect ratio of nano-fiber, and the polymer filler compatibility are the important factors in the NCs fabrication. The interfacial adhesion within the nanomaterial and SP matrix increases the properties (thermal, mechanical) as compared to its individual components (Koshy et al., 2015). Nanomaterials are incorpo-rated into the SP matrix to augment its structural, physical properties and its corresponding functions. Mostly hydrogels and films are used for the fabrication of SP-based NCs. The NCs can be synthesized in two meth-ods that is in situ polymerization and melt mixing method. The former method involves the fusion of nanomaterials and later involves mixing of the nanomaterials but the entangled conformations make this method a challenging task. Several researchers make use of the synthesized nanoma-terials to reinforce with the film or hydrogels forming the NCs. NCs were classified into two types depending on the fillers used.

Soy protein-organic nanocomposites

These include natural sources such as cellulose nanofibers (Chen & Liu, 2008), Starch NPs (Rahman et al., 2016). The starch which is abundant on earth is converted into NPs by the wet method. Using the solvent casting method, the starch NPs synthesized by a wet chemical process of less than 2% were incorporated into SP isolate matrix enhancing the strength and Young's modulus (Zheng et al., 2009). Tian et al., also demonstrated that the starch NPs incorporated into glycerol-SP films form NCs by strong hydrogen bonding. This has enhanced the mechanical strength (Tian & Xu, 2011). In the same manner, the electrospun cellulose fibers interpene-trated with SPI film matrix to enhance the mechanical stability, and the nanocomposite formed was found to be highly translucent. The high aspect ratio and strong hydrogen bonding favored the durable interfacial interac-tions (Chen & Liu, 2008). Mostly nanofillers are used in lower concentra-tions for better homogeneity, enhance the properties (mechanical, thermal

stability) and provide the benefit of components used (Zheng et al., 2009). Besides, such reinforcements have increased the homogeneity and transparency of the films (González & Igarzabal, 2015). To facilitate better bonding or interactions, the cellulose nanocrystals were surface-modified silane coupling agent and ethylene glycol diglycidyl ether is used as a cross-linker for the film formation. The significant chemical cross-linking between amino and epoxy groups led to the fabrication of NCs. This had also increased the thermal and water resistance property (Zhang et al., 2016).

Soy protein-inorganic nanocomposites

The inorganic fillers are widely used for various material science to biomedical applications. These include carbon nanotubes (CNTs) (Zheng et al., 2007b), nanoclay (Kamigaito et al., 1984), NPs of silicates (Xia et al., 2016), hydroxyapatites (Rahman et al., 2016), Ag (Koshy et al., 2015), ZnO (Xiao et al., 2020), TiO_2 (Tian et al., 2018), Cu Nanoclusters (Li et al., 2017a) etc. Similar to organic the reinforcement of inorganic fillers enhances the physical, thermal and mechanical properties. In addition, inorganic NCs have pronounced effects such as antimicrobial and wound dressing properties. These are fabricated by in situ and ex-situ methods. The precursors are added to the SP matrix for the NPs formation (Ray & Okamoto, 2003; Tian, 2012). This enables a better reinforcing effect eventually enhancing the tensile strength and Young's moduli of the NCs (Tian, 2012) Similarly, the SPI film was integrated with the phenyl polyhedral oligomeric silsesquioxanes using the (3-glycidloxypropyl) trimethoxysilane as a cross-linker through ring open polymerization. The amino groups of protein cross-link with the epoxy group of the cross-linker. The in situ fabricated NCs have enhanced the mechanical and water resistance properties (Xia et al., 2016). Ai et al. reported the synthesized silica NPs can be added to the protein matrix to fabricate the NCs. However, to achieve better mechanical strength, the NPs concentration should be below 8% (Ai et al., 2007). Several researchers' attention was dragged toward the clay which was approved by the US Food and Drug Administration for biomedical applications. The most common material present in the clay is Montmorillonite (MMT) which has a good elastic modulus contributing to mechanical property. Mostly, the clay is reinforced into the SP matrix by exfoliation and intercalation. In exfoliation, the nanoclay or silicates layers are discrete and dispersed in the protein matrix. In intercalation, the protein subunits are present between the nanoclay layers. Using the intercalation process, the MMT-SP NCs were fabricated. If the MMT concentration is

below 12% then the MMT is exfoliated and above 12% facilitates intercalation. This is due to the fact that heterogeneous distribution of positive (SP) and negative (MMT) charges for intercalation facilitates the exfoliation. Thus MMT concentration plays a major role in NCs fabrication (Chen & Zhang, 2006). Carbon allotropes like CNTs, graphene have been ideal materials due to their exclusive features like flexibility, high aspect ratio, mechanical strength, thermal and electrical conductivity (Li et al., 2017b; Yu et al., 2007). By compounding the one-dimensional multi–walled CNTs with SP matrix through casting and compression molding method. The protein chain wrapped on the NTs due to strong interactions had improved the mechanical strength (Yu et al., 2007). Li et al. reported the two dimensional, negatively charged graphene and positively charged polyethyleneimine (PEI)-modified cellulose nanocrystals with SP matrix by layer-by-layer assembly. The hydrogen bonding and the multiple interface interactions are responsible for NCs fabrication (Li et al., 2017b). The hydroxyapatite NPs were initially synthesized by the wet chemical method. Further, NPs were allowed for diffusion in the SP resin thereby forming NCs (Rahman et al., 2016). Considering the pharmaceutical and food industry applications, metal and metal oxide NPs were also used as fillers. Silver NPs (AgNPs) are well known for their antimicrobial properties. The SP is the nutrient of microbial growth, there is an immense demand to develop the NCs. In this regard, the AgNPs were prepared by in situ methods by blending the silver (0.1 wt.%) with the protein solution. Further, this AgNPs–protein solution is mixed with SP solution for film fabrication using the solution casting method. In a similar fashion, the titanium oxide NPs were embedded in the SP film. The TiO_2 NPs react with SP resulting in good interfacial adhesion between them that led to increased stress transfer from protein to inorganic fillers ultimately enhancing the tensile strength or the mechanical property (Tian et al., 2018).

4.4 Biomedical applications

Soy protein possesses significant biocompatibility, biodegradability, nontoxicity So, SP is regarded as a promising material to execute several biomedical applications. Soy protein was found to have potential applications as a carrier for the drug or nutraceutical, wound dressing, and tissue engineering.

4.4.1 Drug delivery

SP is one of the fascinating carriers of the drug with the aim of sustained drug release for a longer duration. Such cargos with targeted delivery are

in immense need of the right dosage and to avoid the side effects. This can be achieved by surface modifications, pH or temperature–dependent, and using biomarkers. The amphiphilicity of SP and the balance of hydrophobicity and hydrophilicity permits good interaction with the drug and solvent. This will in turn promote the easy penetration in the tissue of the target. A wide range of drugs is incorporated or immobilized with polar, hydrophobic, and charged amino acids of the protein which can be released upon reaching the site of action. In this regard, SP at both micro and nanoscale has been used as a promising carrier. The SP/zein microspheres were used for the controlled diffusion of riboflavin with zero-order kinetics (Chen et al., 2010). Zheng et al., demonstrated the drug release profile of theophylline using soy alginate microspheres where the equal ratio of carrier composition has shown a better release profile for 10 h at pH 6.8 (intestine) and 7.4 (Colon) (Zheng et al., 2007a). The SP films loaded with rifampicin showed a burst effect and followed zero-order kinetics. This study had shown the greater the cross-linking density lesser will be the erosion thereby increasing the scope of sustained release for longer periods (Chen et al., 2008b). In relation to the above study, SP films reinforced with MMT NPs have shown controlled release of ofloxacin of about 80% at alkaline pH. So, such nanocomposite film can be used for alkaline environments (Nayak et al., 2011). Recently, two polymers were molded using the extrusion method for the theophylline encapsulation. The skin barrier formed by two layers controlled the diffusion of the drug molecules (Vaz et al., 2003b). The SP hydrogels have been used in the drug delivery systems for anticancer. The blended polymers like poly (acrylic acid) (Liu et al., 2009) and (N-isopropylacrylamide) (Liu & Cui, 2011b) were used for pH and temperature stimuli drug release systems respectively. Nanocarriers can easily reach and interact with the targeted tissues and release the drug molecules. Nanocarriers as drug carriers are advantageous owing to stable dispersion, and bioavailability. The encapsulation of the drug molecules augments the bioavailability thus facilitating easy uptake of nanocarriers by the cells. Also, the residence time of drug molecules in the gastrointestinal tract is increased owing to the NP surface obtainable for interaction with the digestive tract (Chen et al., 2006). Thus the drug molecules using nanocarrier provide the platform for plasma bioavailability in high amounts as compared to the naïve drug molecules (Zou et al., 2013). Drug molecules are carried by the SP nanocarriers in three modes shown in Fig. 4.6.

1. Drug incorporation after nanocarrier fabrication (Adsorption)
2. Drug incorporation during nanocarrier fabrication (Layer-by-layer assembly)
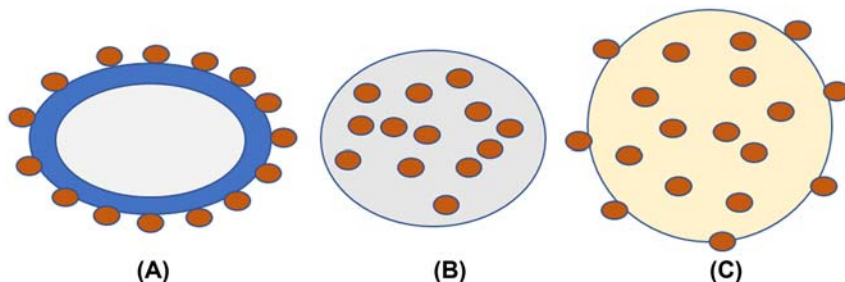
Figure 4.6 Drug molecules are carried by the SP nanocarriers in (A) Adsorption, (B) Entrapment or layer-by-layer assembly, (C) Dispersed along with SP.

3. Drug incorporation before nanocarrier fabrication

In the former, the SP surface amphiphilicity provides the opportunity to interact with both hydrophobic and hydrophilic drug molecules. Such nanocomplexation is mediated by hydrophobic interactions. In this context, Curcumin, a hydrophobic drug was solubilized with organic solvents (Ethanol, Methanol) prior to adsorbing on the nanocarrier. Such transparent nanocomplexation has enhanced the drug's UV stability, aqueous solubility, colloidal stability, and antioxidant activity (Chen et al., 2015a). To increase the loading efficiency of the curcumin Chen et al. had used ultrasonic treatment and achieved 14 wt.% on the SP. Ultrasonication helps the excess drug molecules loaded to disrupt and thus minimize the size of nanocomplexation. The hydrophobic interactions and disulfide bonds of the proteins provide scope for significant drug binding (Chen et al., 2015b). For long-term storage, the SP NPs bound with curcumin were coated with polysaccharide (SAPS) resulting in core-shell nanocarriers formation (Chen, Ou, et al., 2016). This nanocomplexation has improved the acidic pH triggered drug release profile, favorable for the cancer cells (Ochnio et al., 2018). Several water-insoluble and hydrophilic bioactive or drug molecules such as vitamin D (Levinson et al., 2014), resveratrol (Wan et al., 2014), CoQ10 (Chen, Zhang, et al., 2016) and folic acid (Ochnio et al., 2018), Vitamin B12 (Zhang et al., 2013) are delivered respectively. Drug molecules can be encapsulated during nanoparticle preparation by the layer-by-layer assembly. Mostly, the desolvation technique is used for the incorporation of the drug into the interior during the particulation. This offers the advantages of high encapsulation efficiency and maximal payload of the drug, long-term stability, and sustained release of the drug. Hydrophobic drugs like curcumin can also to loaded and released by using biphase (PBS buffer and tween 20). Further, folic acid

is conjugated with drug–loaded NPs for achieving targeted delivery. This advancement has increased Caco-2 cell absorption by 93% (Teng et al., 2013b). Certain bioactive molecules such as β-carotene encapsulated in SP NPs have shown increased cellular antioxidant potential as compared to the drug alone (Yi et al., 2015). Several bioactive molecules such as phytosterols (Cao et al., 2016), linoleic acid (Gao et al., 2014), etc. are delivered by this strategy. The drug molecules are incorporated prior to the self-assembly of SP subunits. The folic acid, polysaccharide, and SP were complexed at acidic pH and subsequently subjected to high–pressure homogenization and heat treatment. This method encapsulated the folic with a good loading amount was used for targeted delivery (Ding & Yao, 2013). The SP is subjected to high alkaline or acidic pH environment to increase the intermolecular repulsive forces between subunits thereby the protein is completely unfolded. Further, the pH is altered back to neutral for self-assembly. This approach is applied where the pH is changed to 12 and ethanolic vitamin D3 is added and subsequent ultrasonication forms the colloidal NPs (Lee et al., 2016). Other than pH shifting, SP is also treated with denaturing agents like urea to break the hydrophobic and hydrogen bonds. So, Wang et al., used this approach by initial heating the SP with high concentrations of urea and followed by dialysis. The pure SP solution is mixed with ethanolic curcumin which will readily form NPs due to hydrophobic interactions (Wang et al., 2018).

4.4.2 Wound dressing

A wound is due to the disruption of anatomical and physiological structure and function. Wound healing is a process that involves hemostasis, inflammation, proliferation, and remodeling stages that vary in their time periods. To repair the wounded tissue, several enzymes, cytokines, growth factors, hormones, and cells are involved. The initiation of healing involves the fibrin clot formation that halts the bleeding. Then the neutrophils in the vicinity ensure the area is free from microbes. Further, the growth factors released from macrophages and fibroblasts repair the wound. The wound type, size, and depth create the difference in the healing. Therefore the wound dressing is necessary to shield the wound from secondary infections either from external or from wound exudates and keep the wound moist. So, several researchers have focused on the fabrication of materials to protect the wound and affix the healing process. Soy protein is one of its kind due to the presence of arginine and glutamine

that are responsible for repair and as an energy source for inflammatory cells respectively. Also, scar formation can be minimized. The biomaterial fabricated from SP in various forms like nanofibers, films, hydrogels etc. is used due to the similarity with the extracellular matrix components. The formaldehyde cross-linked SP films showed biocompatibility and increased proliferation toward human keratinocyte cells (Curt et al., 2009). The glyoxal cross-linked SP films had minimized the biodegradability due to the high concentration of cross-linker (Vaz et al., 2003a). The material with better water vapor transmission is necessary to trap the wound fluids that provide a chance for microbial infection. In this context, SP films with water vapor transmission of about 2300 g/m^2 were fabricated to deliver the gentamicin drug targeting the wound with a bacterial infection (Peles & Zilberman, 2012). The SP scaffold loaded with the gentamicin was examined with guinea pigs for wound dressing. The histological studies for the postburn pigs have shown good re-epithelialization of the wound, epidermis to dermis adherence, and skin appendages (Fig. 4.7) (Egozi et al., 2015). The conventional local treatment is subjected to change frequently and is painful for the patients. To overcome, such scaffolds with good antimicrobial and biocompatibility properties pose a better alternative.

The blend of chitosan with SP membranes is one of the good choices for wound dressing material as they are biocompatible and induce better cell adhesion and proliferation (Silva et al., 2005). In relation, the in vivo studies not only proven the better wound healing ability by reducing the inflammation but also facilitated fresh tissue formation (Santos et al., 2013). The composite, Starch–SP films cross-linked with glutaraldehyde showed wound healing within 20 days in the in vivo studies. They also observed an increase in the uronic acid, collagen, hexosamine quantities in the granulation tissues around the wound (Chien et al., 2013). The prerequisite for wound healing is to retain the moist environment and protect from external mechanical stress. Hydrogels have been one of the solutions that possess water-absorbing nature and mechanical strength. In addition, hydrogels facilitate O_2 permeability into the wound as well as being the blocker for microbial growth. Thus hydrogels have been used for localized drug delivery systems to relieve the pain (Chien et al., 2013). The SP-polyethylene glycol hydrogels were used for transdermal drug delivery and wound healing (Meikle et al., 2012). Similarly, the soft hydrogel was fabricated to limit bacterial infection in open wounds. This material exhibited rapid epithelialization for keratinocytes proliferation and reducing scar formation (Shingel et al., 2006). Thus hydrogel had proven to be biocompatible, induce mineralization, and collagen deposition in fibroblast cells (Meikle et al., 2012).
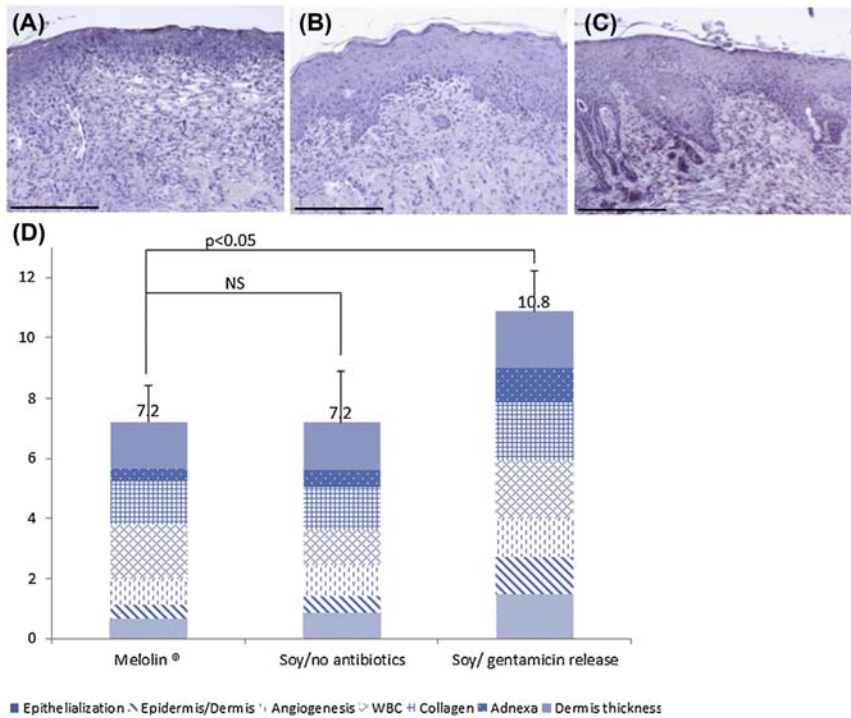
Figure 4.7 Representative histological sections of wound areas were taken from the center of the wound 12 days postburn creation. (A) Melolin-treated group. (B) Soy dressing without antibiotics. (C) Soy dressing with controlled gentamicin release. (D) Scoring of the histological sections of the wound area taken from the center of the wound 12 days postburn creation. *Adapted with permission from Egozi, D., Baranes-Zeevi, M., Ullmann, Y., Gilhar, A., Keren, A., Matanes, E., Berdicevsky, I., Krivoy, N., & Zilberman, M. (2015). Biodegradable soy wound dressings with controlled release of antibiotics: Results from a Guinea pig burn model.* Burns: Journal of the International Society for Burn Injuries, 41(7), 1459–1467. Copyright Elsevier.

4.4.3 Tissue engineering

The plant or naturally derived biomaterials provide immense opportunity to substitute or refabricate the damaged tissues or organs. This could be achieved by the biomaterials that offer cell adhesion, migration, and proliferation. The implants of such biomaterial induce the extracellular matrix to repair. The scaffold for tissue engineering was fabricated by 3D printing and freeze–drying methods. Chien et al. had used the uniform porous 3D SP scaffolds for the in vivo studies in mice where within 14 days have shown thick collagenous fibrous capsules due to better penetration of neutrophils

suggesting good biodegradability. They found good degradability without lingering local immune response at the implanted site of the mice as shown in Fig. 4.8 (Chien et al., 2013). The glyoxal and tannic acid cross-linked SP scaffold did not exhibit any cytotoxicity. In addition, the cell adhesion and proliferation of fibroblast cells were enhanced for seven days (Silva et al., 2003). The freeze-dried 3D porous scaffold using transglutaminase as a cross-linker had shown improved human mesenchymal stem cells seeding ability. This study stated that with an increase in the SP concentration there is an increase in degradation (Chien & Shah, 2012). The SP-chitosan blends mixed with tetraethyl orthosilicate induced apatite formation due to the presence of silanol groups (Silva, 2006). In a related study, the freeze-dried SP-cellulose sponge biomaterial has large pores with thin walls facilitating fibroblast cells proliferation (Luo et al., 2010). Thus such composite scaffolds can be applied for bone and cartilage scaffolds (Luo, 2010; Silva, 2006). The SP-alginate composite hydrogels incorporated with bioactive glass showed good mineralization, osteoconductive and mechanical properties.
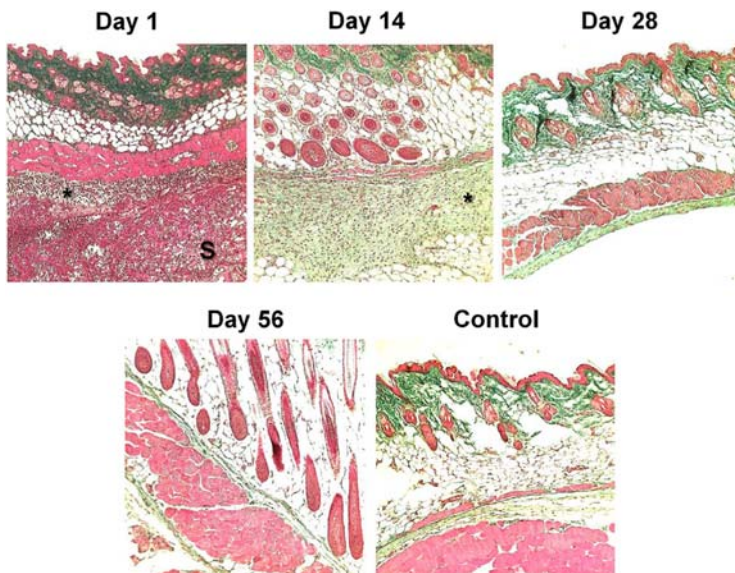
Figure 4.8 Quick degradation of 1% freeze-dried soy protein scaffolds at days 1, 14, 28, and 56 after implantation as observed using Masson's trichrome stain to visualize the fibrous capsule area. Healing of tissue to normal state and reduction of fibrous capsule size was observed after 28 days. S: scaffold; *: fibrous capsule. *Adapted with permission from Chien, K. B., Aguado, B. A., Bryce, P. J., & Shah, R. N. (2013). In vivo acute and humoral response to three-dimensional porous soy protein scaffolds.* Acta Biomaterialia, *9(11), 8983–8990. Copyright Elsevier.*

Such scaffolds are flexible as well thereby tending its ability for soft tissue engineering (Miguez-Pacheco et al., 2015). The SP-based hydrogel cross-linked with genipin was completely absorbed into the tissue at the wound. The cells were surrounded at the site forming ECM eventually a new dermis layer. In this context, the hydrogel revealed the tissue scaffolding abilities (Shevchenko & Santin, 2014). The fibrous scaffold mimics the ECM structure that offers the required milieu for cell proliferation. The electrospun SP nanofibers-based scaffold has good mechanical properties to support the growth of fibroblast cells (Reddy & Yang, 2009).

4.5 Challenges and future prospects

The unique characteristics of SP have extended the wide scope in biomedical applications. However, there are certain challenges in SP forms. The extraction procedures should be precise for obtaining the pure soy protein that is preferred for biomaterial fabrication. The unbalanced globular structure could be a possible reason for the defects in nanofibers eventually causing weak tensile and mechanical strength. So, tuning the protein structure depending on the required dimension is one of the major challenges. In prospect of nanoparticles, the targeting agents should be functionalized to be site-specific or tissue. There are several routes of SP-based biomaterial preparation were executed yet a lot of research to be focused on the large production.

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Details the latest developments in the synthesis, characterization, and biomedical applications of protein biopolymers.

- Covers a range of protein-based biopolymers, including gluten, collagen, keratin, soy, and more
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Protein polymers have garnered increasing focus in the development of biomedical materials, devices, and therapeutics, due to their intrinsic bioactivity, biocompatibility, and biodegradability. This book comprehensively reviews the latest advances in the synthesis, characterization, properties, and applications of protein-based biopolymers; each chapter is dedicated to a single protein class, covering a broad range of proteins, including, silk, collagen, keratin, gluten, and more. The book explores the biomedical potential of these polymers, from tissue engineering to drug delivery and wound healing.

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S M A R T
SUPERCAPACITORS
Fundamentals, Structures, and Applications

Edited by
Chaudhery Mustansar Hussain
M. Basheer Ahamed

Smart Supercapacitors

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Part One

Fundamentals of supercapacitors

General introduction about electrochemistry and supercapacitors

1

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1.1 Electrochemistry

1.1.1 Introduction

Electrochemistry is significant area of contemporary research unifying the knowledge of chemistry and electricity. Initially, this concept was used by Volta in 1793 to make the battery, and then by Nicolson to decompose water into its constituents. Then, Faraday established the quantitative relation between electricity and synthesized product. The electrochemistry deals with electrolytic reaction conducted in electrolytic cell which is made of electrodes and electrolytes that provide electricity. The electrodes are generally fabricated by using metal or semiconducting material, whereas the electrolyte is either aqueous/organic ionic solution or may be in the solid state. Later on, the kinetics and thermodynamic studies of electrochemical reactions were included as an essential part of electrochemistry [1–5]. In the applicability of electrochemistry, the energy conversion and storage have become fundamental requirement of human being now these days. In this regard, fuel cells, ion batteries, capacitors, and supercapacitors (SCs) are being used because of their nonpolluting nature and high power density.

Electrochemistry, a branch of chemistry, deals to chemical reactions involving electrical currents and potentials where spontaneous reactions are capable of generating current for doing useful work. For example, the batteries are used to produce electricity which is converted for useful work. While some chemical reactions are forced to proceed using electrical current. Based on these reactions, several practical products are developed and used for daily life work such as smartphones, automobiles, watches, electronic tablets, pacemakers, and others. Electrochemistry deals with reactions involving electron transfer reactions such as redox reactions and is implemented for purifying or electroplating metals. Electrochemical reactions are conducted into electrolytic cell where the redox reactions are performed at electrodes. For instance, in the battery, there are certain chemicals those react with each other for producing an electrical current. Such batteries are used and thrown but in the current time the

more sophisticated batteries are used those can be reused by recharging with electricity such as batteries used in portable electronic devices like cellphones, computers.

1.1.2   Electrochemistry and energy storage devices

Batteries, full cells, and electrochemical capacitors/SCs are the examples of energy storage and conversion devices, and a schematic sketch of such typical systems is shown in Fig. 1.1. For charge process, such systems have to be connected with an external source (Fig. 1.1), then these systems store a finite charge Q. Due to this, the Q is stored as chemical energy by the conversion of electric energy. During the discharge process, system is connected with an external resistive circuit (Fig. 1.1) where it releases the stored Q and generates a current through the external circuit. The system converts the stored chemical energy into electric energy in discharging process.

1.2   Supercapacitors

1.2.1   Introduction

In scenario of changing global landscape, scientists and researchers of scientific communities have focused on fabricating and refining better efficient energy storage devices (ESDs). For instance, SC which has been ripened and emerged significantly having potential to develop advanced ESDs. SCs are ultra- or electro-chemical ESDs containing electrodes with large surface area and thin electrolytic dielectrics than conventional capacitors for large magnitude of capacitances and have potential to attain greater density of energy [6–10]. Capacitors are electrical charge (EC) storage physical devices without chemical or phase changes with highly reversible process. Electrochemical capacitors are also known as SCs those stock EC in an electric double layer (EDL) between the larger-surface-area containing carbon electrode and liquid electrolyte [6,7]. That's why SCs are also referred as EDL capacitors. A simple SC can be demonstrated as shown in Fig. 1.2 where constructed two conductors are inserted in a container with an electrolyte.
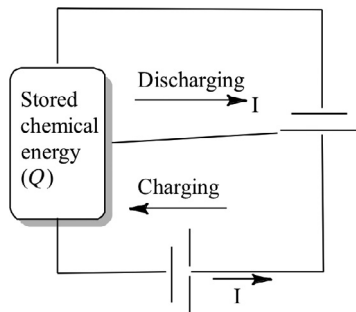

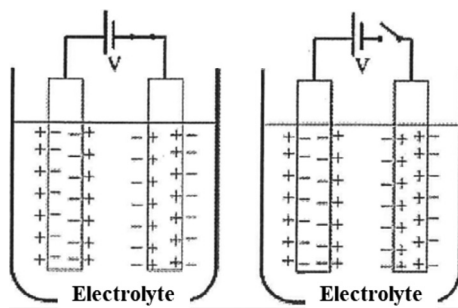**Fig. 1.1** Charging and discharging of a capacitor.

Fig. 1.2 Demonstration of simple supercapacitor.

In such device, initially no voltage, but upon connected through battery the current flow from one rod to the other, and due to which a charge separation is caused at interface of solid (electrode) and liquid (electrolyte). Such series of capacitors are connected through electrolyte where the voltage perseveres after the switch is opened-energy has been stored. At this time, solvated ions present in the electrolyte are attracted to the solid electrode surface by an equal but opposite charge in the solid, and such charge regions in parallel way create double layer. This charge separation and surface area are measured in molecular dimensions like angstroms and m^2g^{-1} of electrode material. For storing energy with a conventional capacitor, the charge carriers (electrons) are removed from one metal plate and depositing them on another metal plate. Due to which the charge separation is occurred and that develop the potential between the two metal plates, that is harnessed to an external circuit. The stored energy depends on the number of stored charges as well as potential between the plates. The earlier is the required function of size and the material used for making electrode whereas later depends upon dielectric failure. Thus, the efficiency of capacitor to store voltages depends on material quality that leads to larger energy densities. Apart from this, SC not require a conventional dielectric where it uses two layers of plates made up by the same material along with their electrical properties refer to EDL. Because of this, the efficient charge separation occurs, although the separation of the layers is very thin in nanometer range. This nanosized separation allows the plates with much larger surface area that develops extremely high capacitances.

1.2.2 Classification of supercapacitors

Based upon Faradaic and non-Faradaic mechanism for storing charge, the SCs are classified in three categories; such as EDL capacitors, pseudocapacitors (PSC), and hybrid capacitors (HCs), as depicted in Fig. 1.3.

1.2.2.1 Electrochemical double-layer capacitors

EDLCs are built by an electrolyte, two carbon electrodes and a separator, and store charges non-Faradaically or electrostatically where charge transfer between
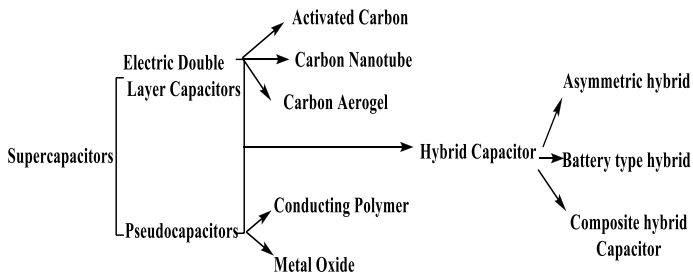
Fig. 1.3 Classification of supercapacitors.

electrolyte and electrodes is prohibited. In such SCs upon applying voltages, the charges are accommodated on the electrodes in form of EDL to store energy. The opposite charge ions are around the separator where the construction of electrode prevent the attraction of the ions, and EDL of charge is developed at each electrode. Such EDLs cause larger surface area and short the distance of electrodes, which leads higher energy densities. Since, the charge transfer as well as chemical change are not occurred between electrolyte and electrode that's why reversible charge transfer process is found. Since the performance of capacitor is measured in terms of charge-discharge cycles (CDC), so the EDLCs has many more CDC as compare to others [6–8]. The nature of electrolyte directs the CDC of EDLCs where aqueous or organic electrolytes may be used. For example, an aqueous electrolyte like KOH and H_2SO_4 decrease the ESR as compare to acetonitrile (an organic electrolyte). Thus, capacitance, ESR, and voltage are the key factors while selecting electrolytes to play important role in applications of SCs [11]. The material used for the fabrication of electrodes also play critical role for the energy storage. For example, material having less surface area are not effective to store energy. Therefore, recently carbon materials in different forms are being used for making electrode because they are low-cost material with larger surface area [11]. Some of them are as follows.

1.2.2.1.1 Activated carbons

It is mostly used carbon based material for the fabrication of electrode due to its low cost with larger surface area as compare to others. It has porous structure with little bit variation like it develops micropores, mesopores and macropores structures with < 20 Å, 20 – 500 Å, >500 Å pore sizes, respectively, leading to larger surface areas. However, the whole surface area of activated carbon is not used for the capacitance because of nature of electrolyte and pore size of activated carbon [11–15]. Since, the higher power and energy densities are associated with larger and smaller pore sizes respectively, therefore, the controlling pore size of activated carbon is a thrust area for designing EDLCs [15].

1.2.2.1.2 Aerogels of carbon

A network of conductive nanoparticles of carbon with specific pore size, develops carbon aerogels without binding agent. Such material shows low ESR on compare

with activated carbons [16], and leads to high power density which is key factor for EDLCs.

1.2.2.1.3 Carbon nanotubes

Carbon nanotube is a current research area for the researcher as electrode material for EDLC because this is a network of interconnected mesopores that increase the charge distribution and high CDC due to high surface area [17–21]. Such interconnected mesopores cause easy diffusion of ions which leads to low ESR with high power density [22–24]. This network of carbon nanotubes grow onto current collector reduces ESR and as a result high energy density as compared to other carbon sources.

1.2.2.2 *Pseudocapacitors (PSC)*

The PSC allows the charge transfer between electrode and electrolyte, and store charge Faradaically via electrosorption, redox and intercalation processes [25,26]. This process develops higher capacitances with high energy densities as compare to EDLCs [27–29] where conducting polymers and metal oxides are used as electrode materials.

1.2.2.2.1 Conducting polymers

Conducting polymers have advantages over carbon-based electrode materials, such as higher capacitance with conductivity and less ESR [12]. For instance, the n-/p-type polymer with great potential energy holds high power densities [30], however, the mechanical stress is a limit for the stability of such PSC during CDC [25–30].

1.2.2.2.2 Metal oxides

The greater conductivity of metal oxides allows them to be used as electrode material for PSC, such as ruthenium oxide [21–33] which is most studied. Ruthenium oxide has amorphous structure which can perform insertion and removal of protons due to which it shows higher capacitance. The hydrous form of ruthenium oxide has comparatively low ESR than others that leads higher energy and power densities for PSC.

1.2.2.3 *Hybrid capacitors*

HCs have relative advantages over EDLCs and PSC because they use both processes to store charge like Faradaic and non-Faradaic processes. Such capacitors have greater energy and power densities without the disturbing CDC and its stability.

1.2.2.3.1 Composite

Such electrodes are fabricated by assimilating carbon-based materials and conducting polymer or metal oxide which leads to have physical and chemical charge storage mechanisms within electrode. Here, the earlier facilitate a EDL of charge by providing large surface area and enhances the interaction between PSC materials and electrolyte whereas the latter increases the capacitance via Faradaic reactions [21,22,25–35]. The example for such electrode is electrode containing carbon nanotubes and polypyrrole (conducting polymer), and used to have higher capacitances than other [22,28,36].

1.2.2.3.2   Asymmetric

The combination of electrodes of EDLC and PSC produces the asymmetric hybrids through Faradaic and non-Faradaic processes, for instance, a combination of activated carbon -ve with conducting polymer +ve electrodes [37–40]. These capacitors have better cycling stability than comparable PSC [38–41].

1.2.2.3.3   Battery type

The battery-type electrodes are made up by coupling of a EDLC electrode and battery electrode. These types of electrodes stand on the need of higher energy SCs [42–45].

1.2.3   Theories

As shown in Fig. 1.4, the conventional capacitor has two electrodes separated by an insulating dielectric material, and upon applying voltage the opposite charges are accumulated on the surfaces of each electrode, and energy is stored.

Generally, the capacitance C is explained as a ratio of stored (positive) charge Q and applied voltage V, such as $C = Q/V$. For a conventional capacitor the relation of capacitance, the surface area of electrode (A), and distance (D) between the electrodes is given as:

$$C = \varepsilon_0 \varepsilon_r \frac{A}{D}$$

ε_0 and ε_r are dielectric constants of free space and insulating material between the electrodes respectively. The stored energy (E) in a capacitor is always directly proportional to C as:
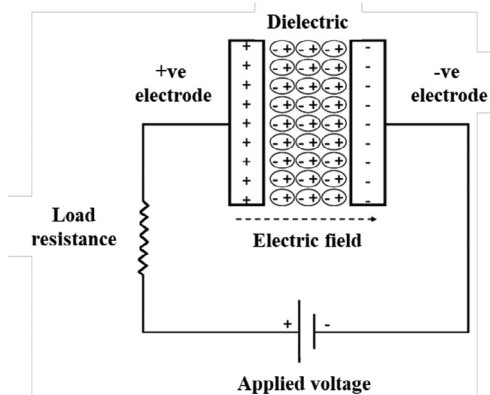
$$E = \frac{1}{2} CV^2$$

Fig. 1.4 Scheme of conventional capacitor.

The illustration of a typically conventional capacitor is shown in Fig. 1.2 where the energy expended per unit time is power (P).

The electrodes and dielectric material are responsible for the resistance from which the voltage during discharge process is determined through these resistances. The maximum power (P) of a capacitor is given by the below equation where ESR is an equivalent series resistance [6–10].

$$P = \frac{V^2}{4 \times ESR}$$

The conventional capacitors have comparatively high power densities, but low energy densities as compared to batteries and other fuel cells. In contrast, the battery has low power and high energy densities as compared to capacitor which leads slow delivery of energy.

The same principle is followed in the case of SCs where they utilize electrodes having high surface areas A with thinner dielectrics which shorten the distance between the electrodes, and enhance the capacitance as well as energy. SCs (typical illustration is shown in Fig. 1.4) maintain low ESR to attain needed power densities and have numerous advantages over batteries and fuel cells, such as high power density, short charging times, and long life cycle [6–9]. Fig. 1.2 provides a schematic diagram of a SC, illustrating some of the physical features described above.

A comparative analysis of battery, conventional capacitor and SC is shown in Fig. 1.5 (Ragone plot) where the power densities of various ESDs are compared with energy densities. The energy density of SCs lies between the energy densities of conventional capacitor and battery.
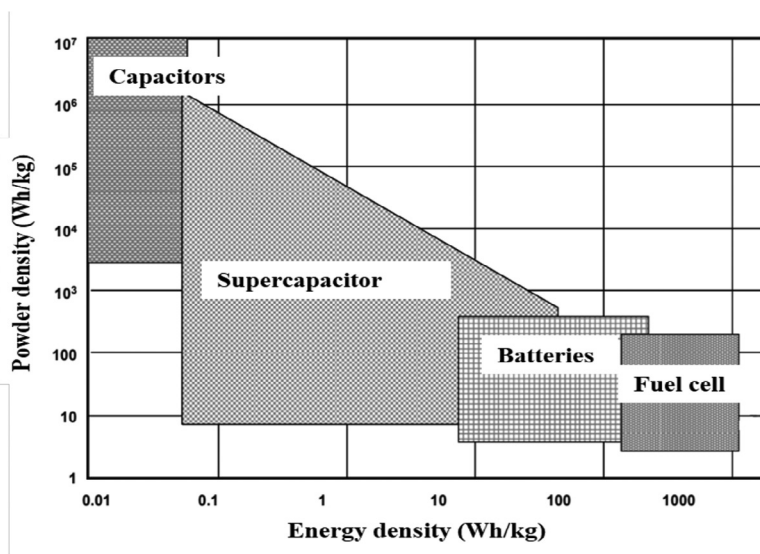

Fig. 1.5 Differentiation of capacitor, batteries, supercapacitor, and fuel cells.

For the understanding of electrochemical capacitance in SCs, the knowledge of EDL and PSC is required that facilitate to know about hybrid or asymmetric capacitors. Many theories have been proposed to understand the mechanism of SCs.

1.2.3.1   Helmholtz theory

A theoretical explanation was given by Helmholtz for the understanding of EDL where two layers of charged ions are developed between the interface of electrolyte and electrode due to applied voltage, as shown in Figs 1.6 and 1.7.

First layer is formed at the electrode's surface contacting the electrolyte whereas another layer is formed from solvated/dissolved ions of electrolyte being attracted by the polarized electrode. These two layers are separated by the monolayer of solvent
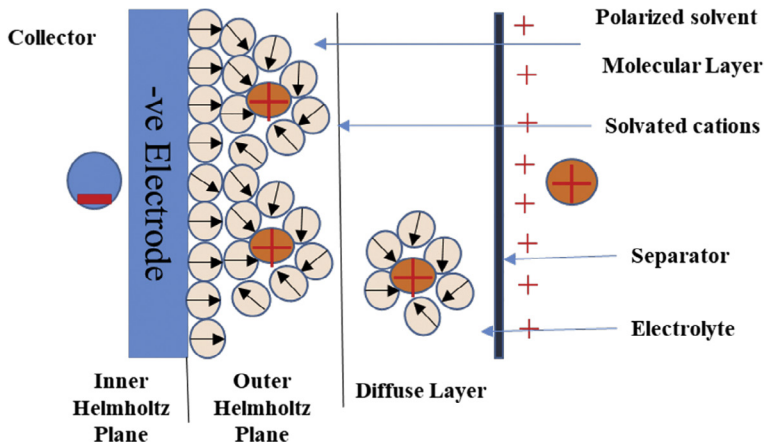

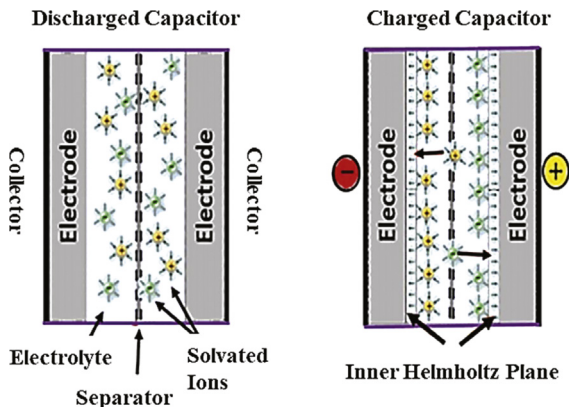**Fig. 1.6** Explanation of electrical double layer.

Fig. 1.7 Process of supercapacitor with electric double layer model.

molecules which is called inner Helmholtz plane (IHP) where it functions like molecular dielectric separator. In outer Helmholtz plane (OHP) the electrode's charges are balanced by opposite ions available in area quantitatively where the strength of applied voltage is accountable for developing a stable electric field in the IHP of the solvent's molecules. The electrode's surface area and the number of adsorbed ions decide the quantity of electric charge accrued in the layers. The EDL capacitance is obtained using the following equation.

$$C_d = \frac{\varepsilon}{4\pi\delta}$$

Here C_d, δ, and ε are differential capacitance, charge layer separation, and dielectric constant, respectively [25–30]. In SCs, the charge of one of the Helmholtz layers at one electrode is opposite to other layers, that's why the resultant capacitance of series two capacitors is total capacitance of SC. Similarly, the total capacitance of symmetrical SC is the average capacitance of two electrodes because the capacitances of both electrodes are similar. Therefore, this theory provides explanation for the ionic layer and electrode in a SC, although this theory could not explain the interactions of electrode with the electrolyte as well as rising the PSC.

1.2.3.2 Gouy Chapman and Stern theory

"The value of capacitance in EDLc is not constant" this observation was studied L.G. Gouy and D.L. Chapman where they noticed that the capacitance varies with the electrolyte's concentration and applied voltage. Therefore, they suggested a diffuse model describing the dependence of the ionic charge distribution on distance from the electrode surface which led to the application of the Maxwell Boltzmann statistics [46]. The Stern layer informs about an impact of the ionic size where the radius of ion is almost equal to the distance of ion and electrode.

1.2.3.3 Grahame theory

Grahame suggested that when ions approach the electrode then some charged or neutral species can pass the layer despite the closeness to electrodes [22]. The attendance of specifically and nonspecifically adsorbed ions the IHP crosses via the centers of the specifically adsorbed ions while OHP is at the distance between solvated ions (adsorbed ions) and the electrode which passes through their centers.

Here the closest inner layer is recognized as Helmholtz layer or compact layer or Stern layer which is consisted by the solvent molecules/ions or specifically adsorbed molecules with IHP and OHP. The OHP is the beginning of the diffuse layer where solvated ions are adsorbed non-specifically, and circulated in the bulk from OHP forming 3D diffuse layer. The thickness of this diffuse layer is found out using ionic concentration, and the capacitance of the EDL is obtained using the following equation.

$$\frac{1}{C_{dl}} = \frac{1}{C_{diff}} + \frac{1}{C_H}$$

The C_{dl}, C_{diff}, and C_{H} are capacitances of the double layer, diffuse region, and Helmholtz region, respectively [47].

1.2.4   Construction of supercapacitor

The ceramic or electrolyte capacitors need a dielectric material while SC does not require this. The SCs is constructed by two porous electrodes with a separator, suitable electrolyte, and current collectors.

1.2.4.1   Current collectors, electrodes electrolytes, and separator

These are built by an aluminum foil instead of titanium or platinum (because aluminum is cheaper) and are coated by the same material which is used for making electrodes. The performance of the electrodes is depending upon the capacitance, so for maximum capacitance the material having larger surface area is used. For this purpose, electrodes are built using highly porous powdered coated material like active carbon or carbon nanotubes. Due to this such material store maximum charges like ions or radical from electrolytes. The electrolyte determines the ESR and can be either aqueous or nonaqueous; however, the nonaqueous electrolytes are given preference due to their high capacity to provide high voltage. Nonaqueous electrolytes consist of ionizable salts in suitable solvents, for instance, acetonitrile or propylene carbonate as solvents and tetraalkylammonium or lithium ions as solutes. The separator is made up of transparent material which is placed in between electrodes, and functions as insulator to prevent from short circuits.

1.2.5   Mechanism of supercapacitor [48–50]

1.2.5.1   Charging process

1. When voltages are applied then ions of opposite charges from electrolyte are attracted by collectors.
2. Attracted ions are collected on the surface of collectors, and charges are developed on each collector.
3. Since due to this two separate layers of charge have been formed, and because of this SC are known as EDLC, as shown in Fig. 1.8.

1.2.5.2   Process of discharging

1. The ions are not attracted by the current collectors.
2. There is a disturbance of ions in the electrolyte.
3. There is a decrement in charges on the surface of collectors.

1.2.6   Merits of supercapacitors

The materials used in construction of SCs as mentioned above, provide high capacitance causing several advantages of SCs over conventional capacitors, for example,
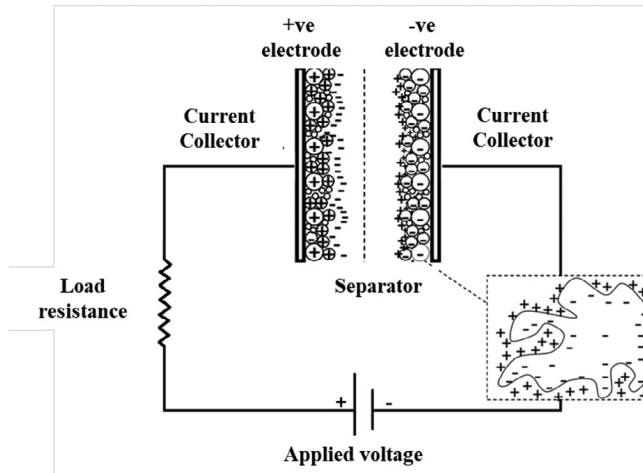
Fig. 1.8 Scheme of an electrochemical double layer capacitor.

high-energy storage capacity, long shelf life. SCs are rechargeable within short time that causes high and frequent power density.

1.2.7 Application of supercapacitors

The unique storage ability makes SCs widely applicable in various things such as UPS, electric drives, electric vehicles, traction, LED flashlights, etc.

1.2.7.1 Hybrid buses

SCs gives biggest market of transportation where electric vehicles are the example for the same due to quick charging. In this regard, in India, in 2017, the emission-free electric buses have been introduced by the Brihan Electric Supply and Transport. Batteries take time to get charged. While braking in vehicles, the back electromotive force is developed by the motors, which is used as regenerative energy for the charging the SCs. This concept has been applied in Shanghai city where buses are running on SCs where they take 1–2 min to charge using regenerative energy produced while braking. The combinations of SCs and batteries in buses has advantage to increase the performance of buses.

1.2.7.2 Automotive

During braking, the back EMF charges the SCs within a short time, this store energy is used to start engine again, and power the vehicle. In India, Tata Motors has recently introduced the first vehicle as Tigor EV for the government run entity Energy Efficiency Services.

1.2.7.3   Traction

The power of vehicle is positive or negative during acceleration, cruising (running at normal speed), and deceleration phases. This variation of power may cause serious trouble like voltage fluctuation or losses in main power supply, etc. To overcome this problem, the SCs are used as a storage device for the short term having interface of dc–dc altering.

1.2.7.4   Electronic applications and renewable energy [51]

Now these days, the SCs are being used in laptops, mobile phones, radio tuners, etc., where bursting power is required. Similarly, three blades containing advanced speed turbines are also used where flexible blades angle is adjusted at operating point. In such system, the blades are driven to 90° position for preventing the mechanical damage of their while failure of power converter. Therefore, in this regard the SCs are used in wind energy to provide power for controlling blade pitch.

1.3   Conclusion

In this chapter, we have focused the construction, working mechanism, and recent developments of SC. The chemistry of effective material for the construction of SC has also been explained. Therefore, this chapter will help the researcher working in the field of energy storage and utilization.

Acknowledgments

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S M A R T
SUPERCAPACITORS
Fundamentals, Structures, and Applications

Supercapacitors are the electrochemical energy storing devices that bridge the gap between fuel cells and rechargeable batteries. Smart Supercapacitors: Fundamentals, Structures and Applications presents the current research and technology on smart supercapacitors and explores their rapidly emerging characteristics and future potential advancements. It begins by describing the basics and fundamentals related to supercapacitors, and their applicability as smart and next generation energy-storing devices. This is followed by a discussion of electrode materials, their fabrication and specific designing techniques, and concludes with a review of the application and commercialization of this technology. This book appeals to the researcher and engineer from both academia and industry and will be a vital resource that will enable them to revolutionize modern supercapacitors.

Key features:

- Explores the potential applications of supercapacitors

- Covers entire spectrum of new advances and recent trends research of supercapacitors

- Explains reliability, safety, economics, and market trends for the use of supercapacitors from a sustainable perspective

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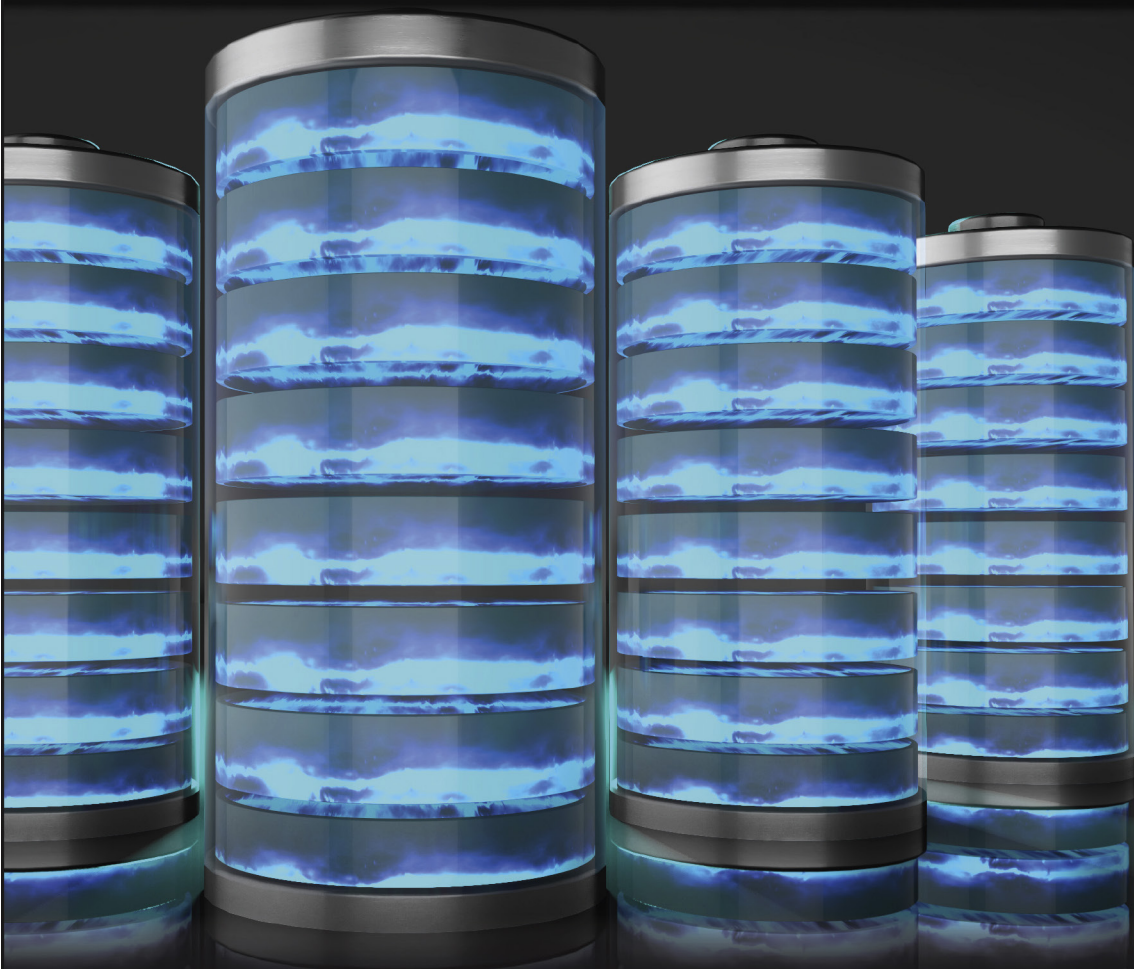
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Part One

Fundamentals of supercapacitors

Supercapacitors— new developments

<div style="text-align:right;font-weight:bold;font-size:2em;">3</div>

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3.1   Introduction

Energy is the prime thrust in today's contemporary world. This requirement can be gratified by the application of clean energy alternatives and efficient energy storage devices (ESDs) like supercapacitors (SCs), fuel cells, batteries, and some other ESDs. SCs are the high-performance sole devices, devoted for energy storage [1] which can enhance the growth rate of low-power electronics and high-power electronics mostly used in the household and military applications respectively. SCs are actually ESDs that are in the range between batteries and capacitors [2]. Fossil fuels are limited and cause serious pollution problems [3], by taking this into consideration many alternative technologies came into existence [4].

In many diverse fields wherever there is need of high and stable energy throughput, SCs are considered as promising candidates in advanced electric-powered devices [5]. Even though SCs are unable to store high level of energy, hence they cannot apply in energy backup devices, but in other ways SCs tie both batteries and conventional dielectric capacitors together [6]. Moreover, SCs are more promising than conventional dielectric capacitors due to their rapid charge/discharge processes, high-power densities, which help in providing reliable power throughputs. Energy and power density (EPD) correlation study needed for the estimation of the energy storage performance of various devices. Subsequently, Ragone plot (Fig. 3.1) graphically described the correlation of energy with power density for various representative ESDs which can be useful for estimating energy storage performance [7]. Fig. 3.2 described the difference in batteries and SCs in their power, charge rate, energy storage and storage mechanism, and charge life limitations [8]. SCs have an extraordinarily high cycle life than batteries and also require 1–10 s recharge times which is very less compared to battery (10–60 min) [8]. Also, in SCs the charge storage mechanisms involve irreversible reactions which resulted in high life cycles. Materials and electrolytes used in SCs for electrochemical processes provide tunable thermal stability to SCs which allow to operate at −40 and 100°C temperature range [9], while batteries can be functioned at −20 and 60°C [10,11]. In Table 3.1, some significant characteristics
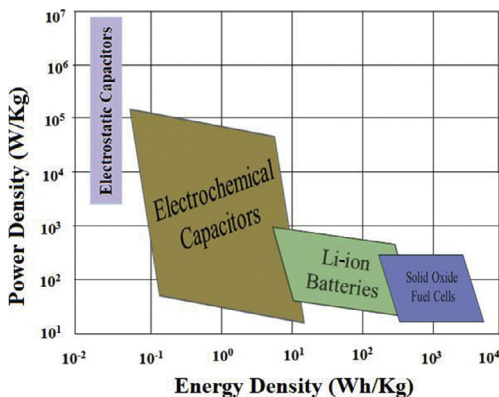
Fig. 3.1 Ragone lots for representative energy storage devices such as batteries, fuel cells, and supercapacitors [7].

such as cycle life, CDg time, specific energy, and charge storage mechanism of SCs, capacitors, and batteries are concisely compared.

To enhance the SCs performance many efforts have been made such as to develop materials with a significantly large effective surface area (ESA) like nano or micro materials and porous materials [12,13], which can improve the exposure of electrode surface to the electrolyte [14] and also to increase the mass transport [15]. Commercially, many types of SCs are available in the market, based on their electrode materials or charging mechanisms, they can be classified into hybrid capacitors (HC) pseudo capacitors (PC), and electric-double layer capacitor (EDLC) as shown in Fig. 3.3 [16]. Every type of SCs differ in their characteristic properties. Cycle stability is a very vital factor for SCs evaluation. Carbon-based electrodes are used in most of the EDLCs which displayed good cycle stability as shown in Table 3.2 [17–29]. Moreover, PC has a smaller power density than EDLCs because faradic processes are sluggish than that of electrostatic processes. PCs and EDLCs have been integrated as HC such as MnO_2-NPG/PPy-NPG for high energy and power density (HEPD) and good operating voltage window [29]. Herein, we have provided roadmap of the evolution, progress, and developments in SCs starting from the evolution of capacitors to the recent developments taken place in SCs (Fig. 3.4).

3.2 Materials for supercapacitor electrodes

Materials used for the SC electrodes are accountable for charge storage and capacitance of SC. Therefore, the ultimate goal for newly designed materials (NDMs) is to have high capacitance compared to conventional materials used in the SC. In general, SCs capacitance mostly rely on ESA, these NDMs provide more ESA which is available for the interaction of electrode and electrolyte. The capacitance is not always proportionate to the ESA [30], where electrochemically accessible area can only be
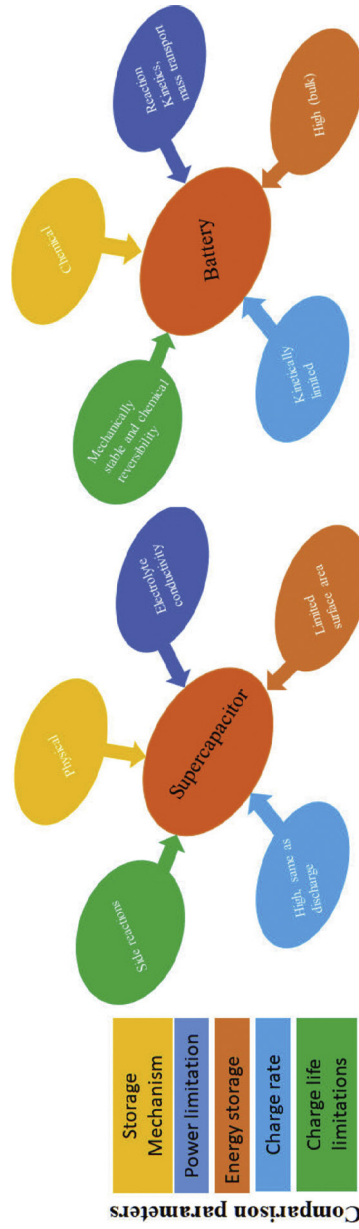
Fig. 3.2 Comparison between batteries and SCs through different operational parameters [8]. SCs, supercapacitors.

Table 3.1 Comparison of the basic performance metrics between different electrochemical energy storage systems.

Order	Characteristics	Capacitor	Supercapacitor	Battery	References
1	Specific energy (Wh kg^{-1})	< 0.1	Up to 1091	Up to 1606	[154,155]
2	Specific power (W kg^{-1})	> 10,000	Up to 19,6000 < 1000	< 1000	[154]
3	Discharge time	$10^{-6}-10^{-3}$ S	s to min	0.03–3 h	[156]
4	Charge time	$10^{-6}-10^{-3}$ S	s to min	1–5 h	[156]
5	Coulombic efficiency (%)	About 100	Up to 99 [157]	70–85	[156]
6	Cycle life	Almost infinite	>500,000	About 1000	[156]
7	Charge storage determinants	Electrode area and dielectric	Microstructure of electrode and electrolyte	Thermo-dynamics and active mass	[156]
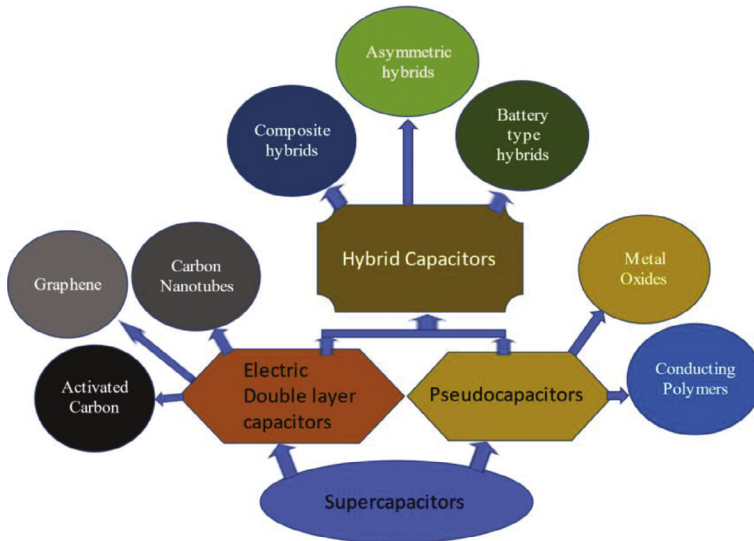
Fig. 3.3 Types of supercapacitors [16].

considered as the electrochemically active surface area (ECASA) [31]. The ECASA is also depends on the pore size and which can be tuned either by making hybrids or composites by introducing nanostructures (graphene, CNT, buckyballs, metal NPs, metal oxide NPs) and conducting polymers (CPs) [32–34].

Table 3.2 Comparison of the key performance metrics between diverse supercapacitors.

Order	Supercapacitor, electrode	S_A (m² g⁻¹)	C_{SP} (F g⁻¹)	E_D (Wh kg⁻¹)	P_D (kW kg⁻¹)	Cycling stability	Measurement done at	Electrolyte	References
a	EDLC								
1	CNS	791.0	560	78	2.8	97% (1000)	10mVs⁻¹	1M HCL	[17]
2	PCNC	1779.3	161	30.2	20.3625	85.4% (5000)	1 Ag⁻¹	1M TEABF₄/AN	[18]
3	CMCNC	1198	194	56.1	61.25	90.6% (100k)	1 Ag⁻¹	1M ionic liquid	[19]
4	CNDHS	2091	183	28.875	37.125	91.1% (100K)	1 Ag⁻¹	1M TEABF₄/AN	[20]
5	AC	2731	311	8.3	18.75	96.4% (5000)	0.5 Ag⁻¹	6M KOH	[21]
6	AC	2731	178	39	6.7	87.5%	0.5 Ag⁻¹	1M TEABF₄/AN	[21]
b	Pseudo								
7	Ni-based MOF	–	705	29.6	0.46	92.1% (5000)	1 Ag⁻¹	6M KOH	[22]
8	Co(OH)₂	–	1287.2	–	–	59.2%	2 Ag⁻¹	1M KOH	[23]
9	Co(OH)₂ on Ni	–	2646	–	–	96.1% (300)	8 Ag⁻¹	2M KOH	[24]
10	Ni(OH)₂	–	2188	–	–	97% (1000)	1mVs⁻¹	1M KOH	[25]
c	Hybrid								
11	Ni(OH)₂/graphite	–	153	35.7	0.490	97% (5000)	5mVs⁻¹	1M KOH	[25]
12	MnO₂–NPG/ PPy-NPG	–	193	86	25	85% (2000)	1.8 V	1M LiClO₄	[26]
13	MnO₂/ NiNTAs@PPy	–	141.9	50.5	–	81.3% (1000)	1.6 V	PVA-LiCl	[27]
14	Ni–Co LDH/PPy films	–	261	61.3	0.65	91% (5000)	1.3 V	1M KOH	[28]
15	VO$_x$.YH₂O/WO₃. ZH₂O.			10.4	2.23		1.9 V	12M LiCl	[29]

MOF, metal–organic framework.

Fig. 3.4 Roadmap of the evolution, progress, and developments in supercapacitors.

3.2.1 Carbon-based electrode materials

Carbon-based electrode materials (CEMs) are promising candidates of ESDs due to their excellent conductivity, chemical/physical stability, and cost-effectivity. CEMs have high surface area which is responsible for excellent capacitance and considered breakthrough in energy storage.
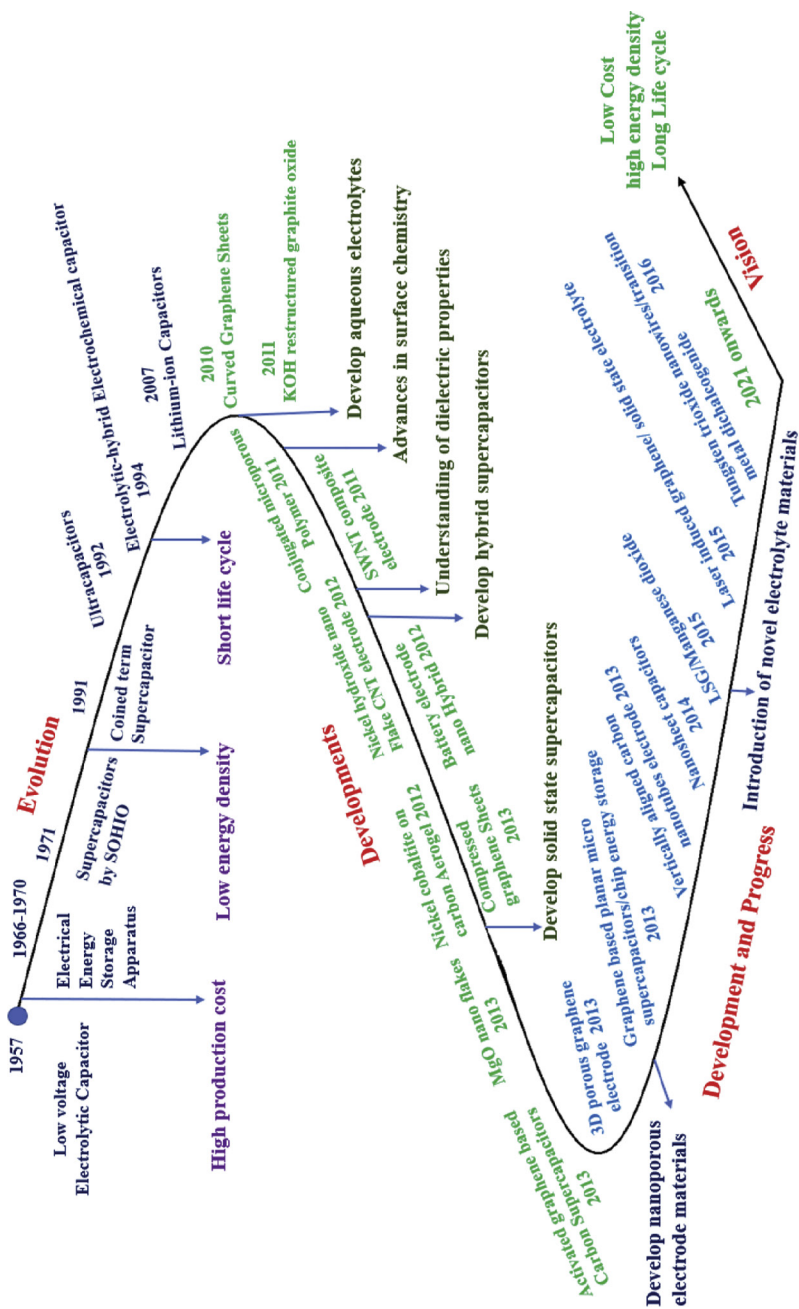
3.2.1.1 Activated carbons

In SCs, activated carbons (ACs) are considered promising materials due to its good electrical performance (GEP), cost effectivity, and high surface area. ACs are mostly obtained from coal, waste, nutshells, and wood as carbonaceous materials by physical and chemical treatments. Physical treatment is conducted at 700–1200°C temperatures with oxidizing and reducing agents such as air, CO_2, and steam whereas chemical compounds such as hydroxide, potash, oxyacid, or metal chloride are used for chemical treatment which is performed at 400–700°C. The use of chemical agents solely depends on precursors and the activation method used for the fabrication. These developments accomplished to attain 3000 m^2 g^{-1} surface area which is high and lead to improvement in the electrochemical properties such as electrical conductivity, energy density, total capacitance, gravimetric capacitance, and real-time constant [35–39].

Recently, Ba et al. [40] reported the chemically activated fig waste as SC electrode which is well organized and with diverse porosity (micro, meso, and macropores). This newly fabricated bioderived and cost-effective material displayed significant 2000 m^2 g^{-1} surface area. Compare to commercially available SCs, fig waste-derived SC has more significant specific capacitances (SPCs) and EPD [41]. In addition, the GEP of the ACs obtained from fig waste is better compare to several bioderived carbon-based nanostructures such as cellulose, sawdust, etc.

3.2.1.2 Carbon nanotubes

Carbon nanotubes (CNTs), the 1D Nobel allotrope of carbon has received much attention due to numerous applications in the diverse field such as energy storage, pollution control, and separation because of their mechanical, structural, and electrical properties [42]. There are two types of CNTs, a one atom thick layer of graphite is graphene which converted into cylinder-shaped form considered as single walled-carbon nanotube (SWCNT) whereas multiwalled carbon nanotube (MWCNT) contains many concentric SWCNTs with different diameters [43,44]. CNTs considered as electrochemical SC electrodes [45–51], due to promising electrochemical properties like low internal resistance, high SPC, and stability under high current loads [52–55].

CNTs are lightweight and have numerous extraordinary properties like high surface area, intrinsic flexibility, and electrical conductivity [56], which can help to make excellent polarizable SC electrodes. However, micropores and internal resistance resulted into the reduction in the actual capacitance compare to the theoretical one. Thus, the carbon fiber-based hybrid electrodes with CNT (CF-CNT) are fabricated to an elastic electrode material for excellent electrochemical performance (ECP) under

varied conditions. Researchers in combination with CF, CNT, and graphene are tried to prepare a 3D composite architecture. Likewise, scientists also reported CF-based reduced graphene oxide (rGO)-CNT which exhibited a SPC of 203 F g^{-1}, nearly four times the pure CF [57].

3.2.1.3   Graphene

Graphene, which is one atom thick single layer, 2D and sp^2 hybridized Nobel allotrope of carbon, global thrust area of research since its inception [58–61]. Graphene has excellent chemical and thermal properties and also has broad potential windows, large surface area, ease of processability with functional groups, and high ECP which makes it an excellent material for ESDs [62–66]. The properties of the graphene can be tuned by making composites of it with variety of CPs, metal and metal oxide nanoparticles.

Electrolytes such as aqueous, organic, and ionic liquid (IL) were used in the graphene-based SCs and found SPCs of 135, 99 [67], and 75 F g^{-1} [68], respectively. In the case of an aqueous electrolyte (AEs), low agglomerated reduced graphene attained a maximum SPC of 205 F g^{-1} with an energy density of 28.5 Wh kg^{-1} [69], whereas hydrazine hydrate treated rGO showed 251 F g^{-1} capacitance [70]. Also, stretchable SCs using rGO 3D-electrodes were found promising candidates for ultra-high-performance SCs in sensitive devices with adjustable volumetric capacitance (VC) [71].

The novel electrostatic self-assembly technique used to prepare MXene/rGO-5 wt% electrode, in aqueous media which exhibited high volumetric energy density (VED) of 32.6 Wh L^{-1} and high VC of 1040 F/cm^3 and maximum cycle life with 61% capacitive retention [72]. Similarly, lithium-ion capacitors were developed by using TiO$_2$ and Li$_3$VO$_4$/CNF with graphene sheets which provided ultra-high energy density (HED) (72 Wh kg^{-1}) and 110 Wh kg^{-1}, respectively, along with good cycling performance [73,74]. These newly prepared SCs can alleviate many difficulties like electrolyte potential difference between conventionally used Li-ion-batteries and upcoming SCs.

3.2.2   Conducting polymers

CPs are a kind of carbon-based polymers with heteroatoms. CPs are electrically conductive due to alternate single and double bond matrix and have reversible Faradaic redox capabilities, HED, and cost effectivity which makes it attractive for the SC applications [75–77]. Among CPs, polypyrrole (PPy), polythiophene (PTP), polyaniline (PANI), and their derivatives are cost effective and highly conductive and have considered and studied as SC electrode materials [78–84]. CPs can be available in several forms such as nanosheets, nanorods, and nanowalls. There morphology optimization is a very significant aspect for the ECP of the subsequent SCs [85].

3.2.2.1   PANI

PANI, a CP which is stable and electrically conductive and requires low cost to synthesize [36,86]. It is reported that PANI composites with nanostructures

(PANI-C-NNs) provide more surface area than its bulk form which makes it more promising SC electrode.

Miao and Luo coworkers independently fabricate PANI-C-NNs and obtain SPC 601 and 488 F g^{-1} [87,88]. In this work, polyamic acid nanofiber is used as a nanofiber template which needs to remove at the end. But during its removal, the structural degradation occurs which limits the approach of fabrication of PANI-C-NNs. However, there are several challenges associated with synthesis of these composites with numerous fabrication routes which can assemble into an electrode.

Electrospinning is a simple technique to fabricate electrospun nanofiber in various morphologies [89]. Unfortunately, PANI is aromatic in nature and has inadequate solubility and ultimately limited dispersion in organic solvents to achieve sufficient viscosity (2000–3000 cP), which makes PANI noncompatible for electrospinning. Chaudhari and coworkers reported novel work by fabricating PANI with carrier polymer (PANI/PEO) which unlocks new electrospinning paths to fabricate PANI nanofibers [90]. In addition, Simotwo et al. [91] reported binder-free approach through a single electrospinning step to fabricate SC electrode with high-purity PANI and PANI-CNT nanofibers.

3.2.2.2   *Polypyrrole*

Polypyrrole (PPy) is a promising candidate for Faradaic PC applications because of its cost-effectivity, quick charging/discharging (CDg), HED, high conductivity, and high thermal stability [92]. Also, these properties make it diverse electrode material for variety of other applications. The PPy monomer is water-soluble and commercially available and also can be easily oxidized [93] which makes it one of the most flourishing CPs. P. Asen et al. [93] reported ternary nanocomposites (reduced graphene oxide/PPy/copper oxide-hydroxide blend) as an electrode material for SCs that showed the advantages of electrochemical double-layer capacitors (EDLC) and pseudocapacitors (PCs). The ternary nanocomposite (reduced graphene oxide/PPy/copper oxide-hydroxide blend) in three electrode systems displayed 997 F g^{-1} SPC.

Moreover, researchers also reported an asymmetric supercapacitors electrode composed of PPy with CNT which showed excellent SPC [94]. Lin and coworkers also reported that SC electrodes have 890 F g^{-1} SPC. In addition, Dubal et al. [95] revealed hybrid (organo-inorganic) materials which showed approximately two times higher capacitances than its pristine one. These hybrid materials constitute 1D PPy nanopipes and heteropolyoxometalate (phosphates of W & Mo). Still, more improved new methods are needed to attain high SPC values.

3.2.3   **Metals oxides**

Nickel oxides (NiO), cobalt oxide (Co$_3$O$_4$), ruthenium oxide (RuO$_2$), and manganese dioxide (MnO$_2$) are transition metal oxides (TMO) that have been considered deliberately as SC electrode materials [96,97]. These mesoporous TMO structures provide high surface area and ordered pore size distributions which make them promising SC materials. SPC is resulted due to easy access of electrode surface for electrolytes

and rapid transport of ionic species. Moreover, TMO is also used with composites of MnO_2 and graphene for ESDs [98].

Though RuO_2 has considerable 905 F g^{-1} SPC but due to high cost it is not used commercially [99]. To avoid that problem, easily available most abundant TMO and metal-based materials have gained huge attention due to their stability and wide stoichiometric composition together with oxides of iron, tin, indium, manganese, vanadium, and cobalt-sulfur (Co_3S_4, CoS_2, Co_9S_8, $Co_{1-x}S$, and Co_xS_y)

3.2.4 Composites

Carbon-based materials (CBM), TMO, and CPs were commonly used as electrode materials but their low cycle stability restricted their practical use in SCs. Electrodes of composite materials (CPM) that have HED can encounter problems associated with CBM, CPs, and TMO electrodes. Moreover, bimetallic heterostructures of gold–nickel composite were fabricated which displayed SPC of 806.1 F g^{-1} and had 500 charge–discharge cycles [100]. Furthermore, modified metal phosphide will also consider as new energy storage materials.

Wang et al. [101] reported SC electrode made from ink of PANI-GO composite which can be used in diverse architectural designs like hollow thin-wall column, column lattice and 3D honeycomb. Among them, a planar (two-dimensional) SC electrode exhibited noteworthy GEP with 1329 mF cm^{-2}. Moreover, Pandey and cow-orkers [102] described the (NiS/G) nanocomposite synthesis for SC electrodes and reported SPC of 187.53 F g^{-1} with minimum impedance and about 1000 life cycles. In addition, Mao et al. described a porous composite of Mn_2TiO_4/TiO_2 exhibited SPC of 98.2 F g^{-1} and about 10,000 cycles stability [103]. In SCs, multiphase graphene composite electrodes are used as versatile materials which can be prepared with metallic matrix, polymeric or ceramic by shear mixing or ball milling type physical processing techniques [104,105]. These revealed that CPM are considered as the best adoptions for SC electrodes based on their excellent performance reports.

3.3 Electrolytes

The electrolyte is a fundamental element of SCs, which provides ionic conductivity in a cell [106]. Electrolyte selection depends upon size and type of ions, concentration, and interaction of ions and solvent and also on electrode materials and the potential window. The operation cell voltage of an electrochemical system (ES) influenced by EPD [106]. Basically, the electrolyte carried out a vital role in creating EDLC and PC as ESDs. The electrolytes can be affected on capacitance, EPD, and cycle-life of the ES. Many electrolytes for ESDs have been reported, they are mainly classified as solid/quasi-solid-state (organic and inorganic) and liquid (aqueous, organic, and IL) electrolytes [107–109]. Till date, the available electrolytes have both practical merits as well as demerits, henceforth there is always room for the development of perfect electrolytes.

For instance, a common issue regarding liquid electrolytes (LQEs) is leakage which is avoided by solid-state electrolytes (SSEs) but they have lesser conductivity than

liquids. The working voltage of AEs is narrow but they can make available high capacitance and conductivity. Moreover, electrolytes (organic and ILs) can be functional at elevated voltages, but they can face poorer ionic conductivity.

Numerous efforts have been taken to produce technically advanced new materials which can have good ionic conductivity, thermal stability, wider operational voltage window, and broader range of working temperature [107,110,111]. These advancements in electrolytes can be evaluated based on their capacitance, EPD, and the CDg processes which will help in experimental and simulation modeling study of electrolytes for the enhancement of ES performance.

3.3.1   Aqueous electrolytes

In general, selection of AEs based on cations (hydro-spheric) size and the movement of respective anions can have effect on ionic conductivity and SPC. In addition, eroding power and electrochemical stable potential window (ESPW) of electrolytes must be noticed during electrolyte selection. Though AEs illustrate conductivity of 0.8 S cm^{-2} for 1M H_2SO_4 at 25°C, which is higher than that of organic electrolytes (OEs) and ILs but due to their small ESPW they are not commercially considered for electrochemical SCs.

Typically, AEs are categorized into acidic, basic, and neutral solutions. Among these AEs sodium sulfate (Na_2SO_4), sulfuric acid (H_2SO_4), and potassium hydroxide (KOH) are deliberately used. Qu et al. [112] reported neutral electrolytes such as lithium sulfate (Li_2SO_4), potassium sulfate (K_2SO_4), and sodium sulfate (Na_2SO_4) and investigated the performance of MnO_2 nanorods. They have tried freshly fabricated AC SC which displayed exceptional cycling behavior with a considerable 17 Wh kg^{-1}energy density and 2 kW kg^{-1}power density. Likewise, $NaMnO_2$ in combination with AC was tested in environmentally benign aqueous solution of sodium sulfate (Na_2SO_4) electrolyte [113], which exhibits 19.5 Wh kg^{-1} energy density and 130 Wh kg^{-1} power density with outstanding life cycles. In SC technology, gels are used to produce a stable, leakage-free electrolytic system. Wang and coworkers [114] reported a novel gel prepared by immersing polyacrylamide gel (PAM-G) into an aqueous solution of 6M KOH for about 60 h. This homogeneous 3D microporous PAM-G electrolytic system provides ionic permeability with exceptional rate capability, cycle stability, and high SPC.

It is also observed that the SSEs for ESDs are more promising candidates due to their durability and long-term GEP. Pan and coworkers [115] reported solid-state matrix electrolytes, prepared by an electro-spun matrix of PVA and GO. This innovative SSEs matrix exhibited less deterioration in ionic conductivity than pure PVA when tested after one month in a steamy atmosphere. SSEs confirmed its superiority from these results than other electrolytes.

3.3.2   Organic electrolytes

OEs provide 2.5–2.8 V operating electric potential window, also allow inexpensive current collectors and packages which make it promising electrolytes to lead in the commercial market [116]. Moreover, conductive salts like tetraethylammonium

tetrafluoroborate (TEABF$_4$) are used in EDLCs as OEs. Propylene carbonate or ACN preferentially used to dissolved these types of conductive salts. However, OEs have many issues such as low conductivity, a small SPC and high cost. Apart from this, they are toxic, volatile, and flammable [117] which restricts their use. Also, moisture found as impurities in OEs which causes performance degradation and self-discharge. Such impurities can be eliminated by standard purification techniques with intricate assembly. In connection with this, SC constituents were fabricated and developed by Hashemi and coworkers [118] for high-performance SCs using 1,4-napthoquinone (NQ) and obtained 4007 F g^{-1} SPC [118].

3.3.3   Ionic liquid electrolytes

Negligible vapor pressure, nonflammability, and wide operating voltage ranges with chemical and thermal stability make ILs promising electrolytes to use in SCs even at high temperatures. Commercially, OEs and AEs have their limitations above 70°C and 80°C temperatures, respectively, [119] as OEs may face ignition and detonation temperature issues whereas AEs have boiling issue at stated temperature.

Haque and coworkers [119] reported thermal (21–150°C) dependent performance of an IL, 1-ethyl-3-methylimidazolium acetate (EMIMAc) as electrolyte and AC as electrode material which displayed 142 F g^{-1} SPC at 150°C. At room temperature, the observed 2.5 Ω cm^2 value of the equivalent series resistance (ESR) resulted due to the AC and EMIMAc compatibility. It has been observed that the electrolyte's ionic conductivity and retention maintenance enhanced at high temperatures whereas ESR decreases at high temperatures.

Moreover, Zarrougui et al. [120] reported six electrolytes of innovative characteristics such as low-viscosity ILs as for SCs where one is planar anion (DCA$^-$) and five anions (PF$_6^-$, BF$_4^-$, TFSI$^-$, TFA$^-$, and OTf$^-$) with nonpolar architectures. These ILs used as electrolytes in ES displayed 135–228 F g^{-1} SPC, considerable HED, and cycle stability. Osti and coworkers [121] applied mixture of ILs (1-ethyl-3methylimidazolium tetrafluoroborate (EmimBF$_4$) +1-ethyl-3methylimidazolium bis(trifluoromethylsulfonyl)imide (EmimTFSI)) as the electrolytes and observed better performance of SCs. They also undergone DFT study and advised to use 4:1 EmimTFSI: EmimBF$_4$ which could offer maximum cations absorption near the electrode surface. The DFT calculations as dry lab findings open new avenues of study for the design and optimum use of electrolyte system.

3.4   Hybrid materials from biowaste for supercapacitors

Biowaste such as biomass and organic waste considered as solid waste, obtained from livestock, wastewater, urban solid waste, forest resources, and agriculture resources causes health risks and environmental pollution. Many efforts have been taken to convert this waste into valuable innovative products like fertilizer materials [122], fodder [123], construction materials [124], catalysts [125], contaminant sorbent materials

[126], energy storage [127–130], and also other environmentally benign applications [131,132]. Fruit skins, animal feathers, and lignocellulosic powder can be castoff for porous carbons [133], composites [134–136], and also graphitic carbon/reduced graphitic carbons for SCs electrodes. The best from waste technology is economic and environmentally benign.

3.5 Modern trends in supercapacitor technology

Technologies have been advanced by taking strong initiatives to grow ultra-compact devices (UCDs) into miniaturized energy-autonomous systems (MEASs). The natural energy transitions are considered as sources such as electromagnetic radiation from the sun, vibration, thermal, and even blood flow and body heat used to harvest energy in MEASs [137–142].

Energy acts like folding, stretching, twisting, and bending as mechanical deformations are also used in MEASs where ESDs need to be used as energy storage component in best-fit UCDs. Recently, 3D porous SCs (3D-PSC), fiber-like SCs (FSC), and paper-like SCs (PSC) are the newest likings seen in ES technology.

In 3D-PSC, 3D architectures of electrodes can offer maximum active sites which can help to have effective chemical reactions, easy electron transport, and ease of accessibility of the current to enhance the electrochemical activity [143,144]. Song et al. [145] reported nitrogen-doped 3D porous graphene electrodes fabricated on polyimide sheet by direct laser induction at room temperature. Herein, doped elemental N increases number of active sites which resulted in excellent PC behavior. PSC can have tunable stretching ability and can be modified into different structures easily for individual applications. Likewise, FSC are fabricated into flexible and ultra-thin fibers for enhanced performance of ESDs. Some advanced architectures proposed or employed are tabulated in Table 3.3.

3.6 Conclusion and future prospects

SCs have potential to tackle today's energy requirements due to its high cycle stability, quick CDg processes, and HEPD. Cost-effective electrode materials, electrolytes are the leading parameters for the industrialization of SCs. Biomass waste-derived SCs could play a vital role in designing and fabricating SCs. In agreement with this, Andrew Burke reported the biomass (biochar) derived electrode is cost-effective than the conventional carbon/carbon SCs electrode [146–148]. In addition, it is also observed that the capacitance performance of conventional SCs and waste-derived SCs are almost same. However, series of expensive chemical agents such as H_2SO_4, H_3PO_4, Na_2SO_4, $ZnCl_2$, NaCl, NaOH, and KOH are used in activation methods. These activation methods can effect on the functions of the electrode which ultimately limit the usage of biomass waste. Activation efficiency could improve by the use of hydrothermal and many other pretreatment methods. In future, scientists will have many

Table 3.3 Summary of some advanced architectures employed or proposed in supercapacitor technology.

Order	Material	Architecture	Electrolyte	Measurement protocol	Electrode configuration	Max. capacitance	References
1	Mn_3O_4 NSs/rGO NSs	Paper-like	6M KOH	GCD = 0.5 A g^{-1}	Three electrode	409 F g^{-1}	[158]
2	Vertically aligned CNF/BDD CNF-PEDOT	Battery-like	1.0M H_2SO_4 0.1M $HClO_4$ in water]/	CV = 10 mV s^{-1}	Three electrode	116.3 mF c m^{-2}	[159]
3	–	Paper-like Three electrode	230 F g^{-1}	[160]			
aceto-nitrile (9/1)							
4	$NG-MnO_2$	Flower-like	1M Na_2SO_4	GCD = 0.5 A g^{-1}	Three electrode	220 F g^{-1}	[161]
5	BPC/MnO_2	Anchored $MnO2$ on biomass	1M Na_2SO_4	GCD = 0.5 A g^{-1}	Three electrode	384.9 F g^{-1}	[162]
6	BPC/Fe_2O_3	Decorated porous carbon with Fe2O3	3M KOH	GCD = 1 A g^{-1}	Three electrode	987.9 F g^{-1}	[163]
7	3D Ni-Co LDH/NiNw	3D cotton-like	6M KOH	GCD = 0.125 A g^{-1}	Three electrode	466.6 F g^{-1}	[164]
8	Ni–Co hydroxide/NF	Flower-like	2M KOH	GCD = 10 mA cm−2	Three electrode	1441.5μA h cm^{-2}	[165]

Order	Material	Architecture	Electrolyte	Measurement protocol	Electrode configuration	Max. capacitance	References
9	MnO$_2$/CNT-web paper	Ultra-thin and stackable	0.5M LiClO$_4$	CV 5 mV s^{-1}	Three electrode	135 mF cm^{-2}	[166]
10	HC/RGO	Belt-like	3M KOH	GCD = 1 A g^{-1}	Three electrode	1662 F g^{-1}	[167]
11	Nitrogen-doped graphene	Bubble-like	2M KOH	GCD = 1 A g^{-1}	Three electrode	481 F g^{-1}	[168]
12	CoMoO$_4$@ Co$_{1.5}$Ni$_{1.5}$S$_4$	Rambutan-like	3M KOH	GCD = 1 A g^{-1}	Three electrode	1405 F g^{-1}	[169]
13	NiCo-LDH@ NiOOH	Battery-like	6M KOH	GCD = 1 A g^{-1}	Three electrode	2622 F g^{-1}	[170]
14	NiCo-S	Highly open nanosheet	2M KOH	GCD = 0.5 A g^{-1}	Two & Three electrode	2553.9 F g^{-1}	[171]
15	N-doped hierarchal carbon	Fiber web, mulberry-like	1.0M H$_2$SO$_4$	GCD = 1 A g^{-1}	Three electrode	298.6 F g^{-1}	[172]
16	Ni$_{0.4}$Co$_{0.6}$(OH)$_2$@ CFC	Peony-like	2M KOH	GCD = 1 A g^{-1}	Three electrode	1816 F g^{-1}	[173]
17	3DPGLS	3D porous	1M TEMABF$_4$/PC)	GCD = 0.2 A g^{-1}	Two electrode	91.15 F g^{-1}	[174]

other cost-effective and environmentally benign replacements like carbon materials which derived from biowaste for energy conservation. Since its inception, many technical problems have been solved which were associated with cost-effectivity and low energy density by introducing hybrid electrolytes (aqueous/nonaqueous electrolytes) and nanostructured electrode materials [149–151]. Recently, flexible solid-state SCs found to be promising candidates for the ESDs by considering their safety, flexibility, HEPD, and easy incorporation with other ESs [152]. Best from the waste approach also used in the fabrication of electrode materials which is highly economical but needs more improvements in commercial point of view. One more attempt toward safe and environmentally friendly SCs is to employ metallic ions to the new quasi-solid-state concept. In connection with this, Wang *et al*. fabricated capacitor of the sodium ion with nanoporous carbon and accomplished 168 Wh Kg^{-1} energy density and good cycle stability [153].

Acknowledgments

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I would like to dedicate this book to
My beloved GOD (*The most glorified, the most high*)
"Meray Pyarey Allah (Subhanahu Wa Ta'ala)"

Dedication by Kabali Vijai Anand
I would like to dedicate this book to
My beloved mother Mrs. Kabali Thulasi
and all other family members

Dedication by Shadpour Mallakpour
I would like to dedicate this handbook to
My wife Mina
My son Iman
My daughters Adeleh and Fereshteh
My granddaughter Termeh

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CHAPTER 11

Graphene-based nanomaterials for antibiotics-independent antibacterial applications

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1. Introduction

Graphene, a 2D single layer sheet of carbon atoms, is the most thrusted area of research [1]. Nanomaterial derivatives of graphene such as graphene oxide (GO), reduced graphene oxide (rGO), and other graphene nanocomposites (GNCs) can be commonly considered as graphene-based nanomaterials (GBNs). GBNs possess vast surface area and also have photothermal effect which results in numerous applications in many areas such as drug delivery [2,3], sensors–biosensors/imaging [4,5], tissue repair/engineering [6,7], mechanical and optical devices, electronics, and cancer therapy [8,9].

Globally, it has been observed that the infectious microorganisms are of great concern for community well-being. It is a well-known fact that with the time and also with the misuse and bad practices of antibiotics, these microorganisms develop resistance to certain drug moieties and its anti–infection is too much difficult. Thus, the common way to tackle such issues, either by synthesizing new drug molecules or also by discovering new drugs, is a time-consuming, hard, and lengthy process. Hence, the advancement of antibiotics–independent antibacterial agents (AIAA) is much more desirable and substantial in the current time. Therefore, GBNs have emerged as AIAA due to their broad-spectrum activities against Gram-positive and Gram-negative bacteria [10,11] distinguishing antibacterial mechanisms.

Among the available GBNs, the most predominant antibacterial nanomaterial GO is due to its sharp edge cutting effect (SECE) [11,12], cell entrapment (CENTP) ability [13], and oxidative stress (OS) [12]. Moreover, the rGO has been utilized as the AIAA due to its robust photothermal effect [14,15]. Therefore, easy availability and cost-effectiveness of GBNs make them the prime candidates for the antibacterial investigation. In addition, the antibacterial activity (AA) of GBNs can be boosted by functional

modifications with other nanomaterials or potential bioactive agents by forming nanocomposites. Herein, we aim to offer a detailed and thoughtful idea of the properties and antibacterial potential of GBNs against various bacterial species. Moreover, we have discussed the probable mechanistic pathways of GBNs to inhibit the growth of bacteria or kill bacteria and also their potential risks.

2. Types of graphene-based nanomaterials (GBNs)

Graphene, 2D honeycomb lattice structural material where carbon atoms are closely arranged [16]. The typical morphology of graphene studied under the electron microscope revealed its hexagonal structural arrangement and permits graphene to acquire outstanding physicochemical properties, such as large surface area (LSA), excellent thermal conductivity (ETC), excellent electrical conductivity (EEC), high mechanical strength (HMS), and zero band gap width [17,18]. GBNs can be differentiated based upon their functions and different properties. In this book chapter, we emphasize on GO, rGO, and their nanocomposites with other nanomaterials and agents.

2.1 Graphene oxide (GO)

Graphene on oxidation gives GO. There are several methods for GO synthesis including Staudenmaier method [19], Brodie method [20], and the most commonly used Hummer's method [21,22]. The structure of GO has a lot of oxygen functional groups (OFG) such as carboxyl groups ($-COOH$), hydroxyl groups ($-OH$) as well as epoxy groups ($-O-$) [23]. The aqueous dispersion of GO can be prepared due to hydrophilic nature of GO which come into existence because of OFG present on its basic nanosheet structure. GO also have exceptional antibacterial potential which persist through OS, SECE, and CENTP type mechanisms. The comprehensive antibacterial performances of GO with their mechanisms are discussed later.

2.2 Reduced graphene oxide (rGO)

Broadly, rGO is obtained by reduction of GO which is more hydrophobic compared to GO. The reduction of oxygen functionalities results in the partial restoration of the electrical conduction and UV–vis absorption of graphene [24]. There are many methods for the reduction of GO to form rGO include reduction by chemicals [25], reduction by electrochemical reactions [26], and reduction by thermal energy [27]. Thermal reduction requires high temperature which is considered as the most suitable and green method among these available methods [28]. Moreover, not only temperature but also alkalinity of the reaction solution plays a vital role in reduction of GO into rGO, which was reported by Hao et al. [29].

Structurally, rGO is analogous to graphene with some defects and have an EEC, HMS, ETC, and photothermal effect (PTE). The PTE of rGO leads to its use in photothermal therapy against cancer and temperature-sensitive controlled drug release [30–32]. Moreover, the PTE of rGO plays a vital role in AA [33,34].

2.3 Graphene-based nanocomposites

The properties of graphene-based nanocomposites (GBNCs) can be tuned by function-alization of GBNs such as GO or rGO with other active agents either by covalent or non-covalent bonding [35]. Herein, we emphasize on graphene-metal/metal oxide nanocomposites (GMNCs) and graphene-polymer nanocomposites (GPNCs).

2.3.1 Graphene-metal/metal oxide nanocomposites

In general, the stabilization and dispersion of the metal/metal oxide nanoparticles can be performed by GMNCs which have LSA and also considered as nanorange building blocks [36]. Compared to individual components, hybridized nanocomposites show better anti-bacterial activity. Silver nanoparticles (AgNPs) have remarkable antibacterial potential which by emitting silver ions from its surface can destroy membrane of the cell and obstruct the cell metabolism [37–39]. But AgNPs aggregation leads to limiting their prac-tical applications [36]. The enhanced AA of GMNCs of GO-AgNPs revealed the role of GO as a reliable carrier, prevents nucleation of AgNPs, and also on the other side these immobilized AgNPs over GO also avoid GO sheets aggregation [40,41]. In addition to this, Zhu et al. stated that the large amount of cations enhanced AA of GO-AgNPs nano-composites by increasing interaction with bacterial cell membranes [42].

Also, metal oxides have been used in the GMNCs such as ZnO used in the GO-ZnO nanocomposite which reported to have good AA [43]. Moreover, other GMNCs such as GO-Fe_3O_4 [44], GO-TiO_2 [45] are commonly used.

2.3.2 Graphene-polymer nanocomposites

Some efficient polymers are used to form GPNCs which increase its water dispersion and also boost up its antibacterial activity. Researchers reported the synthesis of GPNCs of graphene and poly(L-lysine) (PLL) by electrostatic interaction and covalent bonding. It is also observed that the AA of PLL is preserved but due to the presence of graphene de-rivative its biocompatibility gets enhanced [46]. Carpio et al. reported the nanocomposite of polyvinyl-N-carbazole (PVK) with GO as PVK-GO and revealed its enhanced AA [47]. Furthermore, some other polymer-GO nanocomposites with enhanced AA than GO have been developed such as GO-Pluronic [48] and GO-PEG-PHGC [49].

3. History and synthesis of GBNs

The journey of preparation of graphene was started in 1840 and was globally received attention after the isolation of single layer graphene by Geim and Novoselov in 2004 [50]. In this journey, Boehm in 1986 coined the name "graphene" which is used to define a single layer of graphite.

In general, two main types of preparation methods for the GBNs are available such as top-down method (TDM) and bottom-up method (BUM) [51,52]. GBNs can be
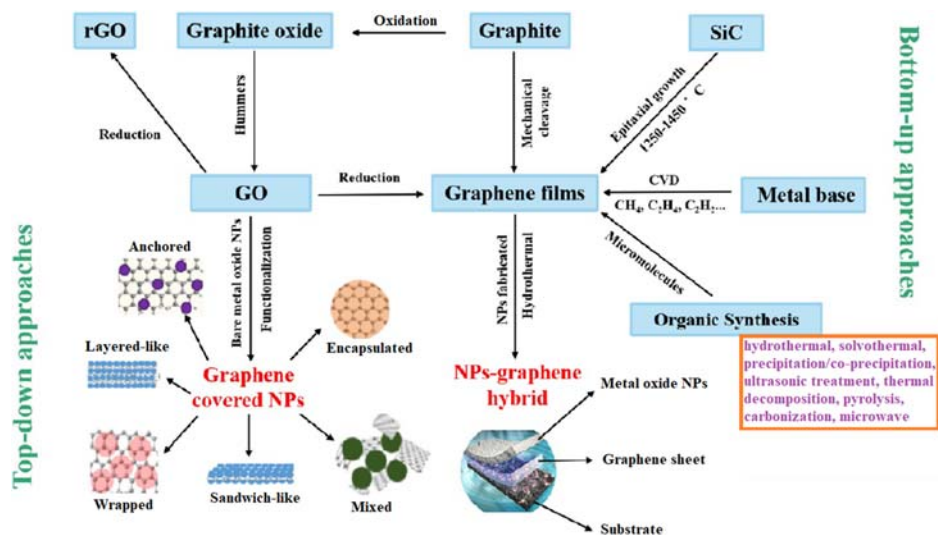
Figure 11.1 Formation of GBNs by different approaches.

synthesized by mechanical cleavage (MC), reduction, and the Hummers' method under TDM approach whereas epitaxial growth, chemical vapor deposition (CVD), arc discharge, and organic synthesis are considered under BUM approach as shown in Fig. 11.1. For the single or multilayer GO and graphene synthesis the BUM approach uses small molecule carbon whereas natural graphite is used in TDM approach as the basic source. Each method has its advantages and disadvantages. Basically, in TDM approach nanomaterials produced satisfy application requirements and hold their basic structure and morphology. Furthermore, the demerits of TDM include expensive raw material, wastage of material, long reaction time, and several reaction conditions. In addition, BUM approach includes saving of materials, nonexpensive raw material, controlled material thickness and morphology, and scalability. The demerit of this approach is that it fails to meet the expected size material. On the other hand, it offers uniform synthesized materials, which is particularly beneficial to their biomedical applications.

4. Structures and properties

Primarily, graphene is considered as the fundamental unit of carbon materials family (CMF) because it can roll into 1D carbon nanotubes (CNT), can make 0D fullerenes by wrapping, and also stacked into 3D graphite. GBNs can be classified based on their chemical elements, OFG, and layers which are responsible for their structural differences, which ultimately decides their physical and chemical properties [53]. Initially, Mermin–Wagner [54] and Landau [55] questioned the thermodynamic stability of 2D materials and researchers considered 2D graphene as an "impractical material." Graphene is a single
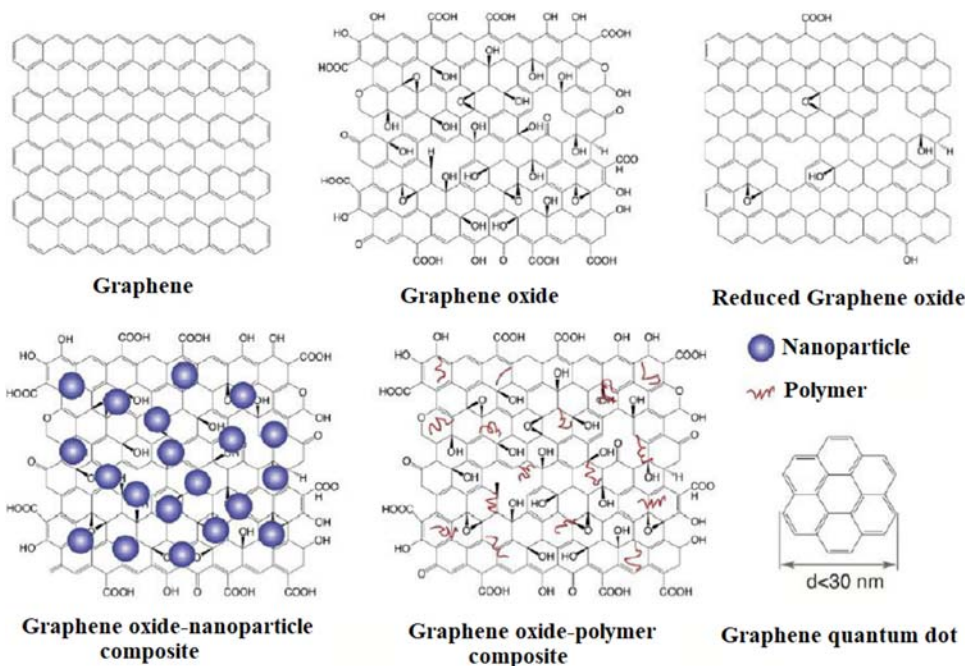
Figure 11.2 Structural representation of GBNs.

atomic layer of sp^2 hybridized carbon atoms which are arranged in the planar hexagons having thickness 0.335 nm. GO is the oxidative form of graphene having many OFG such as hydroxy (−OH), carboxyl (−COOH), ether (−O−), and epoxide [−CH(O) CH−] on the sheet surface (Fig. 11.2) which leads to superior physical and chemical properties [56]. Though the basic structure of graphene and GO is same, additional OFG on the GO surface makes it labile for further surface functional modifications (SFM). The SFM is the major characteristic of GO which leads to its widespread use in numerous fields of applications. Compare to graphene, GO has less conductivity which can be recovered by the chemical or thermal reduction (rGO) of GO or graphite oxide [57]. Recently, GQDs are manufactured using thermal plasma jets which have 1−20 nm diameter size, large surface area, and −OH and −COOH functional groups at their edges which are responsible for surface modification [58].

5. Surface functional modifications (SFM)

GBNs have many applications in numerous fields but the hydrophobic nature of graphene limits its use in biomedical applications. GO get aggregated in physiological condition due to shielding effect of surface functional groups [59]. Apart from this, GO has a strong tendency to adsorb proteins which is in vivo recognized and engulfed by macrophages resulting in inflammation [60]. These shortcomings of GBNs can be avoided by their SFM. Presently,

there are two approaches for SFM such as covalent surface functional modifications (c–SFM) and noncovalent surface functional modifications (nc–SFM) (Table 11.1).

5.1 Covalent and noncovalent modification

In c–SFM, GO is the most solely used GBN because of its abundant OFG. Basically, c–SFM is the introduction of functional groups and even polymers by using free radicals, amides, and other reactions in acidic conditions to form covalent bonds for the required functions. The PEG–GO prepared via an amide bond formed between GO and polyethylene glycol (PEG) can target passively on tumor with prominent efficiency due to its physiological stability [70]. In addition, free radicals can be used to modify graphene by styrene copolymers which significantly improved its dispersion and electrical conductivity [71].

Furthermore, polymers such as polyvinylpyrrolidine (PVP), polyvinyl alcohol (PVA), sodium alginate (SA), amphiphilic copolymer, and chitosan and other functional groups like sulfonic acid (SO_3H), $-OH$, $-CH(O)CH-$, and $-COOH$ can be used for GO functionalization. Sometimes, dual covalent functionalization can be possible which holds the basic form of GO and expands its area of applications. The c–SFM of GO is well-matched for the coupling with variety of molecules specially for biomolecules such as small drug molecules, proteins, and nucleic acids which after functionalization can be employed in several biomedical applications such as imaging, biosensing, drug delivery, tissue engineering, and photothermal therapy.

In nc–SFM approach, comparatively weak forces such as hydrogen bonds, coordination bonds, electrostatic interaction, π–π interaction, and van der Waals forces of attraction are utilized which enhances safety, dispersion, and reactive activation capabilities of GBNs [72]. In general, nc–SFM forces are weaker and do not produce strong chemical bonds, which refers to less stable GBNs both in vitro and in vivo. Chen et al. [73] reported both the covalent and noncovalently modified PVA–GO wherein noncovalently modified graphene retains the original quality with few defects compared to the covalently modified PVA–GO. Both the c–SFM and nc–SFM have their own constraints and rewards. Moreover, nc–SFM of GBNs offers to load with less aromatic anticancer drugs than c–SFM changed GBNs where the most of their conjugated sites favorably bind to the coating polymer. It is also reported that nc–SFM of porphyrin compounds, pyrene, phenanthrene, naphthalene, and gold nanoparticles (AuNPs) with graphene and GO can be possible via π–π interaction [74].

6. Antibacterial activities against various microbial species

Basically, we can categorize microbes into pathogenic and nonpathogenic to humans and such correlation is being gradually established with the progress of human microbiome. Some commonly known pathogenic Gram-negative bacteria are *Escherichia coli, Salmonella typhimurium, Porphyromonas gingivalis, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and

Table 11.1 Functional modification and application of GBNs.

Surface interactions	Nanocomposites	Modified materials	Species	In vitro/in vivo model	Results	References
Covalent	AuPd-rGO/ PDA	PDA, AuPd	PTT	Breast cancer cell (MDAMB231)	Cancer cell ablation was effectively promoted through the synergistic effect of AuPd, rGO and PDA functionalization	[61]
	PVA-rGO/ Bi$_2$S$_3$	PVA	Chemo-PTT (doxorubicin)	BEL-7402 cells, tumor bearing mice	High drug loading ($\sim$500%), perfect photothermal activity in the NIR region	[62]
	GO/PEG- docetaxel	PEG	Drug delivery (docetaxel)	DU-145 prostate cancer cell	Highly active against prostate cancer cells	[63]
	PVP-graphene/ GCE	PVP	Electrochemical sensor	Ascorbic acid, dopamine (DA), uric acid (UA)	The detection limits were greatly increased	[64]
	Amino-N– GQDS– polymers	PEI	Bioimaging	A431 skin cancer cells	71 times less power than required	[65]
Covalent, π–π stacking	GO-PLGA	PLGA	Bone tissue engineering	Rabbit models	Accelerated the proliferations and osteogenic differentiation of BMSCs	[66]
π–π stacking, hydrogen bonding	Streptavidin– biotin– GQDs-GO- cetyltrimethy lammonium bromide–Au nanoparticles	Streptavidin-biotin, Au nanoparticles	Fluorescent nanoprobe	BIOTEG-5′-GGGAGA CAAGGAAAATCC TTCAATGAAGTGG GTCGACA	Successfully applied for the determination of adulterated cocaine samples	[67]

Table 11.1 Functional modification and application of GBNs.—cont'd

Surface interactions	Nanocomposites	Modified materials	Species	In vitro/in vivo model	Results	References
Electrostatic hydrogen bonding	Biomimetic calcium phosphate mineralized GO/chitosan scaffolds	Chitosan	Bone tissue engineering	*Escherichia coli, Staphylococcus epidermidis*	Exceptional adsorbability of nanoparticles, enhancement of osteo inductivity and antibacterial properties	[68]
Hydrogen bonding, $\pi-\pi$ stacking	PPy-GO	PPy	Electrochemical biosensor	Salmonella	The limit of detection was 4.7×10^{-17} M, the detection limit of Salmonella was in the range of $9.6-9.6 \times 10^4$ CFU/mL	[69]

Gram–positive bacteria are *Streptococcus mutans, Staphylococcus aureus* whereas *Candida albicans* is well–known fungi. *Escherichia coli, Staphylococcus aureus* are responsible for the infections in various parts of the body [75—77], gastrointestinal disorders are caused by *Salmonella typhimurium* [78], oral diseases are associated with *Porphyromonas gingivalis, Streptococcus mutans,* and *Candida albicans* [79—83], whereas *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are responsible for nosocomial infections [84,85]. Since the discovery of the first antibiotic *penicillin*, antibiotics have the first priority against bacterial infections. But challenges are constantly increasing due to drug resistance and abuse of antibiotics. So due to the broad antibacterial spectrum of GBNs, they can be considered as antibiotics–independent antibacterial agents (AIA) [86—88]. It is assumed that GBNs have significant potentials to be the AIA against numerous human pathogens which we have summarized in Table 11.2.

7. Antibacterial mechanisms

GBNs are broad–spectrum AIA, their multiple probable mechanisms [89—92] can reveal the understanding of antibacterial activities of GBNs which can guide the design and construction of workable applications of GBNs. Here, we have summarized the well–predictable mechanisms such as OS, SECE, and CENTP (Fig. 11.3).

7.1 Sharp edge-mediated cutting effect

Structurally, GBNs such as rGO and GO are with sharp edges which will adhere on bacterial cells and can cause physical damage to membrane of the cell. Researchers reported that the GO nanosheets physically disrupt bacterial cells, which has been evidenced by TEM image study [93]. Pham et al. deliberated the factors such as edge density (ED) and the contact angle (CA) which can influence the SECE induced AA of GBNs [94]. Here, ED denotes to the density of the edge length of graphene and CA refers to the angle between the sheet and the cell membrane. They conferred the fact that the graphene which has smooth edges leads to high ED and has high AA than rough edges whereas GBNs started to display AA at CA 37 degree and have maximum effect at CA 90 degree. It is also reported that the reduced GBNs have a robust AA effect on membrane of the bacterial cell than the unreduced GBNs [11]. Additionally, an interesting and surprising work has been reported about Langmuir—Blodgett technique which eradicates GO's SECE [95]. This result directs that there will always be other possible mechanisms for the AA of GBNs.

7.2 Oxidative stress

OS ruptures cell structure which results into the loss of viability of the bacteria, occurred due to disproportion of redox reactions [96]. In connection with this, researchers reported that the GBNs are responsible for the peroxidation of lipid which damages the cell membrane and ultimately kills the bacteria [97]. Lipid peroxidation is nothing but oxidation of fatty acids where the active oxygen of bacteria undergoes chain reactions of free radicals in succession leads to the formation of lipid peroxidation. As we have

Table 11.2 Antibacterial characteristics of GBNs.

Graphene nanocomposites	Bacterial strains	Evaluation method	Concentration (µg/mL)	Inhibition	References
Graphene family					
rGO	Escherichia coli (E. coli)	Plate count	100	88	[129]
	Pseudomonas aeruginosa (P. aeruginosa)		175	100	[130]
GO	S. aureus/P. aeruginosa	Agar diffusion assay (ADA)	300	93.7/48%	[131]
	E. coli	Plate count	85	98.5	[132]
GQDs	P. aeruginosa		40	97.7	[133]
	E. coli		175	100	[130]
	Staphylococcus aureus (S. aureus)		200	80/92	[130]
Functionalized with silver NPs					
GO–AgNPs	E. coli	Plate count	10	99.9	[134]
			5	100	[135]
	P. aeruginosa		10	99.9	[136]
	E. coli/S. aureus		10	100	[137]
			45	100	[134]
GO–AgNPs/ polydially ldimethyl ammonium chloride	E. coli		50	100	[137]
GO–AgNPs/ polyamide			10^3	98	[138,139]
GO–AgNPs/ PDA			25	23.7 mm	[140]
rGO–AgNPs			40	100%	[141]
rGO–Ag/Ag$_2$S			N/A	97.76	[108]
GO–AgNPs/ PEI	E. coli/S. aureus		958	99.99	[142]
GO–AgNPs/ aminophenol			500	100	[143]
GO–Ag/TiO$_2$			102	67	[144]
GO–Ag$_3$PO$_4$ NPs			300	92.8/100%	[145]

Table 11.2 Antibacterial characteristics of GBNs.—cont'd

| Graphene nanocomposites	Bacterial strains	Evaluation method	Concentration (μg/mL)	Inhibition	References
Photocatalytic functionalization					
GO-Cds	*E. coli /B. subtilis*	Plate count	200	99.9	[146]
rGO–ZnO NPs	*E. coli*		3×10^3	13/11 mm	[147]
GO–ZnO NPs			31.25/15.62	100	[148,149]
rGO/TiO$_2$	*S. aureus*	ADA	N/A	10.9/10.5 mm	[150]
Functionalization with other metal ions/oxides					
GO-MnFe$_2$O$_4$	*E. coli*	Plate count	10^2	82	[151]
GO-CuNPs/ poly–L–lysine (PLL)			50	99	[152]
GO-Bi$_2$WO$_6$	Mixed culture		250	100	[153]
GO-Fe$_3$O$_4$	*E. coli /S. aureus*		6.6×10^5	91.5	[107]
Functionalization with polymers					
PVA–CS–GO	*E. coli /B. subtilis*	ADA	N/A	1.25/1.40 mm	[154]
GO-CS	*E. coli /S. aureus*	Plate count	3×10^3	100	[155]
rGO– dithiothreitol	*E. coli /S. aureus*		100	86	[156]
GO/PVA			N/A	8.6 mm	[157,158]
GO/PLL/ hyaluronic acid			10^5	66	[152,159]
PDMS–GO– DMA	*E. coli*		N/A	∼40%	[159]
GO-PEG			4×10^3	N/A	[160]
Graphene hydrogels					
BKB/PDA	*E. coli /Listeria*	Plate count	4×10^3	99.3/91	[152,161]
Ag	*E. coli / S. aureus*		2.5×10^3	100	[162]
Functionalized with antibiotics or enzymes					
GO-Lys	*E. coli*	Plate count	32–512	68%	[163]
rGO-Van-nHA	*S. aureus*	King's B medium	1–4% Van	N/A	[164]
GO-cefalexin	*E. coli /S. aureus*	ADA	N/A	6.3/6.9 mm	[165]
GO-PEI- ciprofloxacin	*E. coli*	ADA	1 cm^2 (film)	100%	[166]

[a]*N/A*, not available.
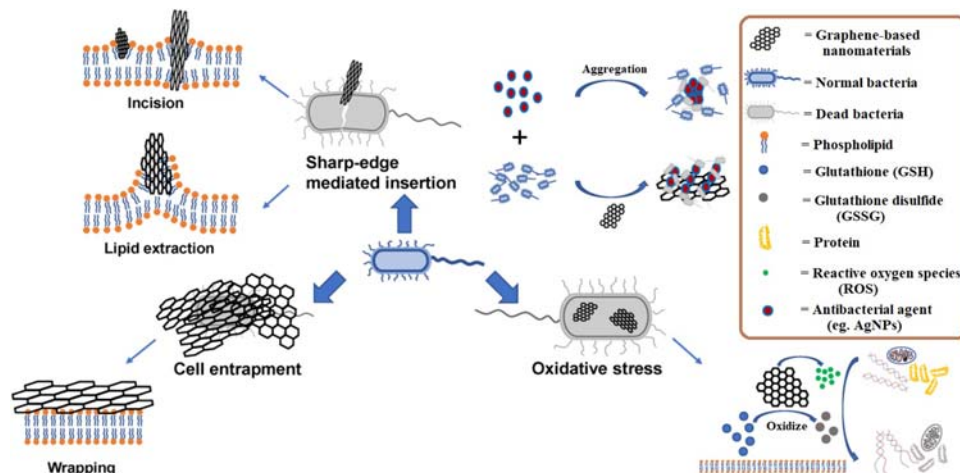
Figure 11.3 GBNs most probable antibacterial mechanisms including sharp-edge cutting effect, oxidative stress, and cell entrapment.

seen that each GBNs has its own characteristics which depends upon its structural orientation. Gurunathan et al. reported that the reactive oxygen species (ROS) portions increased by 3.8-fold and 2.7-fold in *P. aeruginosa* when exposed to GO and rGO, respectively, whereas the reduced glutathione (GSH) content decreased significantly [98]. Therefore, it is inferred that the large production of ROS by GBNs is the cause of OS of bacteria which also gets confirmed from the level of GSH, a reductase in bacteria can oxidize into glutathione disulfide (GSSG) [99]. In contrary to this, Liu et al. stated that after exposure of *E. coli* to four types of GBNs, the decrease of GSH concentration was not in succession with the increase in ROS concentration [12]. The rGO and graphite were shown strong oxidizing behavior toward GSH where they assumed that the conductive GBNs can get electrons from bacterial cell membranes via electron transfer channel which resulted in OS in bacteria. Moreover, Farid et al. noticed that the OS can promote SECE of GO by degrading ATP [100].

7.3 Cell entrapment

CENTP is the trapping of bacteria by GBN sheets which makes them unapproachable from the surroundings and also restricts their nutrition accessibility. In other way, CENTP cannot kill the bacteria but only restricts its growth. CENTP is the key mechanism for the AA of GBNs which can be correlated with the size of GBN sheets. It is observed that the *E. coli* cells were entrapped more efficiently by larger size GO sheets (Fig. 11.4) and the cells could not proliferate due to complete trapping on GO sheets [101]. This has been confirmed by atomic force microscopy (AFM) and inferred that the AA of GO are improved with the increase of its lateral dimensions [91,101].
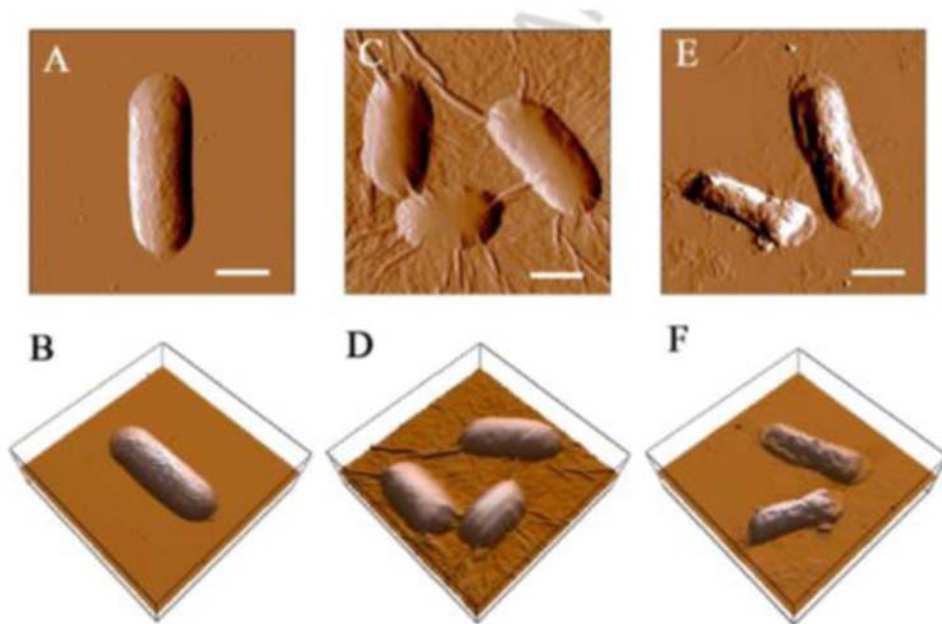
Figure 11.4 AFM images of *E. coli* cells after incubation with deionized water (A, B) or GO with large size (C, D) and small size (E, F). Scale bar: 1 um. (B, D, F) Are modified 3D images of (A, C, E), respectively.

Moreover, it is also found that the entrapped bacteria could be recovered by sonication, which has lost their viability when entrapped by GO sheets [91]. In short, GBNs with small size will be beneficial in the SECE while GBNs with large size is useful in the entrapment effect. In addition, strong UV absorption observed by smaller size GO and have high OS than the GO with the larger size even at the same concentrations. Hence, the antibacterial efficacy of GBNs is size dependent and also affected by other factors such as OS, entrapping, and ED which may guide a further work.

7.4 Other hypotheses

Though, based on the complex interaction of GBNs and bacteria there will be other possible mechanisms which we haven't discussed before. In connection with this, researchers described that the GO sheets and pristine graphene strongly interact with the lipids after penetrating the cell membrane of bacteria and have lipid extraction which destroys the membrane and ultimately the bacterial viability decreases [102]. Also, GBNs can affect protein–protein interactions by interfering with the intracellular signal transduction and biological metabolism [103,104]. Moreover, "suicide effect" another well-known mechanism is proposed where bacteria utilize OFG of GO through a glycolysis-like pathway and become inactivated [93,105]. Herein, the AA efficiency and probable mechanisms of GBNs in respect to some representative pathogens are summarized in Table 11.3.

Table 11.3 Typical examples of antibacterial effects of graphene-based nanomaterials and nanocomposites on representative pathogens.

Bacteria	Material	Antibacterial efficiency	Antibacterial mechanism	Characterization technologies	References
E. coli	GO	90.9% viability loss (100 μg/mL)	Insertion, edge cutting, lipid extraction	TEM, COM	[102]
E. coli	GO	4% survival rate for the exponential–phase cells, 75% for the stationary phase and 50% for the decline phase.	Cell entrapment	XPS, AFM, SEM	[106]
E. coli	MGO–Ag (silver nanoparticle-decorated magnetic graphene oxide)	99.9% viability loss (50 μg/mL)	ROS generation, Ag^+-caused membrane damage	XRD, SEM, TEM, EDS, Raman spectra, magnetic property tests	[107]
E. coli	Ag-RGO	MIC: 20 μg/mL, MBC: 40 μg/mL	Cell entrapment, cell membrane damage, oxidative stress, the bactericidal action of Ag^+	FESEM, XRD, FTIR, Raman spectra, UV–vis absorption spectra, EDS, STEM, SEM	[108]
E. coli	rGO and GO	90% and 98.5% viability loss caused by rGO and GO (85 μg/mL), respectively	Cell membrane damage	AFM, SEM	[109]
E. coli	rGO and GO	91.6% and 76.8% viability loss caused by GO and rGO (80 μg/mL), respectively	Cell membrane damage, ROS-independent oxidative stress	AFM, SEM, XPS, Raman spectra, DLS	[12]

E. coli	GO-SPEEK (sulfonated polyetheretherketone)	77.3% viability loss	Edge cutting, ROS generation, DNA damage, decrease bacteria adhesion	AFM, contact angle, ATR-FTIR, Raman spectra, XPS, SEM	[110]
E. coli	rGO-AgNPs	97% viability loss	Cell membrane damage	SEM, XRD, FTIR, TEM, LSCM	[111]
E. coli	GQD (graphene quantum dots)	IC50 $\leq$ 100 μg/mL	ROS generation, photoexcited killing, cell membrane damage	AFM, TEM	[112]
S. aureus	GO and rGO-poly(dopamine)	rGO-PDA layers: 45% adhesion reduced, 30.5% viability loss in biofilm; bulk GO layers: 55.1% adhesion reduced, 40.7% viability loss in biofilm	Cell membrane damage, ROS generation, electron transfer	AFM, FESEM, micro-Raman spectra, FTIR, XPS	[113]
S. aureus	GO-AgNPs	79.6% viability loss	Cell membrane damage, ROS generation	SEM, TEM, AFM	[114]
S. aureus	GO-PEI-AgNPs	MIC: 3 μg/mL	Cell entrapment, insertion cutting, Ag^+-caused damage to cell structures	TEM, XPS, AFM, FTIR, XRD, TGA	[88]
S. aureus	PLGA/chit-GO-AgNPs	79.4% viability loss	Catalytic oxidation by silver, cell membrane damage, ROS generation	FTIR, SEM, TEM, contact angles, Raman spectra	[115]

Continued

Table 11.3 Typical examples of antibacterial effects of graphene-based nanomaterials and nanocomposites on representative pathogens.—cont'd

Bacteria	Material	Antibacterial efficiency	Antibacterial mechanism	Characterization technologies	References
S. aureus	$Ag-CoFe_2O_4-GO$	97.9% viability loss (12.5 μg/mL)	Cell entrapment, Ag^+-caused DNA damage, lipid oxidization	FTIR, XPS, XRD, Raman spectra, TEM, CV, BET, magnetic property tests	[116]
S. aureus	rGO-Ag/AgCl	MIC: 4 mg/L	ROS generation, cell entrapment	TEM, XPS, SEM, AFM	[117]
MRSA	rGO-IONP	47% viability loss in vivo, 81% viability loss in vivo with NIR	Photothermal effect, cell membrane damage, ROS generation	XRD, FTIR, TEM	[14]
MRSA	Chitosan—iron oxide coated films	100% viability loss	Cell membrane damage, ROS generation, iron oxide NPs-caused antibacterial effect, hydroxyl radical production	FTIR, SEM, XRD, VSM, TGA, contact angle	[118]
MRSA	GQD	IC50 ≤ 100 μg/mL	ROS generation, photoexcited killing, cell membrane damage	AFM, TEM	[112]
P. aeruginosa	GO and rGO	87% and 86% viability loss caused by GO and rGO (75 μg/mL), respectively.	Oxidative stress, ROS generation, DNA fragmentation	UV-vis absorption spectra, XRD, DLS, SEM, Raman spectra	[98]
P. aeruginosa	GO-Ag (43% Ag)	100% viability loss (100 ppm)	Oxidative stress, cell entrapment, the bactericidal action of Ag^+	FESEM, TEM, XRD, UV-vis absorption spectra, FTIR, TGA	[119]

P. aeruginosa	GO	98.49% viability loss (2 μg/mL) under NIR	DNA fragmentation, cell membrane damage	FTIR, Raman spectra, UV–vis absorption spectra, HRTEM, DLS	[109]
P. aeruginosa	Graphene–tin oxide	MIC: 250 μg/mL	Oxidative stress, edge cutting	XRD, Raman spectra, HRTEM, PL spectra	[120]
P. aeruginosa	PLGA/chit-GO-AgNPs	90% viability loss	Catalytic oxidation by silver, cell membrane damage, ROS generation	FTIR, SEM, TEM, contact angles, Raman spectra	[115]
P. aeruginosa	GN-R (graphene nanorough) and GN–S	GN-R: 87.6% viability loss; GN-S: 71.4% viability loss	Cell membrane damage	XRD, EDX, micro-Raman spectra, FESEM, AFM	[94]
S. mutans	GO	100% viability loss in 2 hours (80 μg/mL)	Cell membrane damage, ROS generation	AFM, DLS, FTIR, Raman spectra	[10]
S. mutans	rGO-Ag	MIC: 0.16 mg/mL, MBIC: 0.32 mg/mL	Cell entrapment, the bactericidal action of Ag$^+$	SEM, TEM, TGA, AFM, CLSM	[121]
S. mutans	Titanium modified with minocycline-loaded GO	Over 90% viability loss	The bactericidal action of minocycline hydrochloride, contact killing effect	UV–vis absorption spectra, Raman spectra, XPS, SEM	[122]
S. mutans	ZNGs	99.9% viability reduction (50 μg/mL)	Cell entrapment, edge cutting	FE-SEM, XRD	[123]
C. albicans	CH-GIO films	100% viability loss	Cell membrane damage, ROS generation, iron oxide NPs-caused antibacterial effect, hydroxyl radical production	FTIR, SEM, XRD, VSM, TGA, contact angle	[118]

Table 11.3 Typical examples of antibacterial effects of graphene-based nanomaterials and nanocomposites on representative pathogens.—cont'd

Bacteria	Material	Antibacterial efficiency	Antibacterial mechanism	Characterization technologies	References
C. albicans	GO–AgNPs	77.5% viability loss	Cell membrane damage, oxidative stress	X-ray diffraction, Raman spectra, FTIR, TEM, SEM, AFM	[114]
C. albicans	GO–PEI–Ag	MIC: 4 μg/mL	Cell entrapment, cell membrane damage	TEM, XPS, AFM, FTIR, XRD, TGA	[88]
S. typhimurium	Graphene	MIC: 1 μg/mL	Cell membrane damage, ROS oxidative stress	UV–vis absorption spectra, XRD, FE-SEM, HR–TEM, Raman spectra, FTIR	[124]
S. typhimurium	GO	MIC: 0.25 μg/mL, 0.125 μg/mL with UV irradiation	ROS generation, cell membrane damage	UV–vis absorption spectra, Raman spectra, TEM, AFM, FE-SEM, XPS, UPS	[125]
S. typhimurium	GO–ZnO	MIC: 6.25 μg/mL	ROS generation, lipid extraction, electron transfer	FE-SEM, EDX, AFM, UV–vis absorption spectra, XRD, FTIR, CV	[126]
S. typhimurium	rGO–WS$_2$	83.89% viability loss (250 μg/mL)	ROS-independent oxidative stress, electron transfer, direct contact with membrane	FE-SEM, TEM	[127]
S. typhimurium	GO–AgNPs	100% viability loss	Cell entrapment, cell membrane damage, the bactericidal action of Ag$^+$	XPS, TEM, TGA, FTIR	[128]

8. Conclusion and perspective

GBNS have many biomedical uses such as drug delivery, cancer therapy, and antibacterial activities against several pathogens. Here, in this chapter we discussed about probable GBNs structures and sizes, and their antibiotics–independent antibacterial activities. Moreover, practical problems and their solutions associated with these multifunctional nanomaterials have been discussed. Sharp-edge cutting effect, OS, CENTP, lipid inter-actions, and protein-protein interactions are the possible mechanisms through which GBNs can act as antibacterial agents. They can be used with other bioactive agents in nanocomposites for the enhanced antibacterial activities and physicochemical properties. Currently, bacterial resistance is the most concerning threat which can be overcome with the help of GBNs via the probable antibiotics' independent mechanisms.

On the other hand, GBNs have some issues such as toxicity, adsorption of nonspecific protein, and challenges in targeted drug delivery which can be observed in clinical prac-tices. To avoid non-specific protein adsorption kind of obstacle, the preformed specific protein corona (SPC) on the GBNs surface played a very important role. The SPC can prevent the adsorption of other biomacromolecules which increases the certain interac-tion of GBNs with a certain species of bacteria. This approach ultimately reduces the GBNs toxicity and may direct the design and functional modification of GBNs for clin-ical use.

Acknowledgments

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ANTIMICROBIAL NANOSYSTEMS

Fabrication and Development

Edited by Chaudhery Mustansar Hussain, Kabali Vijai Anand and Shadpour Mallakpour

Antimicrobial Nanosystems: Fabrication and Development provides an in-depth review of nanotechnological advancements in the fields of biotechnology and pharmaceutical industries to counteract bacterial infections and related health issues. Functionalized nanomaterials and their processes are covered, along with the theory and fabrication of antimicrobial nanosystems. The potential applications of antimicrobial nanosystems are also discussed along with their challenges and commercialization.

This book discusses the most frequent problems caused by resistant microorganisms and difficult-to-treat bacteria and highlights the impact of recently developed antimicrobial nanosystems. Various methods to obtain efficient nanomaterials with antimicrobial properties are described, along with their advantages, challenges, and main applications. The design of targeting antimicrobial therapeutics, able to specifically detect pathogenic microorganisms and to act in a very specific manner, is thoroughly investigated.

Antimicrobial Nanosystems: Fabrication and Development is an important reference source for materials scientists, engineers, and pharmaceutical scientists who endeavor to expand their understanding of how nanotechnology is being used to create more efficient antimicrobial treatments.

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Antimicrobial
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Micro and Nano Technologies Series

Antimicrobial
NANOSYSTEMS
FABRICATION AND DEVELOPMENT

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Dedication by Chaudhery Mustansar Hussain
I would like to dedicate this book to
My beloved GOD (*The most glorified, the most high*)
"Meray Pyarey Allah (Subhanahu Wa Ta'ala)"

Dedication by Kabali Vijai Anand
I would like to dedicate this book to
My beloved mother Mrs. Kabali Thulasi
and all other family members

Dedication by Shadpour Mallakpour
I would like to dedicate this handbook to
My wife Mina
My son Iman
My daughters Adeleh and Fereshteh
My granddaughter Termeh

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CHAPTER 7

Nanostructured peptides as potential antimicrobial agent

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1. Introduction to antimicrobial peptides

Recently, the nanometer-sized structures of organic and inorganic compounds like drugs, proteins, and metals have left an impact on scientific research because of their versatile potential from medicinal to material sciences. Apart from material science, the biological compounds such as nucleic acids (DNA/RNA), proteins, microorganisms, and lipids have their sizes in submicron and thus are being considered as biological nanostructures. Using such biological compounds, new nanostructured compounds are being fabricated [1] where peptides are being targeted for such purpose. This is owing to their structure, shapes, relative physicochemical stability, easy preparation in bulk, as well as their intrinsic biocompatibility and biodegradability [2]. These features lead to their utility for making self-assembled nanostructures for medicinal purposes. Thus, various physicochemical properties have been associated with proteins and peptides due to polar side chains and charge variation, hydrophobicity, and hydrophilicity. In this context, the self-assembled peptides are designed considering sequence, number, and type of amino acids where amino acid sequence plays a key role. The tendency of peptides to form specific secondary structure attributes for the preparation of controlled structured nanomaterials with more biocompatibility and biodegradability [3].

Peptides show their antimicrobial properties and that is why they are known as antimicrobial peptides (AMPs). These are synthesized by the varying number of amino acids where they target microorganisms such as bacteria, fungi, viruses, and parasites. AMPs are categorized into anionic, cationic, host defense, and α-helical [4−8]. These have been found effective against wounds and ulcers [9], both Gram–negative and Gram–positive bacteria [10], fungi, and some pathogenic bacteria [11]. Many natural AMPs have been found in prokaryotes and eukaryotes such as fungi, bacteria, plants, and animals [12−15] where tissues and organs are the sources for the same in animals [13]. For instance, more than 250 different AMPs are found in the skin of frog [16] where specific cells

produce them. Similarly, in mammals, the lipopolysaccharide (HEK293 cells) is account-able for the production of AMPs (defensin) which is produced by bacteria [17,18].

1.1 Types of antimicrobial NsAMPs

The antiviral property of AMPs is due to their integrating nature against membrane or envelop of RNA and DNA viruses [19,20] where they cause membrane instability of virus as well as reduce the binding strength of viruses to host cells [21−24]. Not only this but also AMPs block the viral receptors [25,26]; for instance, lactoferrin (α-helical cationic peptides) prevents viral infection via blocking virus−receptor interactions [27−30].

   According to their mechanism of action, first they localize in the cytoplasm and organelles after crossing the cell membrane. It causes the vicissitudes in the host cells by changing the gene expression profile; as a result, the host protection system blocks the viral gene expression. For example, AMP from rabbit neutrophils has been found effective to prevent infection caused by herpes simplex virus type 2 [31].

1.1.1 Antibacterial peptides

The antibacterial property of AMPs is due to their action against bacterial cell membranes by causing disintegration of bilayer of the lipid [32,33]. These AMPs are cationic and amphipathic having hydrophilicophobic domains where they bind to lipid components via hydrophilic−hydrophilic and hydrophobic−hydrophobic interactions. Apart from this some, AMPs also have activity against bacterial DNA and proteins to kill the bacterial cell [34]. For instance, buforin, drosocin, pyrrhocoricin, and apidaecin AMPs have 18−20 amino acid residues and are fallen into this category [35].

1.1.2 Antifungal peptides

For antifungal property of AMPs, they destroy the intracellular components or cell wall of the fungus [36−38]. AMPs bind with chitin that is a component of cell walls and this binding leads to the disruption of the cell wall of fungi [39−43]. The polar and neutral amino acids present in the structure of AMPs are responsible for this activity, for example, indolicin [44] and β-sheet defensin [45].

2. Synthetic and structural aspects of NsAMPs

2.1 Synthesis of NsAMPs

The synthesis of peptides is typically done through amidation reactions where amino group and the carboxylic group are coupled to produce the peptide bond. In this regard,

the insoluble resins were also utilized for the synthesis of peptides and the same technique was used for in solid–phase peptide synthesis [46,47]. Similarly, the enzyme-catalyzed synthesis of peptides is also used [48]. Peptides have provided a biomolecular support in aqueous media for solubilizing and assembling π-conjugated molecules, and their properties are modified by altering the amino acid residues. Kale and Tovar [49] have reported a solid–phase peptide synthesis where they synthesized f DFAG-4 T-GAFD peptide as shown in Fig. 7.1.
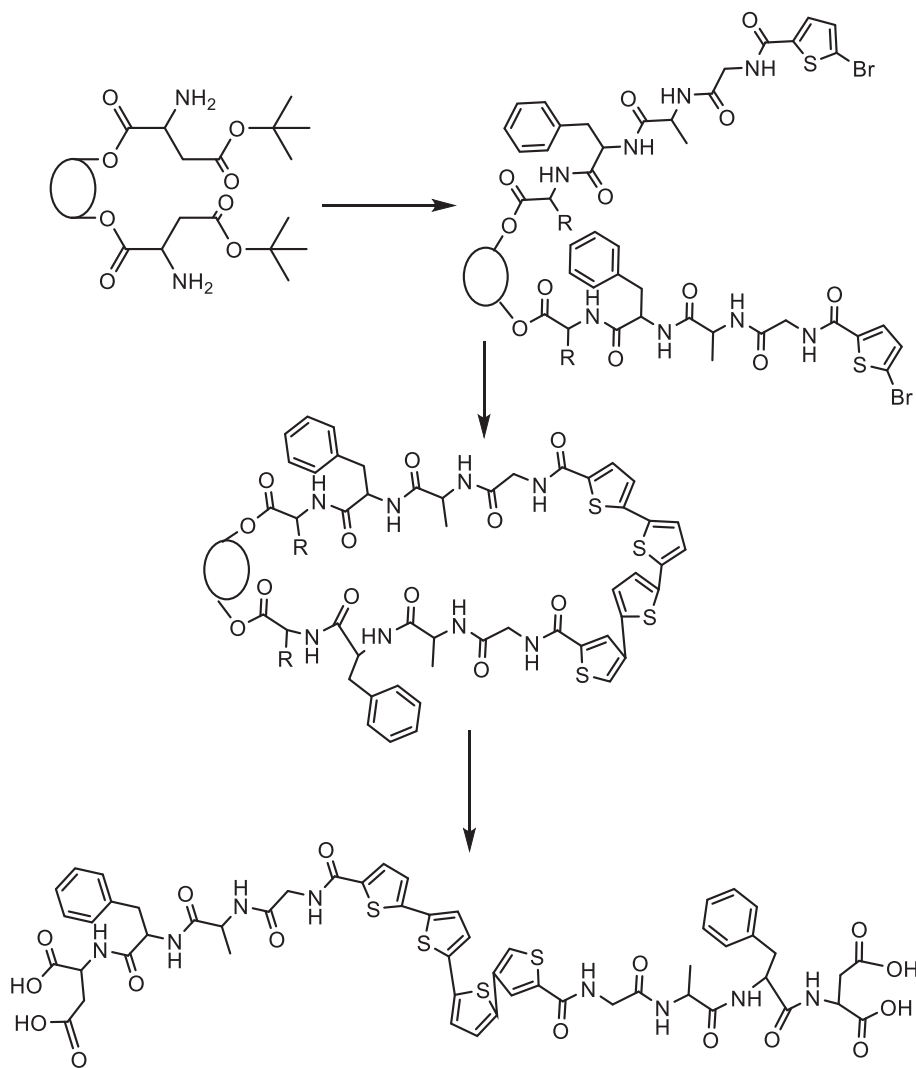

Figure 7.1 Solid-phase peptide synthesis.

2.2 Characterization of NsAMPs

Characterization is very important for structural elucidation of nanostructured antimicrobial peptides (NsAMPs) before starting to work with them for their applications. These characteristics can be determined using several techniques and some of them are as follows.

2.2.1 Atomic force microscopy

Atomic force microscopy imagining techniques can be involved in examining the geometry of the formed structures. In this technique, a potential difference is applied between the tip and the sample where the tip is raised some nanometers above the surface and kept at this distance, and topographical analysis is done.

2.2.2 Electron microscopy

The scanning electron microscopy and transmission electron microscopy (TEM) are widely used characterization tools to find the size and shape in nanodiameter. The surface shape of the formed structures can be readily visualized by such techniques. TEM helps to verify whether the formed structures contain cavities or not as well thermally stable or not. The strength of the structure can also be examined using such techniques.

For the same purpose, additionally, Fourier-transform infrared spectroscopy (FTIR), UV–visible, X-ray diffraction, energy dispersive X-rays, and X-ray photoelectron spectroscopy (XPS) are also used.

3. Nanostructures of antimicrobial peptides

Different kinds of nanostructured peptides have been developed for the betterment in their medicinal properties.

3.1 Tubular form

Scientists have done great progress in the field of nanotechnology and developed nanostructured compounds. In this context, the nanotubes have gained much more attention of researchers because their structure can be controlled in terms of diameter in size due to feasible preparation and self-organization with high efficiency. Nanotubes with a certain inner hole have significant applications in the field of almost all branches of the science like biology, chemistry, and physics. Here these are being used for drug delivery, chemotherapy, molecular separation, catalysis, optics, and electronics [50]. Several methods are being used for the preparation for the hollow bundles of rod-like units, helical structures, and stacked rings of nanotubes. In this regard, the simplest peptide block for self-assembly (diphenylalanine peptide) with a long persistence length such as 100 μm has been reported [51] where hydrogen bonding and π−π stacking were found to be accountable.

3.2 Cyclic form of peptide

The stacking of peptides gives rise to the formation of cyclic peptide nanotubed structure where hydrogen bonds provide the stability. Such peptides have the ability to change their properties and orientation by directing amino acid side chain and carbonyl groups as outward and perpendicular to the ring, respectively. Initially, Ghadiri and coworkers [52] have reported cyclic peptides with heterochirality, based on organic nanotubes having extended β–sheet and cylinders. These were prepared by alternating D– and L–amino acids where hydrogen bonding was key factor for the self-assembly with definite diameter. In acidic medium, this assembly produced nanotube structures which upon hydrogen bonding formed ring-shaped structure of peptides, as shown in Fig. 7.2.

Since in acidic medium the formation of peptide nanotubes is favorable, in the case of alkaline medium, there is repulsion between the negatively charged acidic groups of glutamate, and thus this condition is not favorable. The change in amino acid sequence changes the internal diameter of the nanotubes [53]. Such peptides were found to be very effective for destroying bacterial membrane and showed antibacterial activity [54]. These peptides having hydrophobic surface residues lead to transmembrane channel structure in lipid bilayers through self-assembly process where stacking interactions were accountable for wrapping the lipid bilayer. Similarly, lanreotide octapeptide, a cyclic peptide, was prepared as a growth hormone inhibitor where it was self-assembled into nanotubes [55].
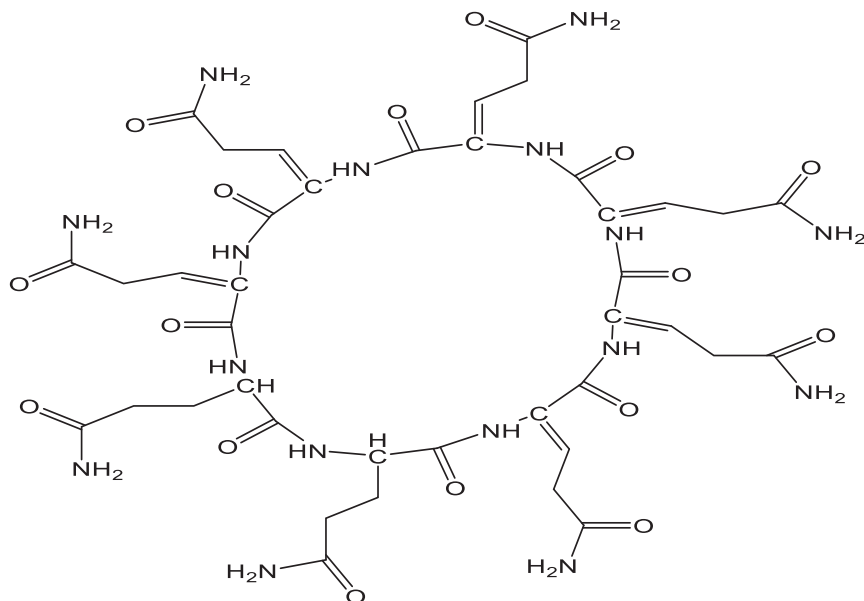

Figure 7.2 Assembly produced nano-sized ring-shaped structure of peptides through hydrogen bonding.

3.3 Hydrophilicophobic nanotubular peptide

Nanotube-like hydrophilicophobic peptides have lipid or surfactant-like properties with tail and head groups and have been developed [56]. The hydrophobic part of such peptides contains a combination of non—polar amino acid residues and hydrocarbon chain and hydrophilic part generally containing a charged amino acid residue where the range is found 50—100 nm [57]. Hydrophobic part containing alanine and valine residue forms a more stable nanotube as well as nanovesicles as compared to isoleucine and leucine [58]. The hydrophilic and phobic properties of such nanostructure peptide are controlled by balancing between size of hydrophobic residues and amino acid sequence. Such peptides have been found as a stabilizer of proteins under thermal conditions where molecular geometry affects the nanostructures [59].

3.4 Nano-sized fibrous peptide

Self-assembling NsAMPs are a crucial point for investigators all around the world because of their utility as potential building blocks, fabrication material, and antimicrobial agents. Hydrophilicophobic peptides form a fiber-like structure having a diameter of less than 100 nm like lipids by self-assembly via hydrophobic interactions at physiological conditions. These can also be formed by mixing two oppositely charged hydrophilicophobic peptides [60]. The hydrophobic part self-assembles into nanofiber structures due to collapsing of hydrophobicity and the formation of β-sheet [61]. Similarly, the β-sheet structure, as secondary structure in proteins, has alternate amino acid side chains with electrostatic and hydrophobic forces that stabilize the sheets [62]. This alternating sequence of amino acids forms close chain packing that leads to the formation of nanofiber [63]. Likewise, short chain containing peptide with an alternating pattern of hydrophobic and hydrophilic amino acids has also been developed having a self-assembled nanofiber structures. Similarly, the nanocapsule-like structure of peptides has also been developed with plentiful of proline, an amino acid [64,65]. The proline containing a pyrrolidine ring side chain and backbone atoms forms a vesicular structure through the self-assembly process, as shown in Fig. 7.3.

The linear and cyclic peptides have different self-assembly process where linear peptides have tryptophan and arginine residues create irregular morphologies, while cyclic peptides form spherical nanostructures. Recently, such nanostructured peptides have also been developed at room temperature in aqueous medium through balancing hydrophobicity and charge (Fig. 7.4) [66].

Similarly, several self-assembled amyloid nanofibrillary structured peptides have been prepared [67] and found to be effective for Alzheimer's disease, type II diabetes, Parkinson's diseases, and many others [68]. These are produced naturally or artificially with β-sheet conformation having 30—40 amino acids.
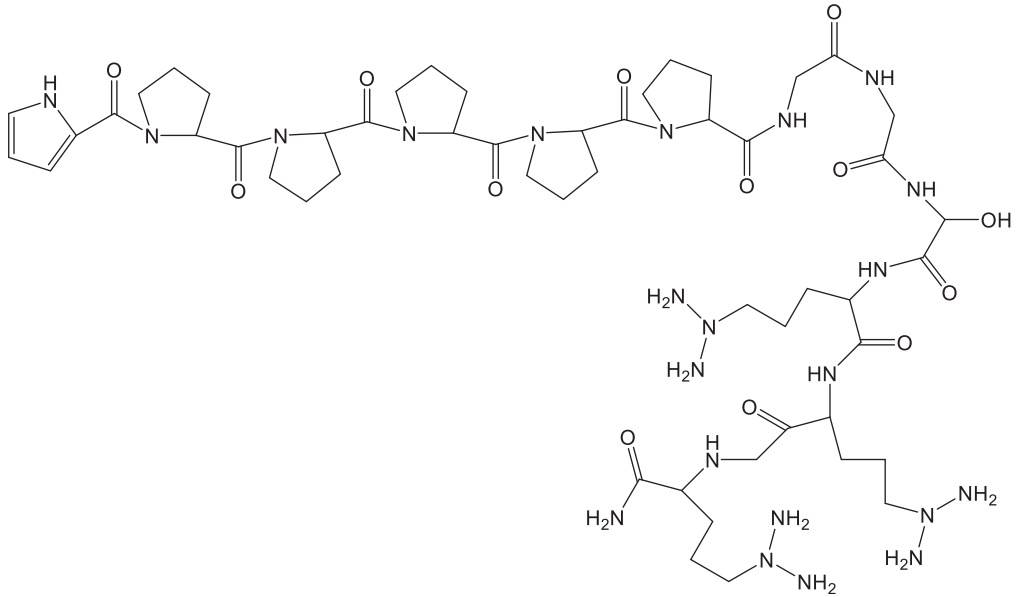
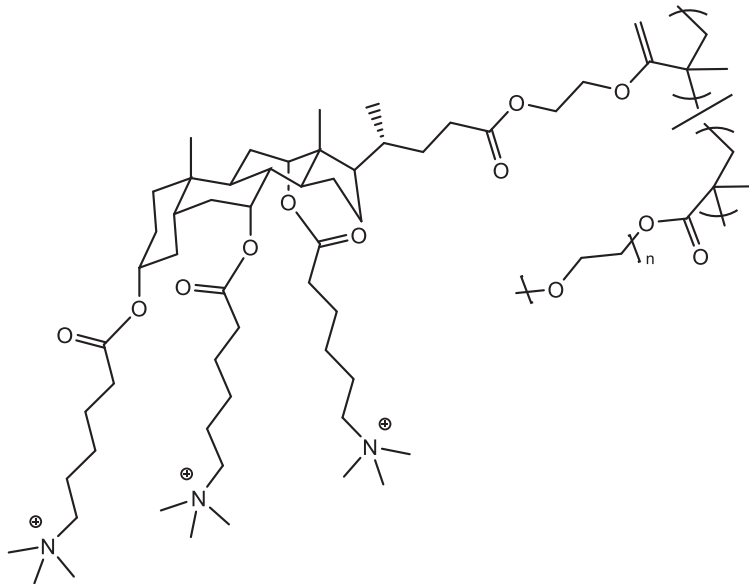**Figure 7.3** Nanocapsule structure of antimicrobial peptides.

Figure 7.4 Cyclic peptides as spherical nanostructures.

4. Development of NsAMPs

The structure of a peptide can be modified into nanostructures via self-assembling in response to physical stimuli such as pH, temperature, strength of ion, and presence of precise enzymes [69]. The modifications in amino acid sequence, the hydrogels composed of nanofibers, or peptide nanotube structures can be adopted. Such NsAMPs show a significant potential for clinical translation; for example, recently, octreotide (a NsAMP) has been granted to treat acromegaly [70]. Such NsAMPs with hydrogel and nanotube structures have been used for hydrogel wound therapy to form building blocks of tissues and skin. Such hydrogels signify the medical device coatings as well as activated to respond to pathogenic stimuli [71,72]. The diversity in the field of biological self-assembly leads to the formation of NsAMPs like nanotubes, nanofibers, and nanoparticles contingent on the formation conditions. NsAMPs are being used as excellent drug for clinical experiments [73] as immune modulating and antiviral agents [74,75]. NsAMPs with small size as well as small pore form proteins that target the membranes of negative bacteria via cell lysis, although few bacteria express their resistance toward NsAMPs due to presence of membranal lipids with positive charge [76]. Especially, in Gram–negative bacteria, the primary binding site of NsAMPs is blocked by modifying the cell wall where NsAMPs perform binding. Despite this, NsAMPs are of interest because NsAMPs can target the membrane damage to intracellular biomolecules and oxidative damage [77,78]. In addition, NsAMPs also have the ability to develop antiresistance activity toward traditional antibiotics [79]. The cationic NsAMPs are found to be effective in controlling the inflammation by neutralizing the endotoxins [80,81]. There are several examples of NsAMPs that have been commercialized such as polymyxin B, colistin, gramicidin, vancomycin, daptomycin, bacitracin, enfuvirtide, and telaprevir. Apart from positive aspects of NsAMPs, they create toxicity also at high doses such as kidney and nervous system damage [82] and other side effects like hypersensitivity [83,84] and low efficiency in contradiction of drug-resistant Gram–positive microorganisms [85]. Currently, numerous NsAMPs are being designed focusing their action against microorganisms as well as for their antiviral and wound healing activity. Especially, scientists are concerned to synthesize Gram–negative active NsAMPs because of the lack of effective NsAMPs in this area. However, there are some restriction factors for developing NsAMPs such as poor pharmacokinetics, constant toxicity [86], hemolytic activity, low hydrosolubility, and rapid renal filtration. To overcome these, several strategies are being adopted like chemical modification of natural AMPs and development of peptidomimetics.

Several features of NsAMPs such as hydrophilicophobic properties, size, net charge, amphipathicity, and helicity affect the antimicrobial effect against bacterial membrane where these all properties have a combined effect to fight with bacteria. Now these days, each property of NsAMPs is being analyzed for antibacterial action; for example,

Gagnon et al. found the antimicrobial activity of NsAMPs was associated with the length of NsAMPs [87]. Similarly, the hydrophobicity of NsAMPs was also specifically analyzed because as the length of amino acid increases, the hydrophobicity as well as net +ve charge also increases. These two parameters define the amphipathicity of NsAMPs that is accountable for the interaction with the membrane of microorganisms [88]. However, many studies suggested that there is no direct relationship between positive net charge and antimicrobial activity; that is why the evaluation of net +ve charge is most important whether it is going to affect the hydrophobicity, hydrophobic moment, amphipathicity and helicity of the NsAMPs. Because the amount of charge creates misbalancing in all mentioned properties of NsAMPs.

4.1 Nanostructured antimicrobial lipopolypeptides

Covalent interaction of cationic or anionic peptide in cultivation process in microorganism produces nanostructured antimicrobial lipopeptides. For instance, the nanostructured antimicrobial lipopolypeptides composed of palmitic acid and cationic peptides containing L- and D-L amino acids have shown their antimicrobial potentials [89]. Such lipopeptides were found in solution as assembled nanostructures having a unique morphology responsible for their biological activity where they have permeated and disintegrated the microorganism's membrane.

4.2 Surface modification of NsAMPs

The antibacterial activity shown by AMPs is due to their surface activity that interacts with bacterial membrane and disrupts it. Therefore, the surface modification to the nano size results in enhancement in the same activity. In this context, the passive and active approaches through the immobilization of polymer micelles decorated with AMPs have been developed for improving antimicrobial efficacy of NsAMPs [90]. AMPs have the potential to fight multidrug-resistant bacteria through membrane disruption process, but the poor stability and short-term activity are attributed to the development of NSAMPs. Thus, the biocompatible self-assembled NsAMPs are stable in vivo and mark their importance. Therefore, nanofibril AMPs are being developed because these are found to be effective for the disruption of microbial membrane [91].

4.3 Battacin-inspired NsAMPs

Despite the discovery of many AMPs, still there is a demand of effective antibiotics because of the expansion of antibiotic confrontation in bacteria. AMPs are self-assembled through hydrogen bonding, hydrophobic, ionic, and $\pi-\pi$ stacking interactions that result in NsAMPs. Such NsAMPs trap the solvent, which results in hydrogels that are very effective in wound healing, drug delivery, and tissue engineering [92]. In this regard, nanostructured antimicrobial hydrogel has been reported by Laverty et al.

which was produced using ultrashort naphthyl–capped cationic peptides. Similarly, the nanostructured antimicrobial hydrogels were produced through self-assembling of peptides with low concentration in buffer solution where it was established by β-sheet—rich nanofibril frameworks [93]. In addition, the NsAMPs have also been prepared via hybrid protein-engineered polymers as supramolecular nanomaterials by way of a dual–assembly process. These synthesized NsAMPs were found to be a groundbreaking tool in the growth of self-assembling nanosystems with potential use for biotechnological and biomedical applications [94].

4.4 Antimicrobial peptide—gold nanoclusters for bacterial targeting and imaging

To enhance antimicrobial activity and decrease the resistance to bacteria, the NsAMPs with metals have been studied [95]. AMPs as reducing ligands have been used for the synthesis of AMP-coated gold nanoclusters avoiding the use of unwanted solvents and reagent. Such clusters express their bacterial binding and photoluminescence topographies useful for the detection and imaging of bacterial contamination.

4.5 Amphiphilicity-induced NsAMPs

Amphiphilicity of NsAMPs has been found very important in addressing an increase in antimicrobial resistance by multidrug-resistant pathogens. In this regard, Rahman et al. [96] reported the facial synthesis of nanostructures amphiphilic copolymer of bile acid with antibacterial activity against Gram +ve and Gram −ve bacteria. Such spheres and rod–shaped NsAMPs were found to be able to preferentially interact with bacterial membranes with less hemolysis against mammalian cells. These NsAMPs were also incorporated with polyethylene glycol for attaining the high stability as well as biocompatibility of NsAMPs.

4.6 Peptide-stabilized emulsions and gels

Nowadays, the hydrogels and stable emulsions are showing importance in the preparation of many functional nanostructured AMPs. Thus, the multifunctional self-assembly and bioactivity properties of a novel surfactant-like peptide having antimicrobial activity are reported recently [97]. These NsAMPs are able to develop hydrogels without changing pH and very capable to stabilize the oil-in-water emulsions. Such NsAMPs have shown discerning antimicrobial activity against −ve bacteria that cause infections, for instance, *Pseudomonas aeruginosa*. These NsAMPs are to be used for formulating AMP emulsions.

4.7 Nanohybrid AMPs

Since AMPs are short and positively charged peptides and discovered from different forms of life. These are effective alternatives of routine antibiotics having the ability to

kill pathogens, for example microcin J25 [98]. Microcin J25 shows impressive antimicrobial activity without toxicity in vivo and in vitro against Gram -ve bacteria such as *Shigella*, *Salmonella*, and *Escherichia coli*. Because of its strong antimicrobial activity, less risk, and high stability, it has been found to be a promising antimicrobial agent. Other than AMPs, the chitosan-based antimicrobials have also been used, but their high concentration is required, and hence there is a need to enhance the antimicrobial activity with low concentration [99]. Therefore, in this context, Yu et al. [100] reported the synthesis and antimicrobial applications of nanoconjugates of chitosan and microcin J25. These conjugates are very effective in treatment of disease caused by multidrug-resistant bacteria without increasing mutation rate and resistance.

4.8 Nanoengineered antimicrobial peptide polymers

For synthesis of peptide polymers having antimicrobial properties, the ring–opening polymerization of amino acid is one of the best methods [101,102]. It provides a facile route for the same to produce macromolecular architectures, like star polymer nanoparticles [103]. Therefore, in this regard, the synthesis of star-shaped peptide polymer nanoparticles with lysine and valine amino acids has been reported recently [104]. These self-assembled nanoengineered AMP polymers are stable up to infinite dilution.

5. Conclusion

This chapter summarizes the biological evaluation of AMPs with their nanostructures. This chapter will be very useful for the readers who are working in this field. It provides very interesting and useful information about nanostructured peptides that may be a base for the researches and scientists for conducting their work.

Acknowledgment

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ANTIMICROBIAL NANOSYSTEMS

Fabrication and Development

Edited by Chaudhery Mustansar Hussain, Kabali Vijai Anand and Shadpour Mallakpour

Antimicrobial Nanosystems: Fabrication and Development provides an in-depth review of nanotechnological advancements in the fields of biotechnology and pharmaceutical industries to counteract bacterial infections and related health issues. Functionalized nanomaterials and their processes are covered, along with the theory and fabrication of antimicrobial nanosystems. The potential applications of antimicrobial nanosystems are also discussed along with their challenges and commercialization.

This book discusses the most frequent problems caused by resistant microorganisms and difficult-to-treat bacteria and highlights the impact of recently developed antimicrobial nanosystems. Various methods to obtain efficient nanomaterials with antimicrobial properties are described, along with their advantages, challenges, and main applications. The design of targeting antimicrobial therapeutics, able to specifically detect pathogenic microorganisms and to act in a very specific manner, is thoroughly investigated.

Antimicrobial Nanosystems: Fabrication and Development is an important reference source for materials scientists, engineers, and pharmaceutical scientists who endeavor to expand their understanding of how nanotechnology is being used to create more efficient antimicrobial treatments.

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शेतकऱ्याची कविता :

शोषण

आणि

जागृती

—प्राचार्य
डॉ. गजानन जाधव

डॉ. गजानन जाधव हे आपल्या संस्थेच्या मोताळा येथील श्री शिवाजी कला, वाणिज्य व विज्ञान महाविद्यालयात प्राचार्य आहेत. ग्रामीण भाग, कष्टकरी वर्ग आणि काबाडकष्ट करणारा शेतकरी ही त्यांची कौटुंबिक पार्श्वभूमी आहे. त्यांचे शेतकऱ्यांच्या कवितेविषयी वैचारिक मांडणी करणारे हे पुस्तक प्रसिद्ध होत आहे. डॉ. पंजाबराव उपाख्य भाऊसाहेब देशमुख यांची कृषिविषयक वैचारिक परंपरा कवितेच्या अंगाने उलगडून दाखविणारे हे लेखन लक्ष वेधून घेणारे आहे. त्यांचे हे लेखनकार्य कौतुकास्पद असल्यामुळे त्याबद्दल मी त्यांचे अभिनंदन करतो.

वास्तविक समाजातील कुठल्याही वर्गापिक्षा शेतकऱ्यांच्या श्रमाला अधिक महत्त्व आहे. मात्र दुर्दैवाने त्याच्या श्रमाला प्रतिष्ठा नाही. त्यांच्या उत्पादनाचे बाजारमूल्य त्यांना मिळत नाही. जो समाजाला जगवतो त्या अन्नदात्याला बहुसंख्य असूनही लोकशाहीमध्ये खरा न्याय मिळत नाही. त्यामुळे शेतकऱ्याची परिस्थिती अत्यंत गंभीर, भीषण झाली आहे. ती बदलणे अत्यंत गरजेचे, अपरिहार्य आहे. त्यासाठी निदान ग्रामीण भागाशी ज्यांची नाळ जोडलेली आहे; त्या सर्वांनी प्रामाणिकपणे प्रयत्न केले पाहिजेत. अन्यथा या कृषिप्रधान भारताची श्रमनिष्ठ संस्कृती त्यांना कधीच माफ करणार नाही. असा संदेश देणारे हे कवितेसंबंधीचे पुस्तक आवर्जून वाचण्यासारखे आहे. पुढील लिखाणासाठी मी त्यांना शुभेच्छा देतो.

- श्री हर्षवर्धन उपाख्य भय्यासाहेब देशमुख

अध्यक्ष

श्री शिवाजी शिक्षण संस्था, अमरावती.