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PREPARATION AND CHARACTERIZATION OF THREE-COMPONENT INTERPOLYELECTROLYTE COMPLEXES OF CHITOSAN

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ABSTRACT

Three-component interpolyelectrolyte complexes based on Bombyx mori chitosan were synthesized, and their compositions and hydrodynamic dimensions were characterized. It was established that, at pH 6.3, binary chitosan/collagen complexes interact with sodium carboxymethylcellulose in a mass ratio of 1:0.2:0.65. Additionally, binary polyelectrolyte complexes composed of Bombyx mori chitosan and collagen were prepared with mass ratios of 1:0.1, 1:0.2, and 1:0.3.

It was observed that during macromolecular interactions, the positive charges of chitosan are partially neutralized by the negative charges of collagen, resulting in a change in the zeta potential from +15.2 mV to +5.67 mV. At this ratio, the complex's surface layer carries a neutral charge (Q(0)). The hydrodynamic particle sizes of the three-component complexes ranged from 136 to 243 nm

Introduction

In recent years, there has been a sustained surge in interest toward the development of novel materials based on polyelectrolyte complexes (PECs), which constitute a distinct class of polymers formed through cooperative, reversible interactions between oppositely charged macroions [1–2].

The formation of PECs occurs in the presence of two or more oppositely charged entities - such as polycations, polyanions, or polyampholytes - where electrostatic interactions facilitate charge neutralization within and between macromolecules. Complexation represents one of the most efficacious strategies for tailoring polymer properties, as the interplay of lyophilizing and blocking agents enables the formation of binary, layered colloidal dispersions and/or films with enhanced functionalities [3].

Polymer blends, characterized by the simple and cost-effective combination of at least two polymers, serve as versatile platforms for generating materials with novel physical attributes without necessitating the synthesis of specialized copolymers. In the context of drug delivery systems, the underlying mechanisms involve intricate processes such as polymer relaxation, chain cleavage, and diffusion. A broad spectrum of biopreparations - ranging from proteins to antibodies - can be incorporated into copolymeric architectures, either through physical entrapment, indirect immobilization, or covalent attachment, depending on their specific properties [4].

The effect of drying temperature (25°C and 45°C) on the physical properties of various forms of composite films composed of gelatin, carboxymethyl cellulose (CMC), and chitosan (CS) in weight ratios ranging from 80:20:0 to 60:30:10 was investigated. It was determined that the gelatin/CMC/CS ratio of 60:30:10 wt.% is optimal for producing food packaging materials, owing to its high vapor permeability and biodegradability rate [5].

Three-component stoichiometric interpolyelectrolyte complexes (IPECs) were synthesized through sequential mixing of a 0.25% pectin solution with chitosan, as well as a 1% trypsin solution, in a mass ratio of 2:1:2. These complexes served as carriers for biologically active substances with prolonged release profiles [6]. Furthermore, interactions among solutions of carboxymethyl chitosan (CMCS), CMC, collagen, and trans-glutaminase (TGase) in various mass ratios (ranging from 40:40:20 to 25:25:50) resulted in the formation of anti-adhesion membranes composed of CMCS/CMC/collagen [7].

It is well established that synthetic sponges based on IPEC comprising collagen, sodium carboxymethyl cellulose (Na-CMC), and glutaraldehyde (GA) in a 2:1:8 wt.% ratio are extensively utilized in bone tissue engineering. To facilitate osteogenesis and cell proliferation, these sponges must possess adequate porosity, rendering them suitable for the repair of bone defects and fractures [8–14]. Using electrospinning techniques, IPEC nanoparticles based on chitosan, collagen, and sodium alginate (Na-Al) were fabricated, with complexes synthesized in a mass ratio of 4:1:1 to generate porous, bioresorbable three-dimensional matrices [15–16]. The collagen/CS/Na-GA IPEC was further recommended as a promising biomaterial for corneal tissue engineering applications [17].

Among various polymer systems, interpolyelectrolyte complexes (IPECs) based on natural polysaccharides have garnered significant interest due to their inherent biodegradability, biocompatibility, and bioavailability. Although numerous IPEC systems have been previously explored - such as collagen/Na-CMC/glutaraldehyde, chitosan/sodium alginate/collagen, and gelatin/CMC/chitosan - the specific advantages of chitosan/collagen/Na-CMC complexes include:

Composed entirely of natural, non-toxic components;

Surface charge and viscosity can be precisely tuned by adjusting component ratios;

Improved film-forming capabilities and structural stability;

Potential for drug delivery applications, owing to hydrogen bonding and electrostatic interactions.

Scientific novelty: This study uniquely investigates the formation of three-component IPECs comprising chitosan, collagen, and Na-CMC, expanding upon previous research. Unlike earlier studies that focused primarily on binary systems, this work systematically characterizes surface charge neutrality ($Q_{(0)}$), diffusion behavior, and morphological properties as functions of component ratios. These insights contribute to a deeper understanding of complex formation mechanisms, structural stability, and their potential applications in biomedical material development.

It is important to emphasize that, for the development of IPECs with bioactive properties aimed at targeted drug delivery, understanding the specifics of their synthesis is crucial. Accordingly, this chapter is dedicated to the production of three-component polyelectrolyte complexes based on chitosan, collagen, and Na-CMC, with the determination of optimal compositions and comprehensive analysis of their physicochemical properties.

Materials and methology

Hydrodynamic parameters were measured using a Litesizer 100 instrument (AntonPaar GmbH, Austria), with calculations performed based on the Einstein-Stokes equation. The dynamic viscosity of solutions of chitosan (CS), sodium carboxymethyl cellulose (Na-CMC), collagen, and their corresponding interpolyelectrolyte complexes (IPECs) was assessed using a HAAKE Viscotester 2 plus rotational viscometer. The surface electrokinetic potential of IPEC particles was

determined via dynamic quasi-elastic light scattering measurements employing a Zetasizer Nano ZS device.

Microscopic studies: Optical images of the samples were captured using a Motic BA210 optical microscope at a scale of 77.20 μm. The morphological analysis of the films was conducted using an Agilent 5500 atomic force microscope (AFM, USA) at 22°C. Silicon cantilevers with a stiffness of 9.5 N/m and a resonance frequency of 145 kHz were employed. The maximum scanning areas for AFM imaging were 15×15 μm² in the X and Y axes, with a vertical (Z) range of 1 μm.

Results and discussions

Hydrodynamic Parameters and Rheological Properties of Three-Component Complexes of CS/Collagen/Na-CMC

Three-component interpolyelectrolyte complexes (IPECs) composed of chitosan (CS), collagen, and sodium carboxymethyl cellulose (Na-CMC) with varying surface charges were synthesized. Their hydrodynamic parameters were subsequently analyzed (Table 1).

Table 1

Dependence of selected hydrodynamic parameters and the dynamic viscosity of solutions on the composition of IPECs: solvent—acetate buffer, pH = 6.3.

№	Samples, mass ratio	d, nm	D, cm^2/sec	μ, dPa·s	ζ potential, mV
1	CS	2380	0,20	13	+15,2
2	Collagen	505	0,74	69	-1,88
3	Na-CMC	203	2,41	75	-2,50
4	CS/collagen=1:0,1 ($Q_{(+)}$)	383	0,09	11	+11,7
5	CS/collagen=1:0,2 ($Q_{(+)}$)	803	0,04	19	+8,47
6	CS/collagen =1:0,3 ($Q_{(+)}$)	250	0,15	17	+5,67
7	CS/collagen/Na-CMC=1:0,1:0,54 ($Q_{(0)}$)	136	0,27	40	0
8	CS/collagen/Na-CMC=1:0,2:0,65 ($Q_{(0)}$)	180	0,21	58	0
9	CS/collagen/Na-CMC=1:0,3:0,75 ($Q_{(0)}$)	243	0,16	96	0

Determination of Zeta Potential of Chitosan and Component Interactions at pH 6.3

The zeta potential (ζ) of chitosan (CS) at pH 6.3 was measured and found to be positive (+15.2 mV). In contrast, solutions of sodium carboxymethyl cellulose (Na-CMC) and collagen exhibited negative ζ potentials, measuring -2.5 mV and -1.88 mV respectively; the isoelectric point of collagen is approximately 6.0.

Upon the addition of 0.1–0.3 wt.% collagen—containing negatively charged -COOH groups—to the CS solution, the zeta potential decreased from +15.2 mV to +5.67 mV. Based on these titration results and ζ potential measurements, it can be concluded that by varying the ratios of CS, collagen, and Na-CMC, stoichiometric three-component IPECs (Q(0)) with balanced surface charges can be obtained. This approach facilitated the development of methods for synthesizing both binary and three-component IPECs with predetermined total surface charge values.

The choice of pH 6.3 for all formulations is based on the isoelectric point of collagen (~6.0), where collagen exhibits minimal net charge. This pH condition promotes efficient electrostatic interactions among the components, enabling optimal complex formation: positively charged chitosan interacts with negatively charged Na-CMC and collagen. Charge balancing at this pH allows the attainment of ζ potential values close to zero, indicating near-neutral surface charge. While achieving zeta potential neutrality ($Q_{(0)}$) at other pH levels is theoretically possible, such adjustments would require modifications to component ratios, which are beyond the scope of this study.

As previously reported, one of the key parameters of IPECs is the diffusion coefficient of their particles, which is inversely proportional to their hydrodynamic radius. The measurements of the hydrodynamic parameters for the initial polyelectrolytes and the resulting complexes reveal that, upon formation of binary and three-component polyelectrolyte complexes, the hydrodynamic diameters decrease by approximately 2-fold and 10-fold, respectively, relative to the original macromolecules.

According to the Einstein-Stokes equation, the molecular diffusion coefficient D characterizes the diffusion process and can be determined from the relation:

$$D = \frac{k \cdot T}{6\pi\eta r} \qquad (1)$$

where: T - is the absolute temperature; η - is the viscosity of the solution; r - is the radius of the diffusing particles; k - is the Boltzmann constant. The data show that the particle diameter is inversely proportional to the diffusion coefficient. With stoichiometric ratios of complexes, the diameter of the particles increases, which accordingly complicates their diffusion and leads to phase separation of the solution (examples No. 5 and 9).

According to the Einstein-Stokes equation, the particle diameter is inversely proportional to the diffusion coefficient, indicating that smaller particles tend to diffuse more rapidly. Phase separation (aggregation) was observed in sample #5 (particle size ~803 nm, $\zeta = +8.47$ mV) and sample 9 (particle size ~243 nm, $\zeta \approx 0$ mV). Despite the smaller size of sample 9, its near-zero zeta potential reduces electrostatic repulsion, thereby increasing the propensity for aggregation - similar to that observed in sample #5. This suggests that aggregation is not solely dependent on particle size but is also significantly influenced by surface charge stability. Other samples with comparable sizes remain stable, likely due to more favorable charge distributions that inhibit coalescence.

The dynamic viscosity of the solutions varied depending on the component ratios. Incorporating collagen into chitosan markedly reduced viscosity (by 5–6 times), which suggests structural changes induced by interaction. Conversely, in three-component systems, viscosity increased with higher collagen and Na-CMC content. This increase is likely attributable to the linearization of collagen's globular structure and the bridging interactions of Na-CMC's carboxyl groups (-COO−) with collagen's amino groups ($-NH_3^+$), resulting in greater molecular entanglement and resistance to flow. Therefore, both collagen and Na-CMC contribute to viscosity modulation.

When Na-CMC is added to the binary CS/collagen complex, the collagen's $-NH_3^+$ groups interact with the $-COO^-$ groups of Na-CMC, positioning the collagen macromolecule between two linear polysaccharide chains. As a result, the dynamic viscosity does not necessarily decrease with variations in the component ratios of chitosan, collagen, and Na-CMC. Notably, at stoichiometric ratios of CS/collagen = 1:0.2 and CS/collagen/Na-CMC = 1:0.3:0.75 (by weight), the measured dynamic viscosities are 19 and 96 dPa·s respectively - values exceeding those observed in other IPEC ratios.

Study of the Morphology of Three-Component Interpolyelectrolyte Complexes of CS/Na-CMC/Collagen with Varying Composition

Using the dry molding method from acetic-ac aqueous solutions of CS/Na-CMC/collagen, films were prepared with different component ratios ranging from 1:0.75:0.01 to 1:0.5:0.08 by mass (Figures 1–3).

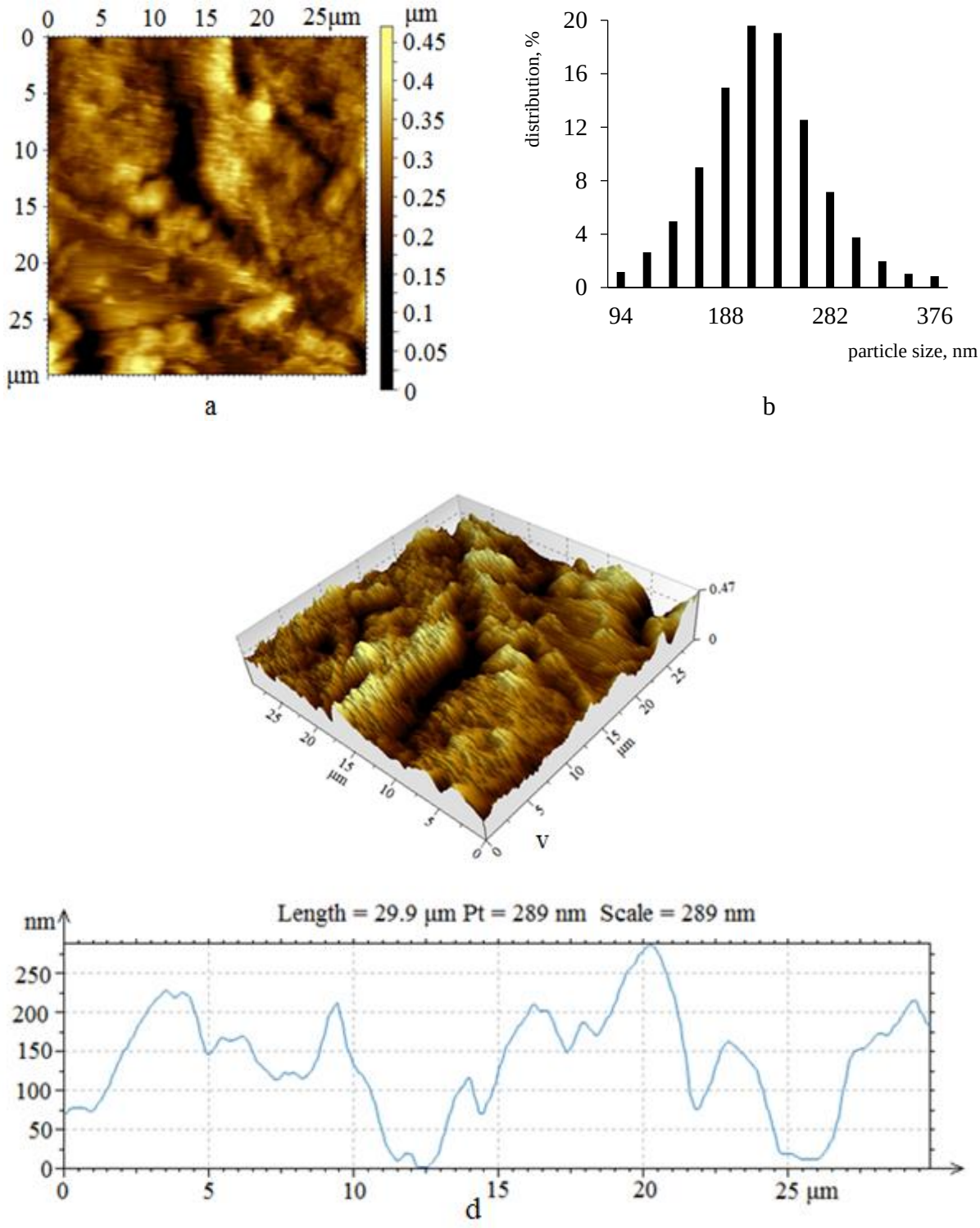

Figure 1. Amplitude (a) and phase (v) AFM images of CS/Na-CMC/collagen samples = 1:0.5:0.08 mass., distribution histogram (b), and surface roughness (d)

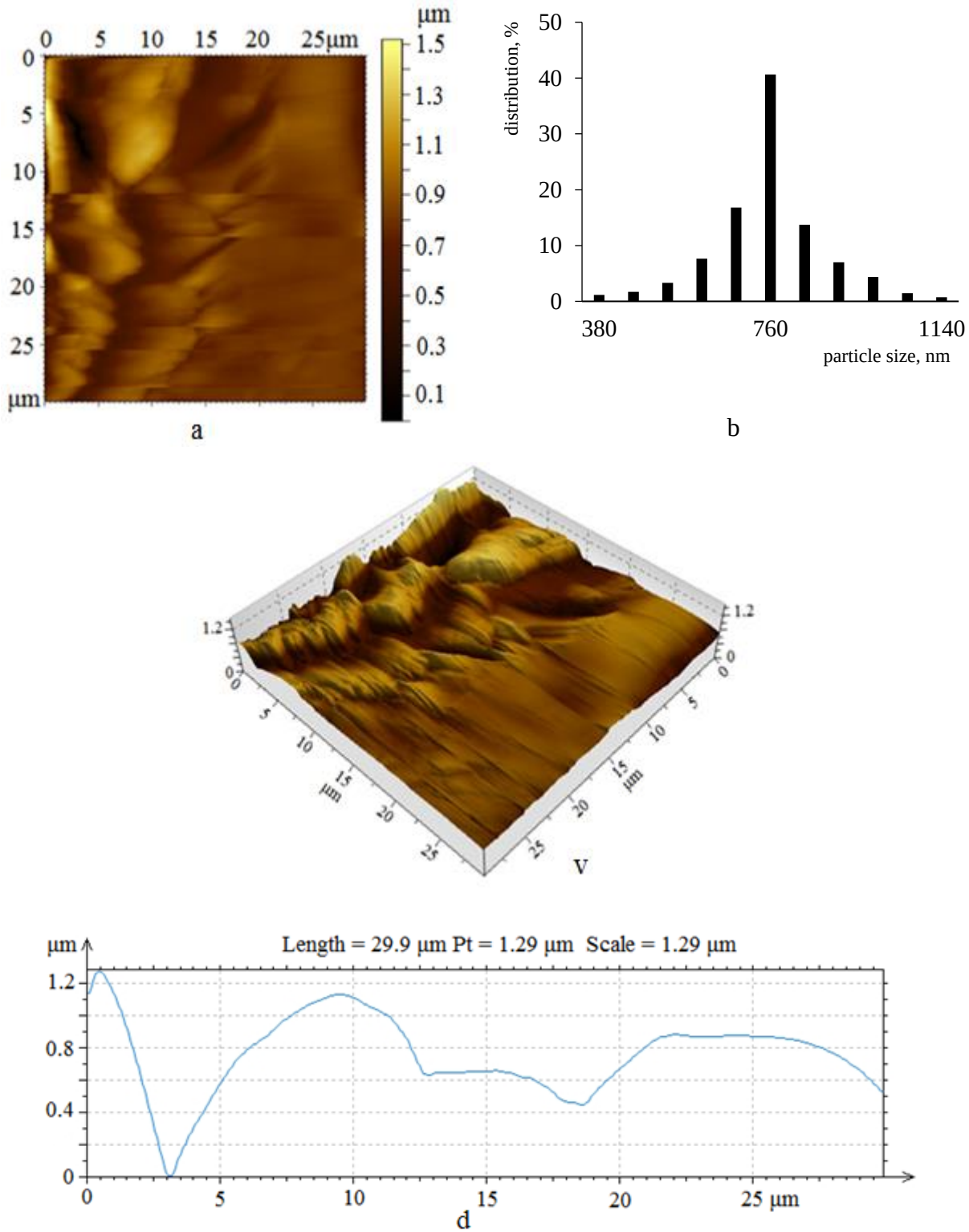

***Figure 2**. Amplitude (a) and phase (v) AFM images of CS/Na-CMC/collagen samples = 1:0.37:0.03 mass., distribution histogram (b), and surface roughness (d)*

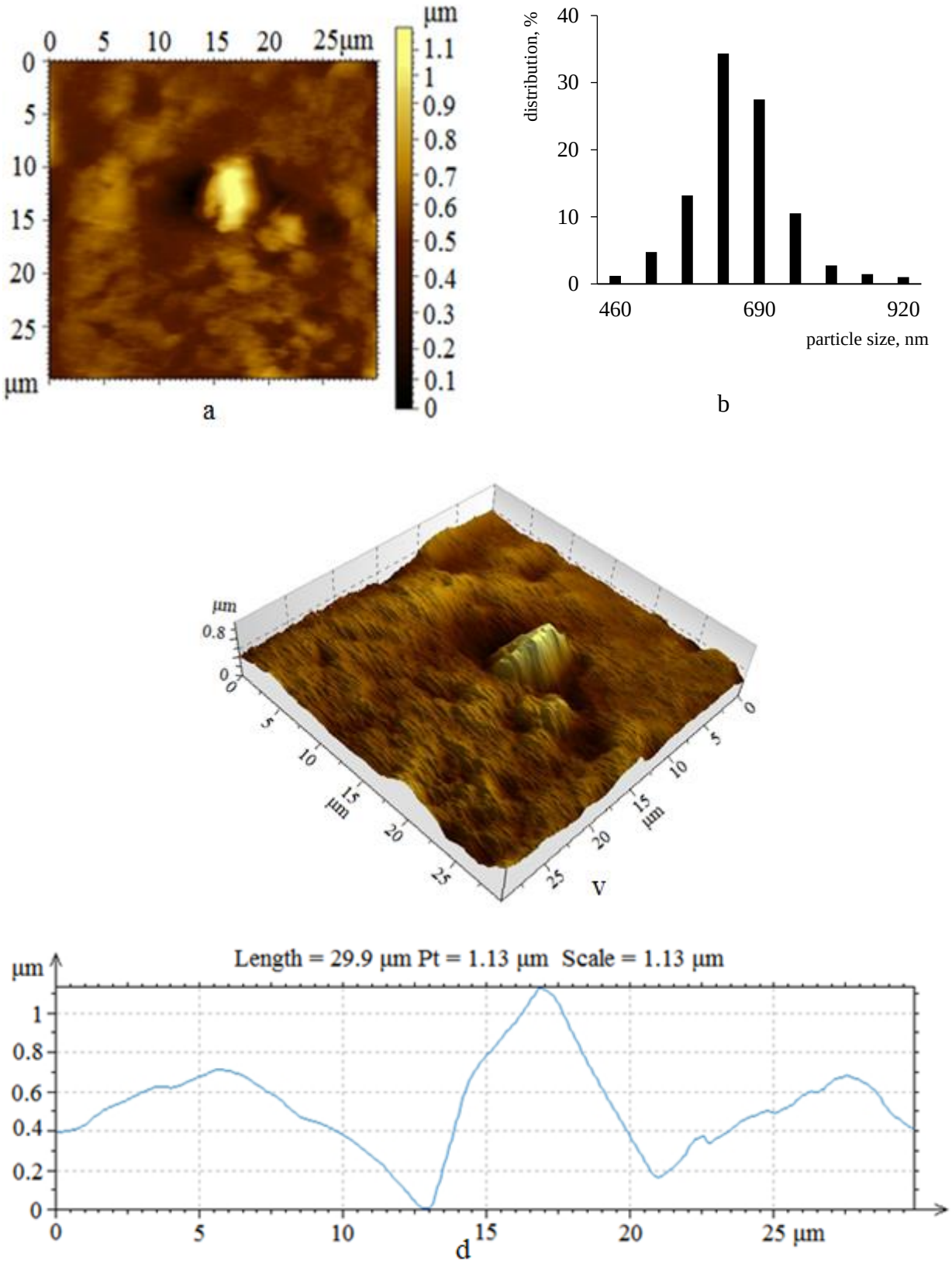

Figure 3. *Amplitude (a) and phase (v) AFM images of CS/Na-CMC/collagen samples = 1:0.75:0.01 mass., distribution histogram (b), and surface roughness (d)*

Microscopic Study of the Morphology of CS/Na-CMC/Collagen Three-Component Complex Films

Microscopic analyses indicate that, regardless of the initial macromolecule ratios, the surface of the films composed of CS/Na-CMC/collagen complexes features non-spherical (shapeless) nanoparticles with a unimodal size distribution, approximately ≤1 μm. Notably, increasing the collagen content in the PEC results in larger particle diameters. For instance, at a ratio of CS/Na-CMC/collagen = 1:0.75:0.01, nanoparticles ranging from 0.1 to 1 μm are formed and are relatively evenly dispersed across the polymer matrix surface. Although AFM images reveal particles within the 380–920 nm range, their local clustering appears to be scale-dependent. Chitosan functions as the matrix component in the film, owing to its higher molecular weight and concentration, while collagen and Na-CMC contribute functional groups that facilitate binding to chitosan, thereby influencing particle distribution. The increase in collagen content appears to promote larger particle formation, likely due to modifications in hydrogen bonding and structural rigidity within the matrix.

Conclusion

In this study, three-component interpolyelectrolyte complexes (IPECs) of CS/collagen/Na-CMC were synthesized by varying the mass ratios of the components. It was established that at a mass ratio of CS/collagen/Na-CMC = 1:0.2:0.65, the functional groups of collagen macromolecules interact maximally with the negatively charged functional groups of Na-CMC through electrostatic interactions and hydrogen bonds (Q(0)).

The hydrodynamic diameters and diffusion coefficients of the initial macromolecules, as well as the binary and three-component IPECs in solution, were determined. Under the selected synthesis conditions, particles with sizes ranging from 136 to 243 nm were formed across all ratios, with a unimodal distribution of particles in solution. These fundamental findings are of significant interest for potential applications of these complexes in medicine.

Additionally, it was confirmed that at a ratio of CS/Na-CMC/collagen = 1:0.75:0.01, the functional groups of Na-CMC interact maximally with the positively charged functional groups of collagen via electrostatic interactions and hydrogen bonds, as verified by DLS, LiteSizer, and AFM analyses. Under the synthesis conditions, nanoparticles ranging from 0.1 to 1 μm were formed and found to be almost uniformly distributed throughout the matrix.

Overall, three-component interpolyelectrolyte complexes based on CS/collagen/Na-CMC and CS/Na-CMC/collagen were successfully synthesized. These complexes have promising potential as drug carriers in medical applications.

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