REVIEW PAPER

# A review study of the use of modified chitosan as a new approach to increase the preservation of blood products (erythrocytes, platelets, and plasma products): 2010-2022

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## ABSTRACT

Due to the unique properties of chitosan (antibacterial and stimulating tissue repair factors) in improving cell function, modified chitosan derivatives are widely used to improve the function of blood products. However, interaction of chitosan positive surface charge with negatively charged blood cells and anionic proteins, increases hemolysis, platelet activation, and dysfunction of plasma proteins, so the use of chitosan in blood applications requires surface modifications. Therefore, in this review study, we review the literature (2010–2022) to determine whether the charged-modified chitosan could eliminate the effects of chitosan on blood products and prepare a platform for more research to improve the preservation of the blood products such as erythrocytes, platelets and plasma proteins (albumin, immunoglobulin (Ig) and factor (FVIII)). Overall, the results of this review study show that negative surface-charged chitosan can increase hematopoiesis and increase the preservation of erythrocytes, platelet, and plasma products. Modified chitosan can be used as an anticoagulant compound for purification and filtration of plasma proteins, gene transfer of FVIII, and to increase the stability of plasma proteins. In addition, due to its antibacterial and hemostatic properties, negatively charged chitosan can stimulate coagulation factors and rapid wound healing and can be used in the production of wound dressings. This review study provides researchers with a new insight into the effectiveness of negative-charged chitosan in improving the preservation of blood products including erythrocytes, platelets, and plasma products (albumin, immunoglobulin, and FVIII) and promises to increase the efficacy of negative-charged chitosan in the future research.

***Keywords:*** *Albumins, Blood platelets, Erythrocytes, Factor VIII, Immunoglobulin G*

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## INTRODUCTION

Chitosan is a polycationic polysaccharide obtained from the alkaline deacetylation of chitin (with varying degrees of deacetylation) [1-4]. Chitosan can be readily obtained from food waste because chitosan is found in the shells of crabs, shrimps, lobsters, krill, squid, and fungi (Fig. 1 and 2) [4-6]. N-acetyl-β-D-glucosamine and d-glucosamine are linked by b-1,4-glycosidic bond to form chitosan. The difference between chitin and chitosan is the presence of the acetyl group at the C-2 position (protonation of the amine group at the C-2 glucose amine position) [6-10]. The conversion of chitin to chitosan takes place through chemical hydrolysis or enzymatic preparation. Thermochemical deacetylation of chitin is performed in solid state under basic conditions (NaOH). Enzymatic hydrolysis is performed in the presence of chitin deacetylase (chitin + $H_2O$ ↔ chitosan + acetate). Bacterial and fungal enzymes also do deacetylation [2, 10, 11]. In 2001, the

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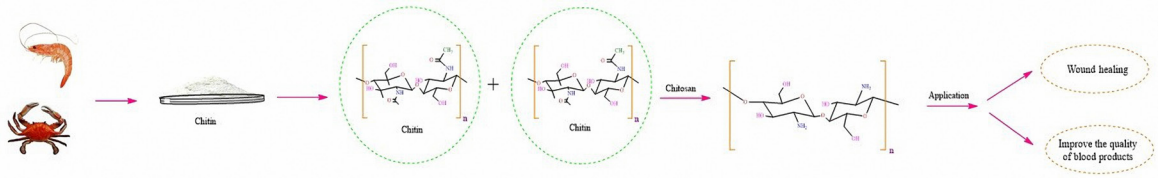


Fig. 1. Chitosan is a polycationic polysaccharide obtained from the alkaline deacetylation of chitin (with varying degrees of deacetylation) [1-4]. Chitosan can be readily obtained from food waste because chitosan is found in the shells of crabs, shrimps, lobsters, krill, squid, and fungi

US Food and Drug Administration recognized chitosan as a safe substance [5]. The properties of chitosan depend on the degree of deacetylation, molecular weight, distribution of acetyl groups, ion concentration, pH, separation, and drying conditions [10]. Chitosan due to pH-dependent solubility, under neutral and base pH can produce stable films on different surfaces. At pH below 6.5, the amine groups are positively charged and chitosan is dissolved. At high pH, the deprotonated amine groups increase and as a result, chitosan becomes insoluble. Amine and hydroxyl groups contribute to chemical modification and the covalent binding of chitosan to other biomolecules and can bind to other polymers and nanoparticles [1, 3]. Biocompatibility, antibacterial properties, hemostatic effect, rapid wound healing, stimulation of bone formation, biodegradability, non-toxicity, high adsorption capacity, collaborator effect immune, anti-lipid activity, reactive active groups, ease of fabrication, ability the construction of various shapes and spatial structures have made chitosan a functional material [6, 7, 9, 11-16].

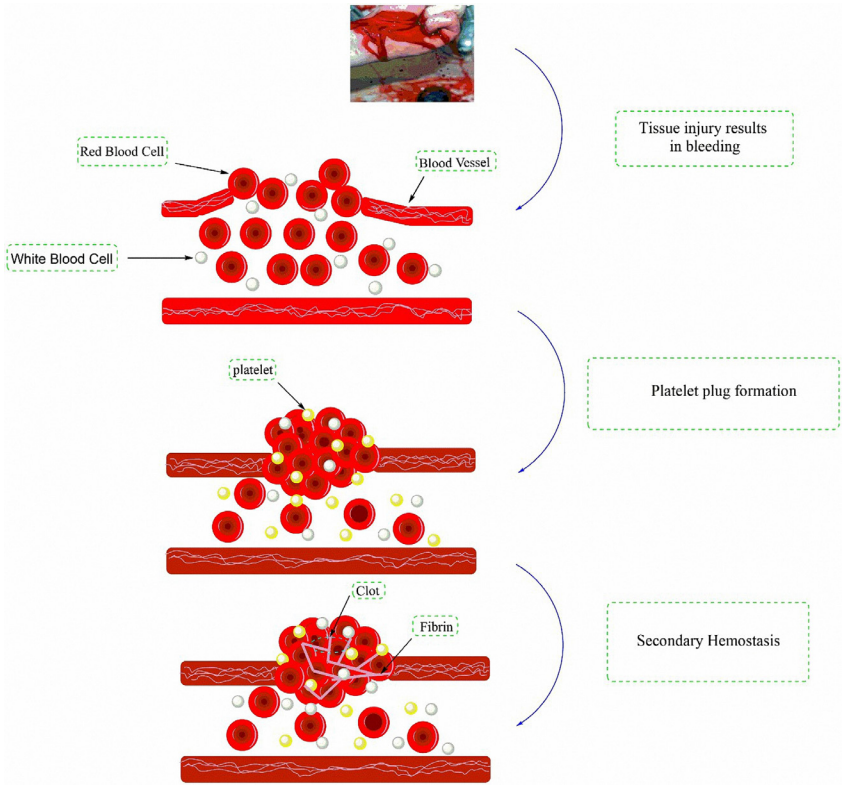


Fig. 2. Ionic gelation and phase separation can be used to make chitosan derivatives [22]. In applications where chitosan is in contact with blood, it can show a high thrombogenic effect due to the positive charge of chitosan, which leads to the absorption and adhesion of plasma proteins, fibrinogen uptake, erythrocyte adhesion, and platelet activation and adhesion to the surface, and eventually causes the destruction of blood bags and processing equipment of blood products. Chitosan activates various coagulation factors and enhances homeostasis (Fig. 2) [1, 7]. Due to the unique properties of chitosan, this polymer can be used to solve problems related to the storage of blood products

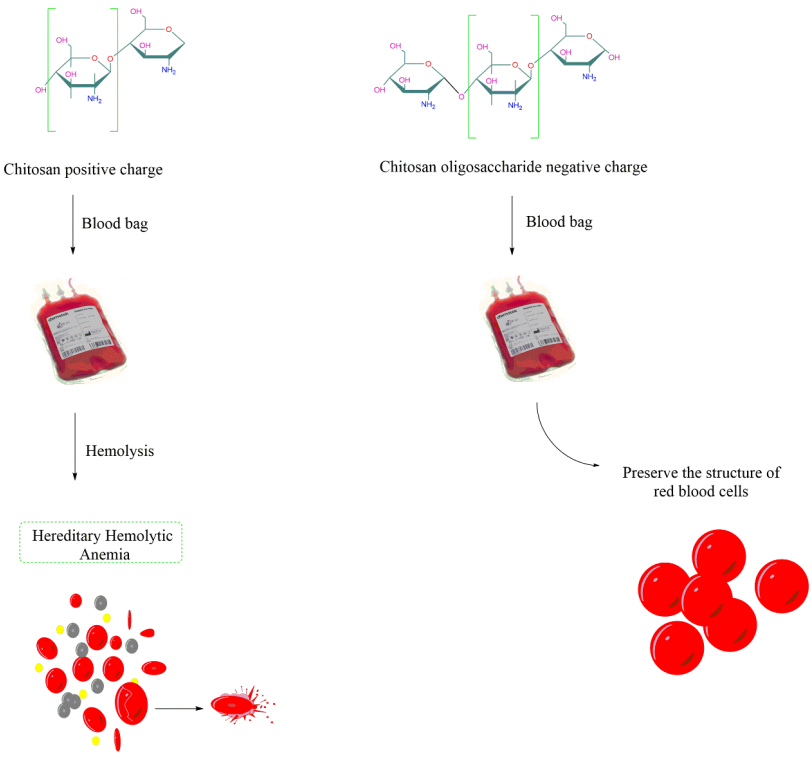


Fig. 3. In chitosan oligosaccharides, due to the reduction in molecular weight compared with chitosan, the positive charge density decreases, thus reducing the interaction between positively charged amine groups and blood products, which prevents damage to the erythrocyte membrane and thus reduces hemolysis (Fig. 3) [24]

Chitosan due to its suitable chemical, physical and mechanical properties is suitable for a variety of biomedical, industrial, food science, technology, and pharmaceutical applications such as artificial skin, wound dressings, hemodialysis membranes, controlled release of drugs, dietary supplements, water refinery, removal of toxins, scaffolding for tissue engineering, semi-permeable membranes, etc (Fig. 2) [1, 3, 6, 13, 17-20]. HemCon®, Celox®, and TraumaStat® are commercially available chitosan-based wound dressings [21]. Ionic gelation and phase separation can be used to make chitosan derivatives [22]. In applications where chitosan is in contact with blood, it can show a high thrombogenic effect due to the positive charge of chitosan, which leads to absorption and adhesion of plasma proteins, fibrinogen uptake, erythrocyte adhesion, and platelet activation and adhesion to the surface and eventually causes the destruction of blood bags and processing equipment of blood products. Chitosan activates various coagulation factors and enhances homeostasis (Fig. 3) [1, 7]. Due to the unique properties of chitosan, this polymer can be used to solve problems related to the storage of blood products.

Negatively charged substances are more biocompatible with blood products such as erythrocytes and platelets, and positively charged substances are less biocompatible. Due to the possibility of surface modifications in polymers, biocompatible derivatives can be produced from them. Chitosan can interact with negatively charged blood cell membranes and anionic proteins due to its positive surface charge. The production of negatively charged chitosan derivatives can increase its biocompatibility [23]. The study of the effect of chitosan on blood products (erythrocytes, platelets, coagulation factors, and proteins) is important in order to use it in various therapeutic applications [24]. The use of nanotechnology can also improve the properties of materials for use in therapeutic applications [25-31].

Today, chitosan hemocompatible derivatives have found a special place in equipment related to blood products. The various products of blood include erythrocytes, platelets, plasma fluid, coagulation factors (including factor VIII (FVIII)), and gamma globulins [32, 33]. Erythrocytes are

the most abundant blood cells that play the role of transporting and releasing oxygen for tissue metabolism. The lifespan of erythrocytes in the blood is 100–120 days [34-38]. Erythrocytes can be stored at 2-6 ℃ for 42 days [23]. Platelets are another group of blood cells that are involved in thrombosis, homeostasis, clot formation, fighting microbial infection, constriction and repair of blood vessels, strengthening atherosclerosis, host defense, and even tumor growth and metastasis. Platelet dysfunction can cause bleeding or thrombosis [39-42]. The lifespan of platelets in the blood is 8-10 days [43]. Platelets last at room temperature of 22–24 ℃ for a maximum of 3 to 7 days and their structure and function are maintained [14, 44, 45]. Albumin is the most abundant plasma soluble protein (52-62%). Albumin has a variety of functions; it is a carrier and distributor protein for endogenous and exogenous substances, fatty acids, steroids, hormones, metal ions (such as copper, zinc, and calcium), and small molecules such as drugs (warfarin, ibuprofen, chlorpromazine, and naproxen), bilirubin and porphyrin. The half-life of albumin is 19 days [46-49]. Coagulation factors and immunoglobulins are two important classes of plasma proteins that play an important role in the homeostasis process, stopping bleeding through the coagulation cascade and immunizing against pathogens [25]. IgG is the major class of immunoglobulins and is the most abundant immunoglobulin in serum [50]. FVIII is a large and complex plasma sialoglycoprotein whose deficiency causes hemophilia A and prevents bleeding [51].

Each of these blood products has specific storage conditions and temperatures and can be used for therapeutic applications. Erythrocytes are used to inject into patients with severe anemia, platelets are used for thrombocytopenic patients, coagulation factors are used to treat patients with hemorrhagic disorders, albumin and immunoglobulins are used for their oncotic and antibody properties, respectively [32, 33]. Maintaining the natural function and metabolic processes of cells and blood products inside and outside the body of living organisms is a major challenge for medical and pharmaceutical fields [52]. Minor changes in the production and composition of blood bags, and storage conditions can have irreparable consequences and impose high costs for reprocessing the blood bank. It is important to take approaches that address the problems associated with storing blood products. The use of polymers and the science of nanotechnology can offer promising solutions [53, 54].

The use of chitosan in the discussion of increasing the preservation of blood products includes a wide range, which on the one hand uses the positive charge of chitosan to accelerate the aggregation of blood cells and proteins in wound healing, and on the other hand, chitosan derivatives with less positive charge are used to prevent blood clotting and to prevent erythrocyte lysis in various therapeutic applications. In this review article, we review the literature (2010-2022) to determine whether the charged-modified chitosan could eliminate the effects of chitosan on blood products and prepare a platform for more research to improve the preservation of the blood products such as erythrocytes, platelets and plasma products (albumin, immunoglobulin (Ig), and factor (FVIII)).

**General effect of chitosan on erythrocytes**

Research shows that the presence of amine groups in the structure of chitosan is the cause of its hemolytic activity. There are several reasons for the lysis of erythrocyte membrane by chitosan, including electrostatic interaction between chitosan and anionic glycoproteins on the surface of erythrocytes, formation of polyelectrolyte complexes from acidic groups of the cellular elements of blood, and amine groups in the structure of chitosan [55]. The strength of hemolysis of chitosan derivatives depends on the number of amines and the degree of protonation of their amines [56]. The presence of primary amines can have a toxic effect on erythrocytes [57]. On the other hand, chitosan nanoparticles synthesized in a neutral medium show less hemolytic activity than chitosan nanoparticles synthesized in an acidic medium [58]. Also, negatively charged chitosan inhibits the hemolysis of erythrocytes.

***Modification of chitosan and application of its derivatives in erythrocyte products***

Due to the hemostatic effect of chitosan on erythrocytes, chitosan and its derivatives can be used to make wound dressings. Gu *et al.* [59] first investigated the effect of fly-larva shell-derived chitosan sponge on rat models with hepatic hemorrhage as a hemostatic agent. They found that chitosan binds to the surface of erythrocytes and causes erythrocytes to deform, thereby causing erythrocytes to accumulate. Chitosan can also activate a high percentage of platelets and increase its hemostatic effect [59]. Dai *et al.* prepared macroporous chitosan-coated mesoporous silica xerogel beads (CSSX) with different concentrations of chitosan and

polyethylene glycol (PEG). Negatively charged blood products interact with positively charged chitosan, and erythrocytes are trapped in a fibrin network. The best performance of CSSX is with 2% chitosan and 5% PEG. Therefore, CSSX can be used as a suitable hemostatic agent for faster wound healing [60, 61]. He *et al.* designed different films of chitosan with varying degrees of protonation and examined the adhesion of erythrocytes. They found that due to the presence of negatively charged neuraminic acid residues in erythrocyte surface glycoproteins, electrostatic interactions between chitosan films with a higher positive charge and erythrocyte are higher [62]. Comparison of chitosan-cotton bandages with different molecular weights and different concentrations of chitosan on erythrocyte showed that the binding of chitosan to erythrocyte was not related to the molecular weight of chitosan, but increasing the concentration of chitosan increased the binding of erythrocyte to it. This is probably due to the increase in protonated groups in it, which increases the chance of erythrocyte membrane binding. Research has shown that combining chitosan with cotton does not cause erythrocytes to accumulate and deform [63]. Comparison of Antarctic krill chitosan (A-Chitosan, with a molecular weight of 157 kDa and a deacetylation degree of 96%) with chitosan obtained from Sigma (S-Chitosan, with a molecular weight similar to A-Chitosan) and the pharmaceutical grade chitosan from a domestic company (P-Chitosan, with a deacetylation degree similar to A-Chitosan) showed that increasing the deacetylation degree of chitosan increases the accumulation and deformation of erythrocyte [64]. Chan *et al*. designed PolySTAT/chitosan bandage to heal wounds faster. Chitosan causes erythrocytes to accumulate due to electrostatic interaction with their membranes; PolySTAT is also a synthetic polymer that increases coagulation by crosslinking fibrin. As a result, PolySTAT/chitosan bandages amend blood clotting and prevent increased arterial pressure through fibrin crosslinks [65]. Zhou *et al.* examined 6-Deoxy-6-(2-aminoethyl) amino chitosan (CS-AEA) and aminoethyl modified CS-AEA (CS-AEA-AEM) in terms of hemolytic activity. The amount of amines in CS-AEA-AEM is higher than in CS-AEA, but because the ratio of primary to secondary amines in CS-AEA is higher than in CS-AEA-AEM, the degree of ionization of CS-AEA amine groups at lower pH is higher and as a result, its hemolytic activity is higher [56]. In comparison of chitosan with its hydrophobic derivative N-alkylated chitosan, it was found that this hydrophobic derivative of chitosan interacts with erythrocytes by hydrophobic interactions. The hydrophobic chains of these particles penetrate the erythrocyte membrane and activate nodes and junctions in the erythrocyte membrane, trapping the cells in a jelly network. Thus N-alkylated chitosan can be used as a hemostatic agent [66]. Wang *et al.* designed a hemostatic bandage using chitosan fibers that reduced homeostasis time in injured rats. Adhesion of chitosan to the wound causes the formation of metal cation-chitosan complexes, which increases the adhesion of erythrocytes and platelets and creates an anionic environment at the wound that helps further clotting [7]. Research shows that chitosan can reduce bleeding time and have a hemostatic effect in patients who have had their teeth extracted [67]. Comparison between chitosan gel and platelet-rich fibrin (PRF) gel in blood clots at the site of tooth extraction showed that the hemostasis effect of chitosan hydrogel was greater and the bleeding stopped in a shorter time than PRF gel. Similar results were obtained by comparing Axiostat (a chitosan-based product) bandage and PRF bandage in the tooth extraction site of heart patients. The function of chitosan is probably due to its positive charge, which interacts with the negative charge of erythrocyte membranes and causes bleeding to stop faster [68, 69]. Thao *et al.* found that N-succinyl chitosan nanoparticles (N-SuC NPs) films have antimicrobial properties and can heal wounds faster and can be used to make wound dressings with antimicrobial properties. The polar carboxylate groups in this nanoparticle reduce the interaction with erythrocytes and reduce their destruction. However, due to increased collagen fouling in the dermis and increased epithelization, it can cause wounds to heal faster [70].

To reduce the effect of the positive charge of chitosan on erythrocyte hemolysis, some work has been done so far. Shelma *et al*. found that binding of the hydrophilic sulfo group by the hydrophobic lauroyl group to the chitosan backbone could reduce the hemolytic activity of chitosan. In the lauroyl sulfated chitosan, due to the negative charge on its surface, the interaction between chitosan and erythrocytes decreases, and as a result, the hemolytic activity of chitosan decreases [55]. Xiong *et al*. investigated two types of hydrophilic chitosan derivatives: N-succinyl-chitosan (NSCS) and N, O-succinyl-chitosan (NOSCS). Due to the negative charge of these two chitosan derivatives, an electrostatic repulsion occurs between the negatively charged erythrocyte membrane and the chitosan derivatives, thereby preventing

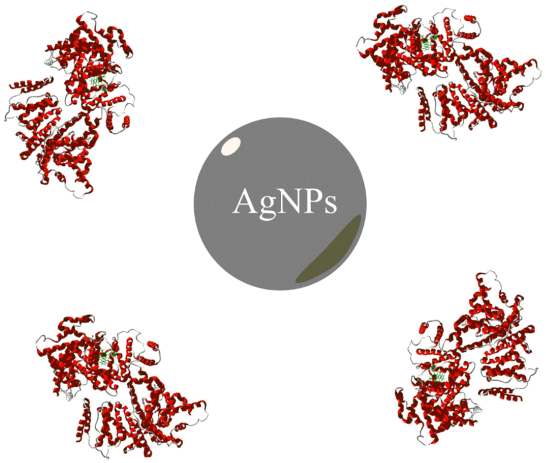


Fig. 4. Sen et al. examined the effect of chitosan-based silver nanoparticles on HSA fibrillation. Interaction between chitosan-based silver nanoparticles and HSA significantly decreases amyloid fibril constitution. Electrostatic interaction between negative charges of HSA and positive charge of nanoparticles decreases the constitution of HSA amyloid-like fibrils and also maintains the structure of HSA optimally. Therefore, these nanoparticles can be used to maintain the structure of albumin products (Fig. 4) [77]

blood cells from coagulating and hemolyzing. It seems that carboxyl groups substituted in C-6 or C-2 hydroxyl group can have an anticoagulant effect [71]. Nadesh *et al*. investigated the effect of solvents (lactic acid and acetic acid) and dispersion medium (acidic medium, saline), surface modifiers (polyethylene glycol (PEG), polyvinyl alcohol (PVA), and ethylenediaminetetraacetic acid (EDTA)) on the blood compatibility of chitosan nanoparticles. They found that surface modifiers had little effect on hemocompatibility, but that EDTA could delay blood clotting to some extent (due to its inherent anticoagulant properties). Nanoparticles dispersed in saline and prepared in lactic acid did not show a hemolytic effect. In glucose metabolism, lactic acid is a common metabolite and is an essential component of lactated Ringer's solution, which can be the reason for its blood compatibility. Surface modifiers make chitosan nanoparticles more uniform, which can lead to better blood compatibility [72]. Bender *et al*. designed two lipid-core nanocapsules stabilized with 80-lecithin polysorbate and uncoated and coated with chitosan and examined them for erythrocyte hemolysis and platelet aggregation in drug delivery systems. At a concentration of 2% (v/v), these two particles had no effect on erythrocyte hemolysis and platelet aggregation, but at a high concentration (10% (v/v)), they could cause erythrocyte hemolysis and platelet aggregation. This suggests that the specific surface area of the colloid could have a greater effect on the chemical nature of the water/particle interface [73]. Benghanem *et al*. examined the grafting of oxidized carboxymethyl cellulose with hydrogen peroxide in the presence of Cu (II) to chitosan (chitosan–OCMC). They found that this hydrogel has antibacterial, antioxidant, non-toxic, and non-hemolytic properties, so based on the results obtained in this article, it seems that this hydrogel can be used in the manufacture of blood bags and blood processing equipment. The maximum hemolytic activity of this film is less than 10%, which indicates its high blood compatibility for use in systems in contact with blood and intravenous administration [74]. Guo *et al*. found that chitosan oligosaccharides reduced the risk of erythrocyte hemolysis compared with chitosan, depending on the dose and molecular weight used, but accumulated irreversibly at higher concentrations. In chitosan oligosaccharides, due to the reduction in molecular weight compared with chitosan, the positive charge density decreases, thus reducing the interaction between positively charged amine groups and blood products, which prevents damage to the erythrocyte membrane and thus reduces hemolysis (Fig. 4) [24]. Yan *et al*. modified the polysulfone membrane (PSf) used in blood purification to improve blood compatibility using 4-(chloromethyl) benzoic acid (CMBA) and sulfonated hydroxypropyl chitosan (SHPCS). In SHPCS24-BAPSf membranes, compared with PSf the hemolytic effect was significantly reduced. Surface modification of the membrane with SHPCS results in the formation of a hydration layer on its surface, which protects the membrane of erythrocytes and thus reduces hemolysis. Therefore, this membrane can be used to make blood purification membranes [75]. Li *et al*. designed chitosan-kappa-carrageenan composite hydrogel (C-K hydrogels) sorbent to remove bacterial and endotoxin contaminants from the blood of patients with sepsis. Endotoxins and chitosan tend to form complexes with each other, which reduces the activity of liposaccharide-induced cytokines. Research has shown that C-K hydrogels have no hemolytic effect on erythrocytes and reduce platelet activation, thus can be used as hemoperfusion sorbents, and helps in the filtration and sterilization of blood products [76].

A summary of the effects of chitosan and its derivatives on erythrocytes is given in Table 1.

**General effect of chitosan on platelets**

Studies show that chitosan increases platelet aggregation and adhesion. The mechanism of

chitosan action is in platelet aggregation and adhesion by increasing intracellular free $Ca^{2+}$ and increasing glycoprotein IIb / IIIa (GPIIb / IIIa) expression on platelet membranes. In the early stages of platelet adhesion, as a result of the production of intracellular signals, thromboxane $A_2$ / ADP is released and GPIIb / IIIa is activated, which increases the accumulation and stability of platelet adhesion. Increased $Ca^{2+}$ activates platelets, and binding of the GPIIb / IIIa complex to fibrinogen causes platelet aggregation and adhesion. Increased $Ca^{2+}$ activates platelets, and binding of the GPIIb / IIIa complex to fibrinogen causes platelet aggregation and adhesion [78]. Research shows that chitosan nanoparticles that are small in size (about 100 nm) and have a low positive charge also have a less anticoagulant and antiplatelet effect [79]. In addition, research has shown that platelet adhesion to chitosan increases with the presence of plasma and extracellular matrix proteins [80].

### *Modification of chitosan and application of its derivatives in platelet products*

Modified chitosan derivatives can be used to make hemostatic agents. Lord *et al*. found that platelet adhesion to protein coated chitosan was by integrins, particularly αIIbβ3. Chitosan alone activates platelets, but proteins coated on chitosan regulate the rate of platelet activation. Platelets that encounter chitosan coated with perlcan and fibrinogen show more activity than platelets that encounter these proteins alone. The activity of platelets that encounter collagen-coated chitosan, collagen, and chitosan alone is similar [80]. Periayah *et al*. examined the hemostatic derivatives of chitosan with different chemical formulas (2% N,O-carboxymethylchitosan (NO-

Table 1. Overview of all studies in the field of effect of chitosan and modified chitosan derivatives in erythrocyte products

| | The name of the modified chitosan nanoparticles or modified chitosan derivatives | Charge of nanoparticles | Size of nanoparticles | Base of nanoparticles or derivatives of chitosan | Type of modification in nanoparticles or derivatives of chitosan | Effects of basic chitosan | Effects of modified nanoparticles, chitosan nanoparticles, or modified chitosan derivatives | Ref. |
|---|---|---|---|---|---|---|---|---|
| 1 | Fly-larva shell-derived chitosan sponge | | | Chitosan | | Erythrocyte hemolysis | Deformation and accumulation of erythrocytes | [59] |
| 2 | Macroporous chitosan-coated mesoporous silica xerogel beads | | | Chitosan | Mesoporous silica xerogel beads | Erythrocyte hemolysis | Erythrocytes are trapped in a fibrin network | [60] |
| 3 | Films of chitosan with varying degrees of protonation | | | Chitosan | Increasing the degree of protonation | Erythrocyte hemolysis | Increased hemolysis and accumulation of erythrocytes | [62] |
| 4 | Chitosan-cotton bandage with high concentration of chitosan | | | Chitosan | Increase in chitosan concentration | Erythrocyte hemolysis | Increased binding of erythrocytes to chitosan | [63] |
| 5 | Antarctic krill chitosan | | | Chitosan | Increase the degree of deacetylation | Erythrocyte hemolysis | Increased hemolysis and accumulation of erythrocytes | [64] |
| 6 | PolySTAT/chitosan bandage | | | Chitosan | PolySTAT | Erythrocyte hemolysis | Increased accumulation of erythrocytes | [65] |
| 7 | 6-Deoxy-6-(2-aminoethyl) amino chitosan | | | Chitosan | 6-Deoxy-6-(2-aminoethyl) amin | Erythrocyte hemolysis | Increased hemolytic activity | [56] |
| 8 | N-alkylated chitosan | | | Chitosan | N-alkylated | Erythrocyte hemolysis | Increased hemostatic activity | [66] |
| 9 | Chitosan fiber bandage | | | Chitosan | | Erythrocyte hemolysis | Increased adhesion of erythrocytes | [7] |
| 10 | Chitosan gel | | | Chitosan | | Erythrocyte hemolysis | Reduce bleeding time in tooth extraction | [67-69] |
| 11 | N-succinyl chitosan nanoparticles | +21±5 mV | 66±9 nm | Chitosan | N-succinyl | Erythrocyte hemolysis | Reduced Erythrocyte hemolysis and destruction | [70] |
| 12 | Lauroyl sulfated chitosan | -6.06mV | 886 nm | Chitosan | Lauroyl sulfated | Erythrocyte hemolysis, platelet aggregation | Reduced Erythrocyte hemolysis, lack of platelet aggregation | [55] |
| 13 | N-succinyl-chitosan and N, O-succinyl-chitosan | | | Chitosan | N-succinyl, N, O-succinyl | Erythrocyte hemolysis | Reduce the absorption of erythrocytes and prevent blood clotting | [71] |
| 14 | EDTA-Chitosan nanoparticles | -5.43±1.1 mV | 118±25 nm | Chitosan | EDTA | Erythrocyte hemolysis and blood clotting | Delay in blood clotting | [72] |
| 15 | Lipid-core nanocapsules stabilized with 80-lecithin polysorbate and coated with chitosan | +9.3±2.5 mV | 133±1.12 | Chitosan | Lipid-core nanocapsules stabilized with 80-lecithin polysorbate | Erythrocyte hemolysis | No effect on erythrocyte hemolysis | [73] |
| 16 | Chitosan–oxidized sodium carboxymethyl cellulose | | | Chitosan | Oxidized sodium carboxymethyl cellulose | Erythrocyte hemolysis | Increased hemocompatibility | [74] |
| 17 | Chitosan oligosaccharides | | | Chitosan | Oligosaccharides | Erythrocyte hemolysis | Decreased hemolysis of erythrocytes | [24] |
| 18 | Polysulfone membrane modified by4-(chloromethyl) benzoic acid (CMBA) and sulfonated hydroxypropyl chitosan (SHPCS) | | | Chitosan | Sulfonated hydroxypropyl | Erythrocyte hemolysis | Decreased hemolysis of erythrocytes | [75] |
| 19 | Chitosan-kappa-carrageenan composite hydrogel | | | Chitosan | Kappa-carrageenan | Erythrocyte hemolysis, platelet aggregation | No hemolytic effect, reduces platelet activation, antibacterial activity | [76] |

CMC), 7% NO-CMC (with 0.45 mL collagen), 8% NO-CMC, NO-CMC-35, Oligo-chitosan (O-C) 52, O-C 53, and 5% O-carboxymethylchitosan (O-CMC) 47). They found that O-C 52 and O-C 53 cause more platelet aggregation and facilitate the clotting process, thus having a greater hemostatic effect. This may be due to the high degree of crystallinity of O-C 53 [81]. Chung *et al*. designed two chitosan nanoparticles functionalized by adenosine diphosphate (ANP) and fibrinogen (FNP) and investigated the mechanism of clot formation. In the sample containing ANP nanoparticles, platelet aggregation was higher at the clot site, which is probably due to the presence of ADP on the surface of ANPs, as it simultaneously activates large numbers of platelets. Research has shown that the fibrin network in the sample containing FNP has a higher density. Thrombin molecules in the blood activate fibrinogen in the blood and on the surface of FNPs, facilitating the formation of a fibrin network, and thereby accelerating clot formation. The group's research showed that ANPs have a higher potential to prevent bleeding [82]. In another study, Periayah *et al*. examined the effect of two chitosan formulations including NO-CMC and O-C in platelet clotting in patients with von Willebrand disease. They found that O-C had smaller pore sizes than NO-CMC, which increased platelet adhesion and increased chemical intermediary reactions. On the other hand, O-C increases the expression of von Willebrand factor (vWF, the first clotting protein that binds to FVIII for constitution of a clot of platelets), FVIII, GpIIb / IIIa, and Thromboxane $A_2$ and thus increases platelet activation and adhesion [83]. Shi *et al*. designed a composite microsphere containing carboxymethyl chitosan, sodium alginate, and collagen (CSCM) to investigate its hemostatic effect. CSCM accelerated platelet aggregation, adhesion, and activation. They suggested that after contact with blood, the CSCM surface area increases due to water absorption, resulting in platelet aggregation occurring in a shorter time [84]. A study by Wu *et al*., comparing the effects of A-Chitosan, S-Chitosan, and P-Chitosan on platelets, showed that the order in which these chitosan derivatives affect platelet uptake and adhesion is A-Chitosan> S-Chitosan> P-Chitosan. Higher molecular weight seems to have a higher hemostatic effect. The higher the water absorption capacity of chitosan, the more platelets adhere to it [64]. In another study, chitosan-cotton bandages were found to have high adhesion to platelets, and the rate at which platelets bind to these bandages depends on chitosan molecular weight and concentration. This bandage also deforms platelets [63]. Comparison of two types of chitosan nanoparticles with different deacetylation degrees (80% and 93%) showed that chitosan nanoparticles with 93% deacetylation degree cause platelet aggregation. This may be due to the higher amount of $NH3^+$ groups due to deacetylation, which increases interaction with the negatively charged platelet surface [85].

In this section, various types of chitosan derivatives with anticoagulant properties that can be used to make bags and platelet processing equipment are examined. Ramtoola *et al*. examined chitosan/poloxamer microparticles, chitosan/PVA microparticles, chitosan/tween 80 microparticles, chitosan-coated 2,5 poly-lactide-co-glycolide nanoparticles, and chitosan-coated 15 poly-lactide-co-glycolide nanoparticles, in terms of platelet aggregation. Due to the structure and properties of chitosans, chitosan nanoparticles were expected to activate platelets, but research has shown that when chitosan is formulated as nanoparticles and microparticles, it has no effect on platelet activation. This may be due to the concentration and surface charge of chitosans in micro and nanoparticles compared with chitosan bandages [86]. Chitosan causes a slight accumulation of platelets *in vitro*, but lauroyl sulfated chitosan (LSCS) has no effect on platelet aggregation. The lack of platelet aggregation in the LSCS sample may be due to the presence of -CH3 side groups and flexible bonds and the presence of $CH_2$ in their structure, which makes the structure more flexible and prevents platelet aggregation. The results show that LSCS has no hemostatic effect [55]. One of the hydrophilic derivatives of chitosan is salicylic acid-chitosan (SA-chitosan), which can reduce platelet adhesion and accumulation. It can be a good alternative to aspirin to prevent thrombosis. This property may be due to the presence of an aromatic ring and acetyl group in the structure of SA-chitosan, which is similar to that found in aspirin [87]. Among PEG-chitosan nanoparticles, PVA-chitosan nanoparticles, and EDTA-chitosan nanoparticles dispersed in saline, PEG-chitosan nanoparticles showed the lowest platelet aggregation; this could be due to the space prevention created by PEG chains in these nanoparticles. Chitosan nanoparticles prepared in an acidic medium cause a strong accumulation of platelets, which can be due to the high positive surface charge of the nanoparticles, which

increases the adhesion of platelet adhesion proteins [72]. Kim *et al*. encapsulated ginseng extract (RG), which has antithrombotic properties, into the chitosan-polyglutamic acid (CS-PGA) nanoparticles, chitosan-fucoidan (CS-Fu) nanoparticles, chitosan-fucoidan, and polyglutamic acid nanoparticles (PF-NPs). Polyglutamic acid and fucoidan also have anticoagulant properties. The results showed that RG could inhibit platelet aggregation well, but when RG was encapsulated inside CS-PGA, CS-Fu, and PF-NPs, its antiplatelet activity increased significantly due to reduced degradation and increased solubility and stability. Therefore, these nanoparticles can be used to make blood bags and blood processing equipment with antiplatelet properties [88, 89]. Guo *et al*. found that chitosan oligosaccharides, in addition to having no effect on platelet activation, also prevented platelet aggregation, thus preventing blood clotting (Fig. 4). This anticoagulant property is probably due to the presence of hydroxyl groups along the molecular chains [24]. Moraes *et al*. found that 2-N-3,6-O-sulfated chitosan reduced platelet uptake due to the presence of negatively charged sulfate groups and the hydrophilic nature of the film, and this shows the blood compatibility of this film, and as a result, this film can be used in medical systems related to blood [90]. As mentioned earlier, Yan *et al*. modified the PSf membrane using SHPCS and CMBA. They found that platelets that attach to the surface of the PSf membrane become deformed and pseudopodia, but in SHPCS24-BAPSf membranes, the presence of SHPCS prevents platelet aggregation and deformation, as a result, it can be used in the manufacture of bags and platelet processing equipment [75]. Arif Asghar *et al*. proposed the green construction of chitosan-functionalized silver nanoparticles using ethanolic bud extract of *Syzygium aromaticum*. This nanoparticle significantly reduces platelet aggregation. The high antiplatelet activity of these nanoparticles may be due to changes in platelet membranes [91]. Ahmed *et al*. compared the chitosan-copper oxide nanocomposite (Cs-CuO-NP's, derived from pomegranate peel extract) with chitosan nanoparticles in terms of biological activity. Cs-CuO-NP's compared with chitosan nanoparticles increase the clotting time of erythrocytes and platelets. *P*. granatum, which is used to synthesize this nanocomposite, contains anthocyanidins, which inhibit cyclooxygenase, possibly reducing fibrinogen levels, and inhibiting coagulation factors and thrombin. As a result, Cs- CuO-NP's have anticoagulant properties and can be used in the manufacture of blood bags, blood processing equipment, and coronary artery stent coverage [92]. Drozd *et al*. functionalized the polyurethane plate surface layer by layer with chitosans and heparin to increase thrombosis resistance. The positive charge of chitosan interacts with the negative charge of heparin and helps to functionalize the polyurethane surface. The anticoagulant property of this surface is due to the presence of unfractionated heparin. Because there is a repulsion between the negative charge of heparin and the negative charge of the blood products, it prevents the adhesion and activation of platelets. Pentasaccharide in heparin also activates antithrombin. As a result, this surface can increase blood-clotting time and can be used in medical applications with anticoagulant properties [93].

Chitosan derivatives can inhibit the antiplatelet activity of protease enzymes and protect platelets against these enzymes. Herfena *et al*. extracted the enzyme bromelain (a protease enzyme with antiplatelet activity) from pineapple cores and loaded it into glutaraldehyde-crosslinked chitosan microspheres (GCM). Stomach acid and intestinal enzymes normally digest this enzyme. Examination of the function of GCM in a platelet-rich plasma shows that the antiplatelet activity of bromelain is significantly inhibited due to encapsulation in GCM [94].

A summary of the effects of chitosan and its derivatives on platelets is given in Table 2.

**General effect of chitosan on albumin**

The study of the behavior of chitosan and BSA shows that the interaction between chitosan and BSA depends largely on the pH and ratio of the two biopolymers. These biopolymers interact through electrostatic interactions between negative charges of BSA and positive charges of chitosan. The interaction of BSA and chitosan changes the secondary structure of BSA and extinguishes the fluorescence property of the tryptophan amino acid of BSA [95]. Bekale *et al*. investigated the interaction of three chitosan nanoparticles with different molecular weights (15, 100, and 200 KDa) and a similar degree of deacetylation with two proteins, HSA and BSA. BSA binds to chitosan nanoparticles through hydrophobic interactions, and by increasing the molecular weight of the

nanoparticles, a more stable bond is formed between BSA and chitosan. However, HSA binds to chitosan nanoparticles through electrostatic interactions, and the order of stability of the bond between these chitosan nanoparticles and HSA is as follows: 100> 200> 15 KDa. Larger nanoparticles cause more changes in the secondary structure of the protein, and the shape of the protein affects the shape of the nanoparticles [96].

### *Modification of chitosan and application of its derivatives in albumin products*

In this section, we examine the effect of chitosan derivatives on increasing the stability of albumin in various applications. Research shows that chitosan and polyvinyl alcohol (PVA) coat on magnetic nanoparticles (PC-Fe3O4) reduce the protein uptake of these nanoparticles. Shagholani *et al*. investigated the interaction between PC-Fe3O4 nanoparticles and BSA protein. They found that the uptake of PC-Fe3O4 nanoparticles for BSA protein was very low. The electrostatic repulsion between the negative charge of PC-Fe3O4 nanoparticles and the negative charge of BSA prevents the adsorption of protein by these nanoparticles. On the other hand, the hydroxyl groups on PVA can be used to bind various targets. Due to the fact that albumin does not adhere to these nanoparticles, they can be used to process albumin products [97]. Sen *et al*. examined the

Table 2. Overview of all studies in the field of effect of chitosan and modified chitosan derivatives in platelet products

| | Name of the modified chitosan nanoparticles or modified chitosan derivatives | Charge of nanoparticles | Size of nanoparticles | Base of nanoparticles or derivatives of chitosan | Type of modification in nanoparticles or derivatives of chitosan | Effects of basic chitosan | Effects of modified chitosan nanoparticles or modified chitosan derivatives | Ref. |
|---|---|---|---|---|---|---|---|---|
| 1 | Chitosan coated with perlcan and fibrinogen | | | Chitosan | Perlcan and fibrinogen | Platelet activation | Increased platelet activation | [80] |
| 2 | Oligo-chitosan | | | Chitosan | | Platelet activation and aggregation | Increases platelet activation and adhesion | [81, 83] |
| 3 | Chitosan nanoparticles functionalized by adenosine diphosphate | +20.6±1.9 mV | 251.0±9.8 nm | Chitosan | Adenosine diphosphate | Platelet aggregation | Increased platelet aggregation | [82] |
| 4 | Chitosan nanoparticles functionalized by fibrinogen | +15.3±1.5 mV | 326.5±14.5 nm | Chitosan | Fibrinogen | Platelet aggregation | Formation of a denser fibrin network | [82] |
| 5 | Composite microsphere containing carboxymethyl chitosan, sodium alginate, and collagen | | | Chitosan | Carboxymethyl | Platelet activation and aggregation | Increased platelet aggregation, adhesion, and activation | [84] |
| 6 | Antarctic krill chitosan | | | Chitosan | Increase the degree of deacetylation | Platelet aggregation | Increased platelet adhesion and accumulation | [64] |
| 7 | Chitosan-cotton dressing | | | Chitosan | Cotton | Platelet adhesion | Increased platelet adhesion | [63] |
| 8 | Chitosan nanoparticles with 93% deacetylation degree | +20.0±6.0 mV | 292±52 nm | Chitosan | 93% deacetylation degree | Platelet aggregation | Increased platelet aggregation | [85] |
| 9 | Chitosan coated %2,5 poly-lactide-co-glycolide nanoparticles | +57 mV | 343 nm | Chitosan | Poly-lactide-co-glycolide | Platelet activation | No platelet activation | [86] |
| 10 | Chitosan coated %15 poly-lactide-co-glycolide nanoparticles | +55 mV | 443 nm | Chitosan | Poly-lactide-co-glycolide | Platelet activation | No platelet activation | [86] |
| 11 | lauroyl sulfated chitosan | -6.06mV | 886 nm | Chitosan | Lauroyl sulfated | Platelet aggregation | Lack of platelet aggregation | [55] |
| 12 | Salicylic acid-chitosan nanoparticles | +52 mV | 292± 2 nm | Chitosan | Salicylic acid | Platelet aggregation | Reduce platelet adhesion and accumulation | [87] |
| 13 | PEG-chitosan nanoparticles | +1.9±1.5 mV | 130±10 nm | Chitosan | PEG | Platelet aggregation | Reduced platelet aggregation | [72] |
| 14 | Chitosan-polyglutamic acid (CS-PGA) nanoparticles | +18.6±1.0 mV | 360±67 nm | Chitosan | Polyglutamic acid | Platelet aggregation | Inhibition of platelet aggregation | [88, 89] |
| 15 | Chitosan-fucoidan (CS-Fu) nanoparticles | +2.8±0.2 mV | 440±44 nm | Chitosan | Fucoidan | Platelet aggregation | Inhibition of platelet aggregation | [88, 89] |
| 16 | Chitosan-fucoidan and polyglutamic acid nanoparticles | -21.5±1.0 mV | 286.7±36.6 nm | Chitosan | Fucoidan and polyglutamic acid | Platelet aggregation | Inhibition of platelet aggregation | [88, 89] |
| 17 | Chitosan oligosaccharides | | | Chitosan | Oligosaccharides | Platelet aggregation | No platelet activation and aggregation | [24] |
| 18 | 2-N-3,6-O- Sulfated chitosan | | | Chitosan | 2-N-3,6-O- Sulfated | Platelet adhesion | Decreased platelet uptake and increased blood compatibility | [90] |
| 19 | Polysulfone membrane modified by4-(chloromethyl) benzoic acid (CMBA) and sulfonated hydroxypropyl chitosan (SHPCS) | | | Chitosan | sulfonated hydroxypropyl | Platelet aggregation | No platelet activation and aggregation | [75] |
| 20 | Chitosan-functionalized silver nanoparticles | Positive | 30–40 nm | Chitosan | Silver | Platelet aggregation | Reduces platelet aggregation | [91] |
| 21 | Chitosan-copper oxide nanocomposite | Positive | 20–30 nm | Chitosan | Copper oxide | Erythrocyte hemolysis, platelet aggregation | Increase the clotting time of erythrocytes and platelets | [92] |
| 22 | Polyurethane plate surface layer by layer with chitosan and heparin | | | Chitosan | | Platelet activation and aggregation | Prevents the adhesion and activation of platelets | [93] |
| 23 | Bromelain loaded on glutaraldehyde-crosslinked chitosan microspheres | | | Chitosan | glutaraldehyde | Platelet activation and aggregation | Maintain the antiplatelet activity of bromelain | [94] |

effect of chitosan-based silver nanoparticles on HSA fibrillation. Interaction between chitosan-based silver nanoparticles and HSA significantly decreases amyloid fibril constitution. Electrostatic interaction between negative charges of HSA and positive charge of nanoparticles decreases the constitution of HSA amyloid-like fibrils and also maintains the structure of HSA optimally. Therefore, these nanoparticles can be used to maintain the structure of albumin products [77]. The colloidal chitosan-zinc-tin oxide nanoparticle binds to BSA via hydrophobic interactions. The binding of this nanoparticle to BSA causes the BSA fluorescence property to be extinguished, which occurs through the formation of a base state complex using a static extinction mechanism. This feature can be used in biosensors and pharmaceutical and biomedical applications [98]. Situ *et al*. examined four chitosan particles with different molecular weights of chitosan and anionic cross-linker with different surface charge densities ($1.5 \times 10^5$ g/mol chitosan- Sodium tripolyphosphate (CS-STPP), $4.0\times 10^5$ g/mol CS-STPP, $1.5 \times 10^5$ g/mol chitosan-sodium hexametaphosphate (CS-SHMP), and $4.0 \times 10^5$ g/mol CS-SHMP), in terms of stability and controlled release of BSA in the intestinal environment. Research has shown that particles with high molecular weight of chitosan and low charge density of cross-linkers, such as $4.0\times 10^5$ g/mol CS-STPP, have weaker nanostructures that leak stored biomaterial. On the other hand, particles with low molecular weight of chitosan and high charge density of cross-linkers, such as $1.5 \times 10^5$ g/mol CS-SHMP, have a very solid structure. This suggests that large size and high charge density both cause leakage of biological material stored in the early stages. Therefore, a more stable particle with a lower charge density, $1.5 \times 10^5$ g/mol CS-STPP, is more suitable for the targeted release of BSA in the intestinal environment [99]. Loading albumin into chitosan spherical nanoparticles can have several benefits, including long-term stability (three months) in powder or suspension, higher enzymatic stability, controlled release of albumin, and higher serum concentrations following oral administration and intraperitoneal injection in healthy rabbits and cirrhotic mice [100]. In a study, Yan *et al*. significantly reduced BSA protein uptake in SHPCS24-BAPSf membranes relative to PSf. The results show that the decrease in protein absorption is due to the increase in surface hydrophobicity. Therefore, this membrane can be used in the processing of albumin products [75].

**General effect of chitosan on Immunoglobulin-G (IGG) products**

Antibodies can bind to chitosan through electrostatic interactions [101]. Decreasing the acetylation degree of chitosan increases the affinity of chitosan for binding to antibodies [102]. Various chemical modifications such as tosylation, acylation, or O-carboxymethylation in the chitosan functional groups, i.e., primary and secondary hydroxylamine groups, help to bind various biomolecules such as antibodies to chitosan [103].

***Modification of chitosan and application of its derivatives in immunoglobulin-G (IGG)* products**

Various studies have been performed on increasing immunoglobulin-G (IgG) absorption by chitosans in order to purify IgG . The difference in IgG adsorption by different adsorbents depends on the adsorbent properties such as porosity, structure, functional groups involved in the reaction, amount of ligand loaded, available surface area, pore size, and pore distribution [104]. Uygun *et al*. examined the absorption of IgG by linoleic acid attached to chitosan beads [poly (LA-Ch)] for IgG purification. IgG interacts with chitosan through hydrophobic interactions, and because the hydrophobicity of poly (LA-Ch) is higher than chitosan, the absorption of IgG by poly (LA-Ch) is higher than chitosan. The increase in IgG absorption by poly (LA-Ch) is due to the presence of specific interactions between the antibody molecule and the hydrophobic aliphatic chain of fatty acids [105]. Comparison of non-functionalized chitosan-alginate adsorbents and chitosan-alginate adsorbents functionalized by Cibacron Blue, Reactive Blue, and Reactive Green showed that IgG adsorption was higher in dye-functionalized adsorbents. Among the adsorbents functionalized by dyes, the chitosan-alginate adsorbent functionalized by Cibacron Blue has the highest IgG absorption. This is probably due to the interaction of Cibacron Blue dye with certain domains of IgG [106]. Epoxide chitosan/alginate composite functionalized by Cibacron Blue F3GA was used to purify IgG. Research has shown, that Cibacron Blue F3GA dye can interact with biomolecules through ionic interactions (due to the presence of sulfonic acid groups) and hydrophobic and hydrogenic interactions (due to the presence of aromatic rings). Changes in buffer and pH can cause changes in IgG absorption. For example, the highest IgG absorption was in the Tris – HCl buffer at pH = 7.8 [107, 108]. Sun *et al*. examined the

IgG adsorption capacity of 2-mercaptopyridine (2-MP) covalent agarose beads embedded agarose–chitosan composite monolithic cryogels (2-MP covalent agarose-chitosan cryogels). The surface area of these cryogels is 350 m2/g. They found that the increase in the adsorption capacity of 2-MP covalent agarose–chitosan cryogels was due to the presence of agarose beads in cryogels [109]. Investigation of IgG absorption on protein Concanavalin A attached Magnetic Chitosan Nanoparticles (Con-A-$Fe_3O_4$-CsNP) showed that Con-A has a strong tendency to interact with IgG due to the presence of carbohydrate groups in its structure. Research has shown that the best nanoparticle size for IgG adsorption is about 200 nm because smaller nanoparticles are likely to create a space barrier, and larger nanoparticles are likely to accumulate and reduce the available surface area [110]. Barroso *et al*. copolymerized chitosan with glycidyl methacrylate (GMA) and then combined it with PVA to improve its mechanical properties. Research has shown that adjustable physicochemical properties, high mechanical strength, and good porosity increase IgG absorption by Chitosan-based monoliths [16]. Khodaei *et al*. examined two carriers of chitosan functionalized by protein A and aldehyde double-branched chitosan functionalized by protein A for IgG absorption. They found that IgG absorption by double-branched chitosan beads was higher; because surface modification of chitosan with tris (2-aminoethyl) amine increases the reaction sites with protein A and thus increases the IgG uptake absorption [111].

Chitosan derivatives can be used to increase the stability of antibodies. Borges *et al*. designed an immunosensor using a gold surface modified by mercaptopropionic acid (MPA) and chitosan and functionalized by F (ab')2 fragments of IgG. The use of chitosan, which is a biocompatible and hydrophilic biopolymer, stabilizes the structure of F (ab')2, and the electrostatic forces created on the surface cause the proper orientation of F (ab')2 [112]. Parween *et al*. designed four paper-based microfluidic systems for the detection of blood groups. Functionalization of these systems was performed by chitosan, chitosan crosslinked with sodium triphosphate pentabasic (chitosan-STPP), chitosan-glutaraldehyde (chitosan-GTA) and chitosan-sodium hydroxide (chitosan-NaOH), respectively. The research of this group showed that the order of stability of anti-blood group antibodies in each of the microfluidic systems functionalized by chitosan at room temperature is as follows: chitosan-NaOH > chitosan = chitosan-STPP> chitosan-GTA. Chitosan-NaOH remains stable for a longer time due to deacetylation and carboxymethylation of chitosan, as this phenomenon increases the ability of chitosan to retain moisture. As a result, the antibodies remain stable for a longer time [113].

Chitosan can be used as a suitable carrier for the transfer of therapeutic antigens and proteins and as an adjunct to vaccines. Fletcher *et al*. investigated the release of IgG and Fab from the chitosan-alginate hydrogel. Alginate hydrogels cause rapid release of antibodies, but the combination of chitosan with this hydrogel causes controlled release of antibodies due to electrostatic interactions between chitosan and antibodies [101]. Mirtajaddini *et al.* used ε-toxin loaded in chitosan nanoparticles to produce antibodies against this antigen in rabbits. Research has shown that these nanoparticles can produce twice as much anti-ε-toxin antibody as the control sample [114].

***General effect of chitosan on gene of factor VIII (FVIII)***

The formation of DNA-carrying chitosan nanoparticles depends on the interaction between the positive charges of amine groups in chitosan and the negative charges of phosphate groups in DNA. Changes in chitosan nanoparticles (such as changes in molecular weight and chitosan chain length, degree of deacetylation, and charge density (N (nitrogen atoms in chitosan) / P (phosphorus atoms in the DNA)) and targeting) are performed to increase DNA-chitosan binding efficiency, improve nanoparticle stability, increase solubility, express protein in a specific tissue, endosomal evaporation, and nuclear localization, for example, high molecular weight (HMW) chitosan is more stable than low molecular weight (LMW) chitosan, but particle aggregation is higher in high molecular weight chitosan and its solubility is lower at biological pH. Intracellular release is better in LMW chitosan. Chitosan does not have pH buffering capacity against endemic destruction. The amine and hydroxyl groups in chitosan help to modify it to increase gene transfer efficiency.

***Modification of chitosan and application of its derivatives in gene transfer of factor VIII (FVIII)***

Much research has been done on the treatment of hemophilia A using oral administration of chitosan nanoparticles carrying the gene of coagulation, FVIII. Bowman *et al*. examined two chitosan nanoparticles carrying DNA FVIII with a similar molecular weight of 390 KDa and a

degree of deacetylation of 83.5% and 70% for oral administration in mice with hemophilia A. Both nanoparticles show a similar level of FVIII release (2-4%) over a month. After one month, a bleeding challenge developed in mice that showed a phenotypic correction of 13/20 mice [115]. Dhadwar *et al*. four chitosan nanoparticles carrying DNA FVIII (0.02% chitosan with 10 µg DNA, 0.02% chitosan with 30 µg DNA, 0.04% chitosan with 10 µg DNA, and 0.04% chitosan with 30 µg DNA) with a degree of deacetylation of more than 84% and a molecular weight of 213 KDa in hemophilia mice. Studies show that 0.02% chitosan nanoparticles with 10 µg DNA show better results due to lack of nanoparticle accumulation. Oral administration of chitosan nanoparticles in hemophilia mice indicates a temporary increase in the expression of FVIII (more than 100 mU after one day) and a slight phenotypic correction [116]. Comparison between four chitosan nanoparticles as FVIII carrier DNA (chitosan chloride salt with a molecular weight of 113 kDa (CL 113), chitosan chloride salt 213 (CL213), chitosan glutamate salt 113 (G113), and chitosan glutamate 213 (G213)) at 50 mM sodium sulfate was performed. The results showed that the gene transfer efficiency by chitosan chloride salt 213 with a charge ratio of 3:1 is higher than other nanoparticles [117]. Yerkesh *et al*. found that adding DNA of immunoregulatory elements (Fc fragment of IgG and IL-10) to DNA encoding FVIII in chitosan nanoparticles could inhibit the formation of anti- FVIII antibodies in mice [118].

A summary of the effects of chitosan and its derivatives on plasma products (Albumin, IgG, and

Table 3. Overview of all studies in the field of the effect of chitosan and modified chitosan derivatives in plasma products (Albumin, IgG, and FVIII)

| | Name of the modified chitosan nanoparticles or modified chitosan derivatives | Charge of nanoparticles | Size of nanoparticles | Base of nanoparticles or derivatives of chitosan | Type of modification in nanoparticles or derivatives of chitosan | Effects of basic chitosan | Effects of modified chitosan nanoparticles or modified chitosan derivatives | Ref. |
|---|---|---|---|---|---|---|---|---|
| 1 | Chitosan and polyvinyl alcohol-coated magnetic nanoparticles | -12.5 mV | 1000 nm | Chitosan and polyvinyl alcohol | $Fe_3O_4$ | BSA absorption | Lack of BSA absorption | [97] |
| 2 | Polysulfone membrane modified by4-(chloromethyl) benzoic acid (CMBA) and sulfonated hydroxypropyl chitosan (SHPCS) | | | Chitosan | Sulfonated hydroxypropyl | BSA absorption | Decreased BSA absorption | [75] |
| 3 | Chitosan nanoparticles with 200 KDa molecular weight | Positive | | Chitosan | | BSA absorption | Increased BSA absorption | [96] |
| 4 | Chitosan nanoparticles with 100 KDa molecular weight | Positive | | Chitosan | | HSA absorption | Increased HSA absorption | [96] |
| 5 | Chitosan-based silver nanoparticles | +40 mV | | Chitosan | Silver | | Decreases the constitution of HSA amyloid-like fibrils | [77] |
| 6 | Chitosan-zinc-tin oxide nanoparticle | | | Chitosan | zinc-tin oxide | BSA absorption | Increased BSA absorption | [98] |
| 7 | Chitosan spherical nanoparticles | 22.4±0.3 - 21.1±0.7 mV | 75.7±3.5 – 78.5±1.8 | Chitosan | | | Controlled release of BSA | [100] |
| 8 | $1.5 \times 10^5$ g/mol chitosan- Sodium tripolyphosphate (CS-STPP) | −1.35 mV | 1085.78±61.45 nm | Chitosan | Sodium tripolyphosphate | | Targeted release of BSA | [99] |
| 9 | Linoleic acid attached to chitosan beads | | | Chitosan | Linoleic acid | Adsorption of antibodies | Increased IgG adsorption | [105] |
| 10 | Chitosan-alginate adsorbents functionalized by Cibacron Blue | | | Chitosan | Cibacron Blue | Adsorption of antibodies | Increased IgG adsorption | [106] |
| 11 | Epoxide chitosan/alginate composite functionalized by Cibacron Blue F3GA | | | Chitosan | Cibacron Blue F3GA | Adsorption of antibodies | Increased IgG adsorption | [107, 108] |
| 12 | 2-mercaptopyridine covalent agarose–chitosan cryogels | | | Chitosan | 2-mercaptopyridine, Agarose | Adsorption of antibodies | Increased IgG adsorption | [109] |
| 13 | Protein Concanavalin A attached Magnetic Chitosan Nanoparticles | | | Chitosan | Protein Concanavalin A | Interaction with antibodies | Increased IgG absorption | [110] |
| 14 | Copolymerized chitosan with glycidyl methacrylate and combined with PVA | | | Chitosan | Glycidyl methacrylate, PVA | Adsorption of antibodies | Increased IgG adsorption | [16] |
| 15 | Aldehyde double-branched chitosan functionalized by protein A | | | Chitosan | protein A | Adsorption of antibodies | Increased IgG adsorption | [111] |
| 16 | Gold surface modified by mercaptopropionic acid (MPA) and chitosan | | | Chitosan | Adsorption of antibodies | | Increased IgG adsorption | [112] |
| 17 | Chitosan-NaOH | | | Chitosan | NaOH | Adsorption of antibodies | Maintaining antibody stability over a long time | [113] |
| 18 | Chitosan-alginate hydrogel | | | Chitosan | alginate | | Controlled release of antibodies | [101] |
| 19 | ε-toxin loaded in chitosan nanoparticles | +32.4 – +48.6 mv | 200-800 nm | Chitosan | Sodium tripolyphosphate, Tween80% | | Production of anti- ε-toxin antibodies in rabbits | [114] |
| 20 | Chitosan nanoparticles with degrees of deacetylation of 83.5% and 70% and molecular weight of 390 KDa | +10 mV | | Chitosan | Degree of deacetylation of 83.5% and 70% | | DNA FVIII carrier | [115] |
| 21 | 0.02% chitosan nanoparticles with 10 µg DNA with a degree of deacetylation of more than 84% | Positive | | Chitosan | Degree of deacetylation of more than 84% | | DNA FVIII carrier, no accumulation of nanoparticles | [116] |
| 22 | Chitosan chloride salt 213 nanoparticles | Positive | | Chitosan | Degree of deacetylation of more than 84% | | Increase gene transfer efficiency | [117] |
| 23 | Chitosan nanoparticles with DNA encoding FVIII and DNA of immunoregulatory elements | Positive | | Chitosan | | | Inhibit the formation of anti- FVIII antibodies | [118] |

FVIII) is given in Table 3.

## CONCLUSION

In this review article, we examined the surface modification of chitosan and the production of new derivatives with optimal properties to increase the preservation of blood products including erythrocytes, platelets, and plasma products (albumin, immunoglobulin (Ig), and factor (FVIII)). PolySTAT / chitosan dressing, N-alkyl chitosan nanoparticles, and chitosan activated by adenosine diphosphate can be used as effective wound dressings due to their antimicrobial properties and increased accumulation of erythrocytes and platelets at the wound site. Chitosan-fucoidan and polyglutamic acid nanoparticles can be used in blood processing equipment due to the lack of platelet aggregation and reduced erythrocyte hemolysis. SHPCS24-BAPSf membranes can be used in blood purification products due to reduced hemolysis and platelet aggregation and lack of BSA uptake. Chitosan spherical nanoparticles contribute to the stability of albumin and its targeted release. Chitosan-NaOH increases the stability of antibodies. Chitosan /alginate composite functionalized by Cibacron helps increase IgG absorption in chromatographic columns to purify IgG due to ionic, hydrogen, and hydrophobic bonds between Cibacron and IgG. Chitosan chloride salt 213 nanoparticles interact with the negative charge of DNA and by protecting DNA, helps transfer the FVIII gene to hemophilia patients. The results show that the modified chitosan derivatives can be used to increase the preservation of blood products in wound dressings, blood bags, and blood processing equipment, increase the stability and purification of plasma proteins, and transfer of the FVIII gene. This review article could open a new horizon for researchers to use modified chitosan derivatives to increase the preservation of blood products and pave the way for the use of chitosan and its derivatives in future research.

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## CONFLICTS OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

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