Applications of Coacervates

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Coacervates are one of the most intriguing systems in colloid chemistry. The term comes from the Latin words “co” (jointly) and “acerv” (a mound). Coacervation is a phenomenon in which a colloidal solution gets separated into colloid-rich and colloid-poor phases.

polyelectrolytes polymers surfactants protein peptide coacervates

1. Perspective

Coacervation is a phenomenon in which a colloidal solution gets separated into colloid-rich and colloid-poor phases. The viscous colloid-rich phase is the coacervate which generally stays at the bottom. The upper colloid-poor phase is a dilute equilibrium phase. Generally, two types of “coacervation” are talked about, the simple or self and complex coacervation. In simple coacervation, one type of polymer or surfactant is used in a solvent. The addition of salt, alcohol or a change in pH or temperature results in the separation of phases. In the case of complex coacervation, two oppositely charged polyelectrolytes interact with each other [1]. In this case, also pH, ionic strength, charge ratio, molecular weight, charge density, and some other characteristics of polymers (polyelectrolytes) are important factors. The formation of coacervates is the outcome of a complex balance of electrostatics, hydrophobics, excluded volume, van der Waals, and other contributions to overall system stability. In simple coacervation, soluble complex formation occurs, and they may be called lyophilic colloids. Gelatin, ethyl cellulose, etc., are good examples of polymeric substances which show simple coacervation. Gum Arabic, chitosan, etc., together show complex coacervation. Coacervating agents in the first case are ethanol, water, etc., whereas in the second case, mixed liquids (solutions) are often used. Two polyelectrolytes of different charges are present in the case of complex coacervation and the electrostatic polyelectrolyte–polyelectrolyte interaction leads to an almost neutral charge, i.e., zero, and coacervates start to form. However, it should be noted that quite often, entropic factors are more important than electrostatic interaction in complex coacervation [1][2]. It can be comprehensively stated that simple coacervation involves species such as polyelectrolytes, proteins, peptides, surfactants, and additives such as salts, alcohols, etc. Complex coacervation involves polyelectrolytes, proteins, and surfactants of opposite charges with or without additives.

Coacervates are one of the most intriguing systems in colloid chemistry. The term comes from the Latin words “co” (jointly) and “acerv” (a mound). Colloidal molecules which intend to phase-separate from an aqueous medium in the formation of a second aqueous layer are referred to as “Acerv” [3]. Bungenberg de Jong and Kruyt defined in their original 1929 paper that “the coacervate consists of liquid which in a more or less degree has lost its free mobility” [4]. Recently, Prifits and Tirrell proposed that the formation of coacervates involves a network of dense

polyanion and polycation chains kept together by fluctuating electrostatic interactions that are neither strong nor fixed [5]. Primarily, coacervation occurs when a colloidal dispersion separates into two immiscible liquid phases in the same solvent media. These two liquid phases are incompatible and immiscible in the same solvent. Their separation from the liquid to the solid phase causes precipitation. This coacervate phase has higher viscosity and concentration than the preliminary solution, and it has a variety of distinctive characteristics. In fact, depending on its density, the coacervate phase can stay as a turbid suspension of amorphous droplets or coalesce into a top or bottom liquid phase.

2. Applications of Coacervates

Coacervates are very useful in many facets of industry and biology. As mentioned earlier, they are used as medium for the synthesis of nanomaterials, as emulsifiers, in drug delivery, in cosmetic formulation (for example, body and skincare products), as additives in food processing, and viscosity modifiers. Complex coacervation has been used to drive ordered block copolymer gels, stimuli-responsive sensory materials, etc. It has been used for protein purification, wastewater treatment, and many others. Herein, researchers briefly discuss some uses and applications of coacervates.

2.1. Wastewater Treatment

The need for a cost-effective and environmentally as well as economically sustainable method of removing contaminants from large amounts of wastewater is very worthy of consideration. Coacervation-based extraction is widely used in the enrichment and separation of compounds from aqueous systems, and it has a number of advantages, including low organic-solvent consumption, nonvolatility and nonflammability, simple and time-/energy-/cost-effective procedures, and high extraction efficiency [6]. Surfactants are used in the majority of cases reported thus far.

Because of the potential toxicities of many surfactants, the use of alternative macromolecule-based coacervates has acquired interest in recent years. Immobilizing coacervate onto the porous solid surfaces to adsorb organic contaminants in wastewater is one of the ways. The coacervate of poly(diallyldimethylammonium chloride) (PDADMAC) with mixed low-toxic-surfactant micelles have been applied to glass and quartz sand, and have shown excellent interception of orange OT at high ionic strength [7]. Zhao et al. [8] developed a similar system from cationic gemini surfactant hexamethylene-1,6-bis(dodecyldimethylammonium bromide) and 10% hydrolyzed polyacrylamide (HPAM) in neutral conditions. The coacervates exhibit preferable adsorption of anionic methyl orange (MO) over cationic MB (methylene blue) due to the synergy of hydrophobic, electrostatic, and cation–π interaction achieving an extraction efficiency of more than 95% with a polyacrylamide (PAM) concentration ranging from 0.05 to 0.5 wt.%. The coacervate formed by HPAM and a dynamic covalent cationic single-chain surfactant has also been used to recycle dyes in wastewater. Chiappisi et al. [9] showed that a highly pH-sensitive system based on cationic polysaccharide chitosan and the anionic surfactant nonaoxyethyleneoleylether carboxylic acid can be used for the removal of dyes, metal ions, etc. Surfactant-free extraction systems are also the aim of development. Zhao and Zacharia [10] set three pairs of oppositely charged polyelectrolytes to generate complex

coacervation, that is, cationic PEI and anionic poly(vinyl sulfonate), poly(acrylic acid) or poly(4-styrenesulfonic acid) (SPS), and compared their sequestration capacity for MB. As a result, only PEI/SPS showed good extraction efficiency (>80%) over a range of MB at its optimal pH (≈1.3), while the other two pairs performed not so well, especially at low MB concentration. This indicated that electrostatic interaction, and the hydrophobic nature of the coacervate phase, might be insufficient to extract target compounds from the water-rich phase, and it is necessary to introduce other strong interactions such as π–π interaction.

Ballesteros-Gomez et al. showed that vesicular coacervates can be used for wastewater treatment [11]. Multifunctional supramolecular solvents (SUPRASs) were created in aqueous solutions comprising carboxylic acid and carboxylate combinations that self-assembled and coacervated when tetraalkylammonium ions were added. SUPRAS were made up of huge unilamellar vesicular aggregates connected by tetraalkylammonium ions in coacervate droplets. The SUPRASs produced were tested for their usefulness as multifunctional extractants in water treatment. At room temperature, the SUPRASs were found to remove all contaminants, including anionic, cationic, and ionizable dyes, as well as polyaromatic hydrocarbons (PAHs). The SUPRAS-based treatment was shown to be effective in removing colors from textile effluents and benzo(a) pyrene from tap water. SUPRASs, or coacervate droplets, have a great potential for use in complete wastewater treatment.

2.2. Protein Purification

Due to the many advantages mentioned in previous sections, coacervation-based purification has potential in a benign way without affecting the structure of the proteins. In this respect, complex coacervation has been considered a useful strategy. Proteins with low isoelectric pH (Ip) favorably interact with cationic polyelectrolytes more than those withhigh Ip. The net charge of the protein is not the only factor. The surface charge duration (i.e., charge anisotropy) plays an important role [12][13]. From a 1:1 mixture of β-lactoglobulin (BLG, Ip ≈ 5.2) and bovine serum albumin (BSA, Ip ≈ 4.9), Xu et al. [14] extracted BLG employing cationic PDADMAC for its higher affinity to BLG than BSA in light of its negativity. At pH 7.0 and ionic strength = 0.1, the formed coacervate contained 90% BLG, and the associated polymer was totally removed by redissolution of the coacervate at pH 3.5, and subsequent ultrafiltration. Inverse protein selectivity has been realized with the anionic polysaccharide hyaluronic acid, the main difference being that coacervation is induced at pH 3.5 and dissolved at pH 7.0 [15]. In addition, the choice of polyelectrolytes can be extended to polysaccharides (chitosan and carrageenan) or protein (gelatin and lactoferrin) having biocompatibility for separating various protein mixtures including whey proteins pea whey proteins, etc. [16][17][18].Contributions of Kapelner and Obermeyer [19] and Lyons et al. [20] in this area, employing natural and model proteins of varied amino acid sequences under different environmental conditions, can be referred to herein.

2.3. Food Formulation

Microencapsulation provides food systems with a variety of benefits, including the preservation of delicate agents in harsh environments, the extending of shelf life, the masking of disagreeable odors or tastes, the ease of handling and transportation, and so on [21]. Because of their nontoxicity, biocompatibility, and biodegradability,

oppositely charged polysaccharides, proteins, etc., are commonly used as shell materials. The process usually consists of five steps: (i) dispersion of core materials in protein solution, (ii) creation of coacervate by adding polysaccharide solution, (iii) coacervate deposition around the core, (iv) hardening/crosslinking of the shell, and (v) drying of microcapsules into powders. Step (v) may be skipped in some cases. Many factors influence the size and appearance of the resulting microcapsules (e.g., formulation, temperature, pH, stirring rate, etc.). Mononuclear droplets, for example, form at low stirring rates, while multinuclear droplets form at high swirling rates [22][23].

The coacervation-based microencapsulation technology has been proven to be suitable for hydrophobic substances. Vanillin is a prevalent taste in dairy products; however, due to its volatile nature, it has a short shelf life. Hasanv and Rafe [24] adopted β-cyclodextrin to afford an inclusion complex with vanillin first and then encapsulated it in the coacervate of rice bran protein and flaxseed gum. As a result, the rate of vanillin degradation was substantially slower, with the initial amount of vanillin staying at 75% after 30 days at room temperature. Nutritional oils are high in polyunsaturated fatty acids and have a variety of health benefits, although they are prone to oxidation. To address this issue, soya protein and chitosan were used by Yuan et al. [25] for encapsulating algal oil, considering that the coacervate shell shall function as a barrier to O_2 penetration where chitosan can function as a secondary oxidant. Additionally, oil-based microencapsulation of gelatin/cashew gum crosslinked by tannic acid, and gelatin/gum Arabic crosslinked by glutaraldehyde are reported [26][27]. Stable microcapsules of anchovy oil using gelatin and sodium hexametaphosphate were produced by Xia et al. [28].

Edible oils are found to be useful for increasing their partition in the coacervate phase. Rocha-Selmi et al. [29] employed both gum Arabic and gelatin to improve the heat resistance of aspartame. Calderón-Oliver et al. [30] studied the coacervate of collagen with pectin or alginate which was used to encapsulate antimicrobial peptide nisin, and antioxidant fromavocado peel extract. At pH 3, the two shell matters showed different optimum protein–polysaccharide ratios (1:1 for collagen/alginate and 4:1 for collagen/pectin) although their encapsulation outcomes were nearly identical. Microorganisms such asprobiotics are used in functional foods. Coacervate from gum Arabic and whey protein isolate was developed by Bosnea et al. [31] with reference to providing effective protection to live *L. Paracasei* cells in relation to yoghurt food production, and its storage in adverse environmental conditions. After 45 days at 4 °C, the polymer shell allowed the free passage of nutrients and metabolites for cell activity in the yogurt matrix, and about 97 percent cell viability was maintained. When cells were exposed to simulated stomach fluid (pH = 2) for 3 h, their survival was unaffected, and their decrease profile remained consistent throughout storage. Meanwhile, the integration of coacervate (3 wt.% polymers) with a concentration less than 10 wt.% appeared to have no effect on the rheological properties of yogurts, which is more appealing in practical applications.

2.4. Cellular Mimics

Coacervateis an abiotic cellular analog (i.e., protocell) with anability to explore the mechanism of cell function, and related prebiotic evolution [32][33]. Compartmentalization provides benefits to different intracellular processes such asinterior reaction rate and specificity, responding (1) to subtle environmental change, (2) inhibiting exterior reaction, (3) buffering the concentration of the molecule, etc. [34][35]. A protocell comprising carboxymethyl dextran

sodium salt and poly-*L*-lysine was created in relation to supporting RNA catalysis by Drobot et al. [36]. Kojima and Takayama [37] constructed coacervates using ATP and PDDA mixed with an aqueous two-phase system comprising dextran and PEG. The system showed a mitigation of substrate inhibition effect for dextranase. A ternary protocell consortia endowed with antagonistic enzyme-mediated interaction was designed by Qiao et al. [38]. The designed response–retaliation pathway has potential for programming population dynamics of interacting protocells.

Another significant problem in protocell production has been achieving reversible coacervation and disintegration as needed. In recent years, a variety of techniques have been examined, ranging from tuning external parameters such as temperature, pH, and ionic strength to manipulating phase-forming macromolecule concentration by synthesis and destruction, or their characteristics by post-translational modifications [39][40][41]. A complex coacervate using polyuridylic acid, RNA, and short cationic peptide was planned by Aumiller and Keating et al. [42], and thereby manipulated for phase separation by the enzymatic phosphorylation/dephosphorylation process. The assembly/disassembly behavior of the system-based single-stranded RNA and synthetic peptide was reported by Banerjee et al. [43]. They suggested a possible underlying supramolecular dynamical mechanism of ribonucleoprotein granules. Light-responsive coacervate droplets were prepared by Martin et al. [44] using double-stranded DNA and trans-azobenzene trimethylammonium bromide. The droplets breakdown under UV light and reassemble under blue light; the process is also thermal-sensitive. The photoswitchable protocell, when combined with the coacervate's ability to promote gene expression, could be used to trigger signaling pathways in populations.

2.5. Nanoparticle Synthesis

Coacervates have been found to be useful in the synthesis of nanoparticles. Nanoparticles are particles where at least one dimension of the particle is less than 100 nm. These particles have properties which are different from the corresponding bulk properties. The coassemblies of ionic-neutral block copolymers with oppositely charged species are called complex coacervate core micelles (C3Ms).These C3Ms can be used to synthesize nanoparticles because they are good nano reactors and scaffolds. Size and shape can be easily controlled. This is a straight forward, versatile synthetic method. These are water-based systems; organic solvents are not used and, hence, environmentally friendly. Metal, metal oxides, and quantum dots have been synthesized using C3Ms. The coacervate core can also be used for the biomimetic mineralization of silica, barium carbonate, and calcium carbonate [45]. The spherical, stable gelatin nanoparticles were prepared by Mohanty et al. [46] by using a simple coacervation process. Type B gelatin was used with ethanol as the coacervating liquid. Turbid solution was obtained which separated into two liquid phases. The upper supernatant dilute phase contained the gelatin nanoparticles which were characterized by dynamic light scattering, SANS, TEM, etc. Additionally, starch nanoparticles were prepared by addition of aqueous solution of synthesized positively charged (i.e., amine-group-containing) starch (PosSt) to aqueous solution of negatively (i.e., carboxylic-group-containing) charged starch (NegSt) under constant stirring at room temperature. Starch nanoparticles were formed spontaneously. These were characterized by particle size, size distribution (PDI), and ζ-potential measurements. TEM was used for morphology determination. Particle sizes were 140 to 350 nm, and had a ζ-potential ranging from −10 to −35 mV, depending on the formation conditions. The experimental details are available in original publication by Barthold et

al. [47]. Gelatinnano-/submicron particles (GN/SPs) were synthesized usingthe binary nonsolvent-aided coacervation (BNAC) method. This coacervation method yields about a threefold lower particle size and higher average yield. In this research, 0.5% (*w*/*v*) gelatin aqueous solution with a binary nonsolvent system of acetone and ethanol was used. Particle size, zeta potential, swelling ratio, etc., were determined by Patra et al. [48].

2.6. Delivery Carrier

Complex coacervates used in controlled delivery systems are mostly in the form of either micro-/nanocapsules/beads or stimuli-responsive membranes for controlled delivery systems. As mentioned above [37], gelatin nanoparticles made by using the coacervation technique can be used for drug delivery. Nitrofurazone was used as a model drug to study drug-loading efficiency and its rate of release. The results indicate a potential for it in wound management. Barthold et al. [47] have shown that starch nanoparticles synthesized by the coacervation method can be used for the pulmonary delivery of proteins. Proteins which are “active pharmaceutical ingredient” such as Insulin, IgG1, RNAse, etc., were used. Coacervate drug delivery is gaining momentum because of the controlled, sustained release of its cargo for long periods. Coacervates are also useful because both drug solubility and protein stability increase [49][50]. Different materials have been used as a platform for the delivery of drugs, proteins, RNA, DNA, and nutraceuticals.

Heparin forms coacervates with biodegradable polycation, poly(ethylene argininylaspartatediglyceride) (PEAD) and is a good carrier for growth factor (GF). These have high loading efficiency (>90%) and good bioactivity and biocompatibility; as it gives sustained release, the half-life increases and the administration of dose frequency decreases. Depending on the binding affinity of PEAD with heparin at various physiological conditions, the hydrolysis of PEAD can last for a few days to a month [51]. Injectable and biodegradable hydrogels, the entrapment medium, can be added to this which prolong the retention of coacervates in vivo [41]. The heparin/PEAD system has been widely used to deliver GF in case of heart repair, bone regeneration, wound healing, etc. [51][52][53][54]. Furthermore, “ibuprofen” was encapsulated in a system made of poly(allylamine hydrochloride) (PAH) and multivalent anion tripolyphosphate (TPP). The coacervate shows gel-like properties because of high electrostatic interaction. It has high load capacity and shows sustained release. The presence of sodium dodecylsulphate, however, makes the network permeable to small molecules making its release time tunable [55].

A UCST-type polymer was synthesized by Zhang et al. [56] by functionalizing a side group of poly(*N*-(2-hydroxypropyl)methacrylamide) with glycolamide, which formed a coacervate at decreasing temperatures from 50 °C to room temperature. The hydrophilic protein bovine serum albumin (BSA) could be accommodated in the formed coacervate. The coacervate, however, transformed to a soluble state with time and, hence, BSA was released in vivo over a period of four days.

Redox potential, pH, temperature, and glucose level are important considerations in determining targeted drug delivery platforms. The inflammatory and tumor sites are quite often acidic and, hence, one needs a pH-responsive system to target these sites. Nishida et al. [57] formed a pH-responsive LCST-type β-cyclodextrin-threaded polyrotaxane for the targeted delivery of therapeutic proteins. A glucose-responsive insulin delivery system is,

again, very important. Insulin and glucose oxidase were incorporated in coacervate microdroplets with stable to neutral or alkaline pH. The insulin release rate was found to be a function of glucose level [58].

pH-responsive carboxymethyl chitosan (CMCS) complex coacervate has been studied for oral drug delivery. The CMCS self-coacervates were made near its isoelectric point by adjusting pH. The FTIR results indicate the presence of electrostatic interactions, hydrogen bonding, and hydrophobic interactions in the CMCS self-coacervation, which remained stable in the pH range of 3.0–6.0. These coacervates were found to be pH-responsive and stable over a wide range of ionic strengths. Lactoferrin (LF) was encapsulated and used in oral delivery. The encapsulation efficiency was 94.79 ± 0.49% with a loading capacity (LC) of 26.29 ± 0.52% when 2 mg LF was present. Various experiments show that CMCS protects LF (>80%) from hydrolysis and remains bioactive [59].

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