

REVIEW

RECENT ADVANCES IN THE ELECTROPHORETIC DEPOSITION OF CHITOSAN-SILVER NANOCOMPOSITE COATINGS ON METALLIC IMPLANTS WITH ENHANCED BIOCIDAL PROPERTIES

Bożena Łosiewicz^{1,a,*}, Patrycja Osak^{1,b}, Delfina Nowińska¹

¹ – Institute of Materials Engineering, Faculty of Science and Technology, University of Silesia in Katowice, 75 Pułku Piechoty 1A Str., 41-500 Chorzów, Poland

^a – ORCID: 0000-0001-9472-1893; ^b – ORCID: 0000-0002-0628-6367

*corresponding author: bozena.losiewicz@us.edu.pl

Abstract

This review covers the latest issues related to the development of medical technologies based on chitosan coatings. The progress of modern implantology requires medicine to adapt constantly to new opportunities and challenges. Infections may occur at the implantation site or in the body and may require treatment with antibiotics or surgery to resolve them. Due to its unique properties, such as biocompatibility, biodegradability, non-toxicity and antibacterial activity, chitosan has been proposed to be co-deposited with biocidal silver nanoparticles in the form of nanocomposite coatings on the surface of metallic implants to extinguish local inflammations. The mechanism of co-deposition of chitosan with metal ions and particles using electrophoretic deposition has not yet been thoroughly explained. This, among others, results from a number of factors that influence the process and their mutual relations. Therefore, the present review aims at a deeper understanding of the mechanism of creating chitosan-silver nanocomposite coatings.

Keywords: biocide, chitosan, electrophoretic deposition, metallic implants, nanocomposite, silver nanoparticles

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1. Introduction

Metallic biomaterials are one of the fastest developing groups of biomaterials due to their biocompatibility, high corrosion resistance in the environment of bodily fluids, good mechanical properties, the possibility of modifying properties by creating alloys, the possibility of modifying the structure by changing the size of grains and precipitates, and the possibility of modifying the surface by selecting the appropriate roughness or applying coatings [1–4]. Metallic biomaterials are used in orthopaedics, craniofacial surgery, dentistry, heart surgery and endovascular surgery [4]. They are mainly used to produce implants to stabilise bone fragments and to treat bone defects, and also as heart valves, stents, endoprostheses and dental prostheses. Some of the most commonly used metals and alloys for implants include titanium [5, 6] and titanium alloys [7–11], cobalt-chromium alloys [12], austenitic steel [13], nickel-titanium alloy [14, 15] and magnesium alloys [16]. The choice of metallic biomaterial for an implant depends on various factors, including the specific application, the patient's medical history and the surgeon's experience and preferences.

The biocompatibility of metallic biomaterials can be increased by using chitosan (Chit) coatings; Chit is one of the most promising natural polymers, especially in tissue engineering and regenerative medicine [17–21]. On an industrial scale, Chit is produced by deacetylation of chitin [18, 22, 23]. The sources of chitin – the shells of red crabs, shrimp, krill and other crustaceans – are obtained during processing sea fishing for food. Chit can also be isolated from the cell walls of the filamentous fungi belonging to the class Zygomycetes (the *Mucor*, *Absidia*, *Rhizopus* and *Gongronella* genera) [24]. By adding the appropriate enzymes to the fungi and using the optimal composition of the medium, with the help of alkaline separation of the insoluble fraction in bases, it is possible to separate Chit from fungal biomass. Thanks to continuous mushroom cultivation, it is possible to obtain a large amount of raw material for the production of Chit in a relatively short time.

In an environment with low ionic strength, Chit takes the form of an extended structure of macromolecules due to electrostatic repulsion between segments of the chain. When the ionic strength tends to infinity, it completely neutralises the proton groups, causing the electrostatic repulsion forces to disappear, and the structure of Chit becomes a compact zone regardless of differences in molecular weight. The presence of free protonated amino groups in the Chit structure allows the formation of complexes with derivatives with a negative charge, such as polymers, proteins or dyes. In addition, Chit may bind selectively to cholesterol, fat, cancer cells, DNA and RNA. Chit also has the ability to form chelates with metal ions, which requires the special involvement of –OH and –O– groups in D-glucosamine residues, and amine ions in Chit complex metal ions more effectively than acetyl groups in chitin [17].

Chit is used in medicine as a component of dressing materials, as it stops bleeding; accelerates wound healing; and exerts anti-inflammatory, analgesic, antiviral, antifungal and antibacterial effects [17, 18, 25–27]. Chit has the ability to adsorb strongly on mucous membranes, which allows it to be used as a carrier in mucoadhesive drug-delivery systems, thanks to which drugs are bound to the site of their action, that is, mucous membranes [28]. This natural biopolymer also has the ability to create a variety of morphological structures such as films, membranes, nanoparticles, fibres and knitted and nonwoven fabrics made from them, and hydrogels [17–22, 28]. Moreover, the chemical composition of Chit is similar to the composition of the extracellular matrix of biological systems [29]. In addition to biocompatibility, Chit also shows biodegradability, bioactivity, non-toxicity, water wettability, the ability to chelate and bind metal ions and organic substances, easy miscibility, and can also be a matrix for dispersed solid particles [17–19, 21, 28, 30–32]. Particularly interesting is the possibility of producing Chit-based

nanocomposite coatings with dispersed silver nanoparticles (Ag NPs), which show high antibacterial activity [33]. Ag NPs production methods, their properties and their possible mechanisms of antibacterial activity have been discussed in detail in previous studies [33–36]. Ag NPs used in medicine are nanomaterials that have a size of 1–100 nm in at least one dimension or at least 50% of the particles in the particle size distribution are in the nanometre scale. They exhibit significant antibacterial activity due to their high surface-area-to-volume ratio, which increases as their size decreases [34, 35]. Smaller Ag NPs show an increased active surface, which consequently leads to greater antibacterial activity. Moreover, by selecting appropriate reaction conditions, the shape of Ag NPs can be controlled. Ag NPs in the form of spheres (e.g. a diameter of 5–100 nm [35], 40–80 nm and 120–180 nm [34]), platelets (e.g. 20–60 nm [34]), cubes (e.g. 140–180 nm [34]), rods (e.g. a diameter of 80–120 nm and a length of > 1000 nm [34]) and triangular (e.g. 31 nm [35]), have been prepared. The published reports regarding the influence of the shape of Ag NPs on their antibacterial activity are contradictory [34–36]. Some authors claim that among different shapes of Ag NPs, those in the triangular form have the highest antibacterial activity due to their sharp edges and vertices, and the largest active surface area [36]. Others have proved that spherical Ag NPs have the highest antibacterial activity [34, 35]. Co-deposition of Ag NPs with these unique physicochemical properties and the Chit matrix on metallic substrates using electrophoretic deposition (EPD) enhances the biocidal properties of Chit-Ag NP nanocomposite coatings due to the synergistic effect of both the Chit matrix and the embedded Ag NPs composite component [37, 38].

This review discusses the latest research of Chit-Ag NP nanocomposite coatings on metallic implants, especially dental implants, obtained using the EPD method. The main goal of this review is to provide a deeper understanding of the mechanism by Chit-Ag NP nanocomposite coatings are formed; it has yet to be fully understood due to numerous factors that influence the EPD process. The studies included in this review were retrieved from the Scopus database, with a publication range covering the last 5 years.

2. Inflammation and Complications After Implantation

Biomaterials should be biocompatible, which means harmony of interactions within living matter [1–4]. Biocompatible implants do not initiate toxicological and immunological reactions and do not irritate tissues in the host's body. However, inflammation occurs after each implantation because mechanisms are stimulated in a living organism to reject the implant as a foreign body [2, 3, 20]. After the implant is detected by the immune system, the body starts producing antibodies that have a strong oxidising effect. As a result of the body's defensive reaction, proteins accumulate near the implant and are absorbed into it, which initiates the degradation of the implant. Protein dissociation and reactions at the cellular level cause changes in the pH of the blood and bodily fluids, which constitute a strong corrosive environment with a high concentration of chloride anions. Bodily fluids are buffer solutions with a stable pH in the range of 7.00–7.35. After implanting a metal biomaterial into the bone, the pH in the area surrounding the implant decreases to approximately 5.20 and returns to the physiological value of 7.35–7.45 only after 2 weeks. Additionally, the concentration of dissolved oxygen in bodily fluids is unfavourable – four times lower than the concentration in air – which delays the regeneration of the self-passive oxide layer and increases the amount of metal ions released from the surface of the metal implant into the body [4–9, 12–15, 30, 31]. Other reasons why inflammation may occur after implantation include surgical trauma causing tissue damage, infection as a result of bacteria or other pathogens entering the body during the implantation process, an allergic reaction to the biomaterials used in the implant or the biomaterials applied

during the surgical procedure, and implant malposition leading to irritation or pressure on surrounding tissues [1, 3, 4].

Although implantation has a high success rate of up to 98%, the invasiveness of commonly performed implant procedures also carries the risk of complications after implantation, which is an inherent element of the osseointegration process [39]. Complications may occur at the early and late stages of osseointegration. Complications in the early stages include infection; swelling; haematomas; petechiae; and bleeding occurring in the maxilla and mandible, sinuses and soft tissues around the implant, which are caused by a surgical approach that is too traumatic, overheating of bone tissues during the procedure or bacterial infection of the wound. Late-stage complications are a consequence of complications of the early stage of osseointegration and are manifested by mucosal perforation, maxillary sinusitis, mandibular fractures, failure of osseointegration, bone defects and periapical implant failure. Of note, bacterial contamination during the procedure and improper oral hygiene after implantation increase the risk of complications. Peri-implant infections are initiated mainly by bacteria attaching to implants in the form of biofilm and can be classified based on the route of spread, the time of implant contamination, the period of symptom onset and the course of infection [39–41].

Post-implantation complications can be divided into mechanical, technical and biological [40]. The main cause of mechanical complications after implantation implant placement that is too tight, implant displacement or excessive force loading the implant, as is the case, for example, in the case of bruxism (Figure 1a) [42]. One of the most common mechanical complications after implantation is overloading the implants, leading to their loosening or fracture. Implant fractures occur due to manufacturing defects or as a result of loading the implant too quickly with a prosthesis or bridge.

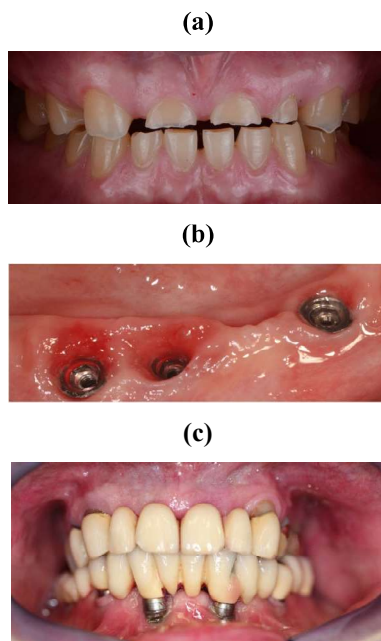


Figure 1. (a) Chipped and worn teeth as a result of bruxism [42] (the image is from stock.adobe.com and has been reproduced under the terms and conditions of a standard license), (b) peri-implant mucositis [41] (this image has been reproduced under the terms and conditions of a Creative Commons Attribution [CC BY] license) and (c) peri-implantitis [43].

When occlusal forces are too high – approximately 200–300 N, there is damage to the implant connector and subsequent loosening of the shaft [40]. Technical complications often occur with implant-supported fixed partial dentures and include fractures of the veneering porcelain and fractures of the framework. The group of biological complications includes sensory disturbance, progressive bone loss, formation of bacterial plaque and bacterial infections.

Peri-implant diseases include inflammation of the mucosa around implants with clinical symptoms of redness, swelling and bleeding [41, 44]. They are classified into curable peri-implant mucositis and incurable peri-implantitis. Figure 1b shows a clinical case of peri-implant mucositis with inflammation induced by abutment loosening [41]. Marginal inflammation of the peri-implant mucosa is visible without loss of supporting bone after initial bone remodelling. When the extent of inflammation increases and exceeds the gum–bone border with bone destruction, the disease progresses to peri-implantitis (Figure 1c) [43]. This destructive inflammatory process affects both soft and hard tissues surrounding dental implants. The longer peri-implantitis lasts, the greater the bone loss, which may lead to the loss of implants along with the prosthetic restoration. Therefore, there is a need to develop innovative dental implants with enhanced osteoinductive properties with the possibility of intelligent and controlled drug delivery directly to the site of inflammation.

3. Production Methods of Chitosan-Silver Nanocomposite Coatings

Chit-Ag NP nanocomposite coatings are biodegradable, biocompatible and environmentally friendly biomaterials that have attracted significant attention in recent years due to their potential applications in medicine [37, 38]. The production of these coatings involves the incorporation of Ag NPs into the Chit matrix to enhance their properties, such as mechanical strength, thermal stability and barrier properties. Table 1 lists several methods developed for the production of Chit-Ag NP nanocomposite coatings along with a description of the basis of each method.

Each method has its advantages and disadvantages, and the choice of method depends on the specific application and desired properties of the nanocomposite coating. Chit-Ag NP nanocomposites are advanced and multifunctional biomaterials composed of Ag NPs dispersed in a polymer matrix and/or covered with a polymer (core shell) [45]. Regarding the use of EPD to produce Chit-Ag NP nanocomposite coatings, the advantages are it requires relatively inexpensive and simple equipment; it is easy to control the properties, thickness and porosity of thin films by changing EPD parameters; sensitive biomaterials such as Chit can be co-deposited at room temperature; substrates with complex shapes can be coated homogeneously; it requires a short amount of time; easy scalability; and high production efficiency. The main disadvantage of EPD include Ag NP agglomeration in the suspension bath during the production of Chit-Ag NP nanocomposite coatings, which requires the use of surfactants and nanoparticle stabilisers [17].

Table 1. Methods for the production of chitosan silver nanoparticle (Chit-Ag NP) nanocomposite coatings.

Type of method	Description of the method	Reference
Solvent casting	Chit and Ag NPs are dispersed in a suitable solvent, such as acetic acid or water, to form a homogeneous solution, which is then cast onto a substrate and allowed to dry, forming a thin nanocomposite coating. The solvent is evaporated, leaving behind the nanocomposite coating on the substrate.	[46]
Electrospinning	An electric field is used to generate a jet of Chit-Ag NP solution, which is then collected on a grounded collector to form a nanofibrous mat. The electrospinning process allows for the formation of nanofibers with high surface area and tunable pore size, which can be tailored for specific applications.	[46]
Layer-by-layer assembly	Alternating deposition of Chit and Ag NPs onto a substrate is used to form a multilayer coating. The process is typically carried out by dipping the substrate into a solution containing Chit and Ag NPs, followed by rinsing with a suitable solvent to remove any unbound species. The desired number of layers is achieved by repeating the process.	[46]
In situ polymerisation	Chit is mixed with a monomer and a cross-linker, and Ag NPs are added to the mixture. The mixture is then heated or irradiated to initiate the polymerisation process, resulting in the formation of a nanocomposite coating. This method can be used to prepare coatings with high mechanical strength and improved barrier properties.	[46]
Co-precipitation	Chit and Ag NPs are mixed in a suitable solvent, and a precipitating agent is added to form a nanocomposite precipitate, which is then collected by filtration, washed with a suitable solvent, and dried to form a nanocomposite coating.	[46]
Spray-drying	Dispersion of Chit and Ag NPs in a suitable solvent is used, followed by the introduction of the solution into a spray dryer. The solvent is evaporated in the spray dryer, forming a powder of the nanocomposite coating. The powder can then be collected and used for various applications, such as encapsulation or coating.	[46]
Electrophoretic deposition	Synthesis of thin nanocomposite Chit-Ag NPs films and coatings on various substrates is available. This method relies on the movement of charged particles under an electric field to deposit them on a metallic electrode.	[38, 45–49]

4. Chitosan-Silver Structure

Chit-Ag NPs are a nanocomposite biomaterial where Ag NPs are embedded into a Chit matrix (Figure 2). When the natural polymer Chit is combined with Ag NPs, which have antimicrobial properties, the resulting Chit-Ag NP nanocomposite exhibits enhanced antibacterial activity [50–52]. The structure of the Chit-Ag NP nanocomposite involves Ag NPs distributed within the Chit polymer matrix [53].

Chit is a linear polysaccharide composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine (a deacetylated unit) and N-acetyl-D-glucosamine (an acetylated unit). The presence of amine groups is responsible for many of the unique properties of Chit, including biocompatibility, biodegradability and the ability to form films [45, 54]. The structure does not indicate the degree of polymerisation or the ratio of deacetylated to acetylated units, which affect the physicochemical properties of Chit. The presence of amino and hydroxyl groups in the Chit structure allows it to form stable interactions with Ag NPs [53]. Chit's affinity for Ag NPs may be due to the formation of chemical bonds between electron-rich nitrogen atoms and the lone pairs in the silver orbitals, which is important for creating new Chit-Ag NP nanocomposite structure. The amino groups of Chit can change the permeability of bacterial cells by reacting with anionic groups on the surface of bacterial cells [19, 20, 22, 23, 45].

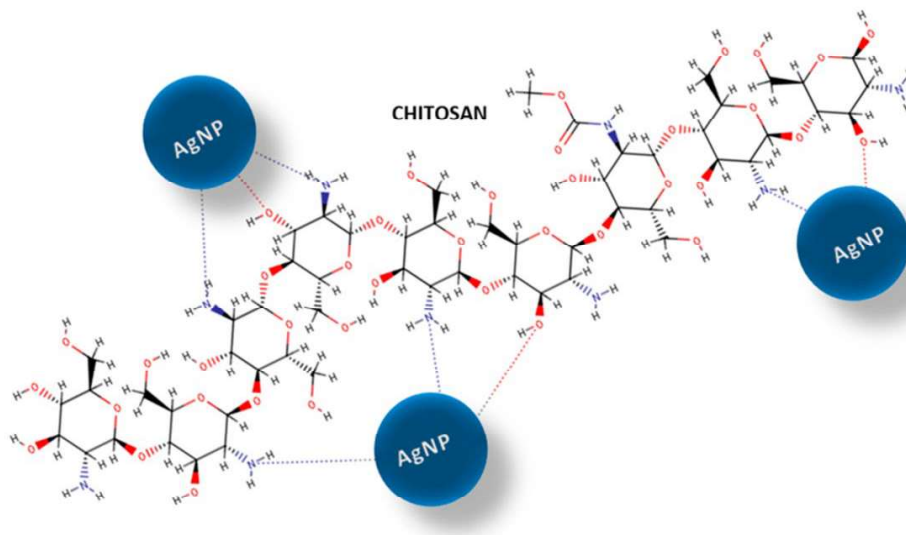


Figure 2. A schematic illustration of how silver nanoparticles (Ag NPs) are embedded into the chitosan (Chit) matrix [53]. This figure has been reproduced under the terms and conditions of a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>)).

Such properties of Chit-Ag NP nanocomposites result in a growing interest in these biomaterials for the needs of modern medicine. Chit-Ag NP nanocomposites may be used in the future as therapeutic agents to fight pathogenic microorganisms whose drug resistance is increasing. According to the literature, Chit-Ag NP nanocomposites have biocidal properties against *Escherichia coli*, *Escherichia bacillus*, *Enterococcus faecium*, *Candida albicans*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, among other microorganisms [45, 51]. There is also a need to develop innovative metallic implants with increased osteoinductive properties with the possibility of intelligent

and controlled drug delivery directly to the site of inflammation. The use of such implants would result in a gentler course of treatment for patients and a reduction in the amount of anti-inflammatory drugs they take. This is especially important for older people, who are often additionally burdened with oral drug supplementation, which is required in the treatment of other age-related diseases, such as hypertension, diabetes, rheumatological diseases and others [1–3, 6, 8–13].

5. Electrophoretic Deposition Mechanism of Chitosan-Silver Nanocomposite Coatings

EPD is used to deposit particles, such as colloids or suspensions, onto a substrate using an electric field [55]. The particles to be deposited are suspended in a liquid medium, such as water, and the mixture is stirred or agitated to obtain a homogenous suspension (Figure 3a). The pH of the suspension is also adjusted to ensure that the particles have stable electrophoretic mobility.

Researchers have proposed EPD for green synthesis of Chit-Ag NP nanocomposite coatings based on chemical reduction from a dilute acetic acid using silver nitrate (AgNO_3) as a precursor to obtain Ag NPs [31, 37, 50, 56]. According to research, citric acid yields a more compact Chit matrix structure compared with acetic acid due to less intense electroevolution of hydrogen [57–59]. The crystalline component of the Chit-Ag NP nanocomposite has also been used in the form of Ag NPs [37, 38, 60, 61].

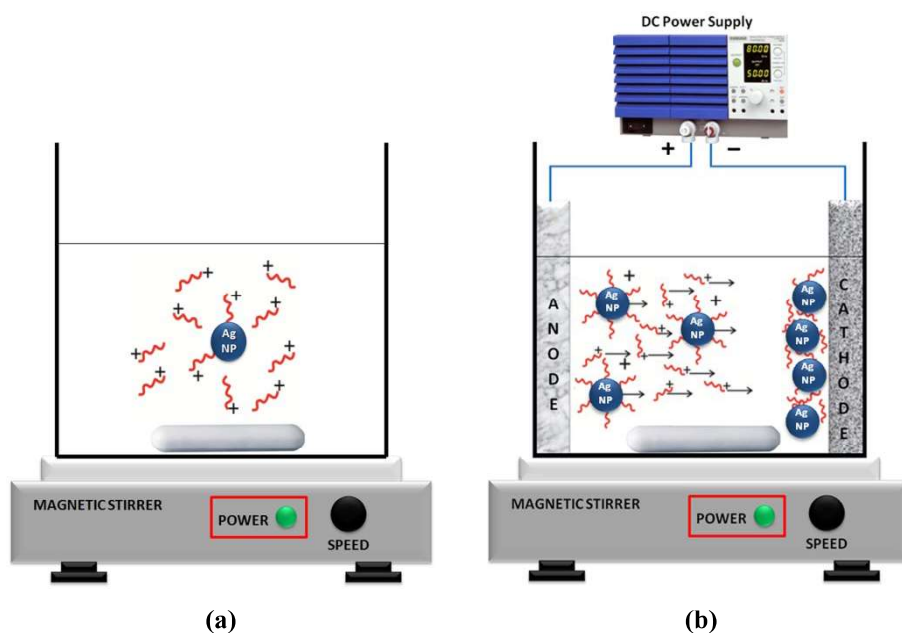


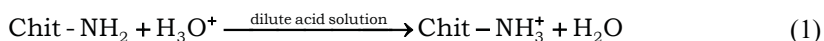
Figure 3. A schematic illustration of (a) chitosan (Chit) protonation and Chit adsorption on silver nanoparticles (Ag NPs) in a dilute solution of acid and (b) electrophoretic co-deposition of Ag NPs with Chit as a cationic polymer.

Several factors can influence the efficiency and quality of EPD, including the key EPD process parameters: the concentration of Ag NPs in the suspension (e.g. 0.05 g of Ag NPs with an average powder grain 30 nm dispersed in 1 dm³ of 1% acetic acid [38],

and 0.005 or 0.01 g Ag NPs with an average powder grain 30 nm dispersed in 100 ml of 1 vol% acetic acid [61]), the applied voltage (e.g. 10 V [38, 61], 15 V [60], 20 V [38] and 30 V [61]), the duration of the EPD process (e.g. 1 min [38, 61], 3 min [61], 10 min [60] and 30 min [31]), the pH of the suspension (e.g. 4.6 [60]) and the substrate type (e.g. Co-Cr-Mo alloy [31], a nanotubular TiO₂ layer on the Ti13Zr13Nb alloy [38], additively manufactured Ti6Al4V ELI alloy [60] and Ti Grade 2 [61]). The effects of key EPD process parameters on the improvement of microstructural homogeneity; mechanical and electronic properties; adhesion; and *in vitro* corrosion resistance, biocompatibility and biological activity of the Chit-Ag NP nanocomposite coatings on metallic implants have been reported [31, 38, 60, 61]. The EPD method has allowed researchers to produce Chit-Ag NP nanocomposite coatings with about 2 wt% Ag, which ensures maximum antibacterial activity [31], and to develop drug-delivery systems, for example, the Chit/Eudragit E 100/Ag NP nanocomposite coating [61].

An adsorption mechanism can be proposed to explain co-deposition of Ag NPs and amorphous Chit matrix on the metallic substrate (Figure 3b). Electrode reactions do not participate in the EPD of solid particles, despite the fact that the solid particles in a colloidal solution may have a positive or negative charge depending on the pH of the solution [62]. Solid particles in a colloidal solution behave like charge carriers and provide current flow. The electric field strength in the EPD process is too weak to overcome the mutual repulsion of particles, but it is large enough for a higher number of particles to accumulate near the electrode with the opposite charge [63]. The introduction of dispersing agents or polyelectrolytes into the solution can provide the right charge for colloidal particles and ensure their correct dispersion in the solution. In cataphoresis, polyelectrolytes with a protonated –NH₂ group, such as Chit, are used; they are applied during cataphoretic co-deposition of solid particles. Chit is used as a binding agent for Ag NPs, which enable their co-deposition to obtain composite coatings on the surface of a metallic electrode. Polyelectrolytes show significant non-electrostatic interactions with solid particle surfaces [64]. Therefore, adsorption of Chit onto Ag NPs in the entire suspension volume can be assumed. Figure 3a presents a schematic illustration of the protonation of Chit and Chit adsorption on Ag NPs in a dilute acid solution. Chit adsorption may be associated with the formation of Ag-Chit chelates. Ag NPs can also interact with amino groups, between which hydrogen bonds are formed and are tightly wrapped in Chit chains (Figure 2). Ag NPs surrounded by Chit chains have a positive charge; therefore, in the EPD process under the influence of applied voltage they move towards the cathode, on which they are embedded in the coating (Figure 3b). It is worth noting that increasing the pH of the cathode area during cataphoresis may reduce electrostatic repulsion and promote chelation in the obtained coatings [62].

Based on the above, the mechanism of co-deposition of the Chit matrix with Ag NPs on a metallic substrate proceeds in two stages. In the first stage, polymer protonation occurs in the colloidal solution – a mixture of well-dispersed Ag NPs and Chit – to generate Chit macromolecules with a positive charge (Figure 3a). Chit amino groups are only protonated at pH < 6.5 [19]. Only under such conditions does Chit become a cationic polyelectrolyte according to reaction (1):



Then, the Chit chains are adsorbed onto the surface of Ag NPs, giving them a positive charge. In the EPD process, an electric field applied from an external power source causes the electrophoretic movement of positively charged Ag NPs with adsorbed Chit on the surface and free polymer chains, which move towards the cathode, on the surface of which they are co-deposited in the form of a Chit-Ag NP nanocomposite coating.

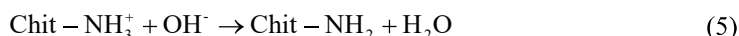
The deprotonation of positively charged Chit chains occurs at the cathode, and in the electrode space there is a local pH increase associated with the water electrolysis according to reaction (2) [65]:



Reactions (3) and (4) occur on the cathode surface:



An increase in the pH of the solution results in a decrease in charge and at pH = 6.5, the amino groups of Chit are deprotonated according to reaction (5):



As a result of neutralisation of the protonated amino groups of the Chit chain in the presence of OH^- ions, an insoluble nanocomposite coating with the Chit matrix and co-deposited crystalline component in the form of Ag NPs forms on the surface of the cathode. The deposition efficiency and quality of the deposited Chit-Ag NP nanocomposite coatings are affected by the cataphoresis conditions. It can be expected that as the concentration of Ag NPs in the colloidal solution increases, the mass of deposited polymer and co-deposited Ag NPs increases, and thus the thickness of the deposited coating increases.

6. Conclusions

Chit, a biopolymer derived from chitin, has been widely studied for its biocidal properties and biocompatibility. Ag NPs have also been recognised for their strong antimicrobial activity. There has been a marked increase in the attention directed towards the use of Chit-Ag NP nanocomposite coatings due to their potential applications in modern medicine. The combination of Chit and Ag NPs in a nanocomposite coating has shown enhanced biocidal properties, making it a promising biomaterial for antimicrobial applications to modulate inflammation and to prevent complications after implantation. Among the production methods of Chit-Ag NP nanocomposite coatings, EPD seems to be a low-cost, effective, easily scalable and promising method due to its ability to produce uniform and adherent coatings on substrates with a complex shape. However, there is a need for further research to optimise the EPD process and to understand the relationship between the deposition parameters and the biocidal properties of the Chit-Ag NP nanocomposite coatings. Considering that the antibacterial activity of Ag NPs is influenced by their shape, size, concentration, synthesis time and surface charge, the incorporation of a composite component in the form of Ag with a particle size below 1 nm, silver Ångström particles (Ag ÅPs), into the Chit matrix would generate a new Chit-Ag ÅP composite with superior antibacterial activity due to higher effective contact and anticancer properties of Ag ÅPs for applications in tissue engineering and drug-delivery systems.

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