Original paper

The cysteine-silver sol at different pH: influence of silver salts chemical nature

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Abstract: The effect of the chemical nature of silver salts (silver nitrate, silver nitrite, silver acetate) used as a precursor in the synthesis of cysteine-silver sols on their behavior at different medium acidities was investigated. The visual analysis revealed a similar behavior of all systems: a change in the *pH* of the medium from an acidic value to an alkaline one in cysteine-silver sols leads to the transition from a homogeneous solution to a turbid one and homogeneous again. Turbidimetric titration and electrophoretic light scattering (zeta-potential measurement) measurements showed that all the systems exhibit an isoelectric point, the value of which depends on the sample preparation history, namely, on the chemical nature of the silver salt. This behavior is associated with the presence of amino and carboxyl groups located on the surface of cysteine-silver sol nanoparticles. Based on previously reported data and the results obtained in this study, a structural model of nanoparticles forming the corresponding sols is proposed.

Keywords: cysteine-silver sol, pH, isoelectric point, turbidity, zeta-potential.

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Оригинальная статья

Цистеин-серебряный золь при различных pH: влияние химической природы солей серебра

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Аннотация: Исследовано влияние химической природы солей серебра (нитрат серебра, нитрит серебра, ацетат серебра) – прекурсора для синтеза цистеин-серебряных золей – на поведение последнего при различных значениях кислотности среды. Визуальный анализ выявил схожее поведение всех систем: изменение *pH* среды от кислого значения к щелочному в цистеин-серебряных золях ведет к переходу от гомогенного раствора к мутному и снова гомогенному. С использованием методов турбидиметрического титрования и электрофоретического рассеяния света (измерения дзета-потенциала) было показано, что все системы обладают изоэлектрической точкой, величина которой зависит от предыстории приготовления образца - химической природы соли серебра. Такое поведение связано с наличием амино и

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карбоксильных групп, расположенных на поверхности наночастиц цистеин-серебряных золей. На основе предыдущих результатов и данных, полученных в настоящей работе, была предложена структура наночастиц, формирующих соответствующий золь.
Ключевые слова: цистеин-серебряный золь, pH, изоэлектрическая точка, мутность, дзета-потенциал.

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1. Introduction

Low-molecular-weight gelators (LMWGs) are a distinctive class of molecular systems capable of forming nanostructured supramolecular gels through non-covalent interactions, including electrostatic interactions, hydrogen bonding, and $\pi-\pi$ stacking [1, 2]. The ability of such systems to undergo dynamic self-assembly opens up opportunities for the development of new materials with considerable application potential [3]. Among these materials, there are hydrogels based on various amino acids [4-11]. Here, one of the unusual examples of self-assembly are gels based on sulfur-containing amino acids and salts of some metals – Ag, Au, Cu [12-16]. Recently, we have shown that such systems exhibit a range of functional properties, including antibacterial and antibiofilm activity, anticancer activity, photoactivity, and film-forming ability [17-24].

In most cases, gel formation proceeds in two stages. For example, the interaction of aqueous solutions of *L*-cysteine with silver nitrate first leads to the formation of a so-called cysteine-silver sol (CSS), a colloidal solution of nanoparticles [18]. Gelation then occurs upon the addition of various low-molecular-weight electrolytes, such as metal salts, to the CSS [17]. Recently, computer simulations have shown that hydrogen bonds between protonated amino groups and carboxyl/carboxylate groups in the CSS are responsible for the formation of the gel network [23]. We subsequently confirmed this finding indirectly in experiments involving pH variation in the CSS [24]. Thus, the ability of these systems to undergo gelation depends on the surface structure of the nanoparticles in the initial sol.

The present work investigates the effect of the chemical nature of silver salts ($AgNO_3$, $AgNO_2$, or $AgOOCCH_3$) on the behavior of the corresponding *L*-cysteine-based hydrosols at different medium acidities.

2. The experimental part

General procedure for CSS synthesis

CSS samples were prepared according to the procedure described previously [18]. An aqueous solution of the silver AgX salt ($AgNO_3$, > 99%, RedOx; $AgNO_2$, > 99%, MACKLIN; $AgOOCCH_3$, > 99,5%, Aladdin; 0,01 wt.%) was added to an aqueous solution of *L*-cysteine (*L*-cys, > 99%, MACKLIN; 0,01 wt.%). The molar ratio of the initial components, *L*-cys: AgX, was 1:1,25. The resulting slightly opalescent mixtures were stirred at room temperature (25°C) for 5 min and then kept in the dark for 3 h. As a result, transparent solutions ranging in color from brown to yellowish green were obtained; in the case of $AgNO_2$, a gel was formed. The gel was diluted twofold. Different amounts of KOH (PA grade) were then added to the CSS samples, and the solutions were mixed at room temperature for 1 min. The resulting mixtures were analyzed using various instrumental techniques.

Methods

The pH of the solutions was measured using a Seven Multi S70 pH meter (Mettler Toledo). Turbidimetric titration was performed using a Hach 2100N turbidimeter (Hach). The electrophoretic mobility of aggregates in the samples was measured with a Zetasizer Nano ZS analyzer (Malvern) equipped with a $He-Ne$ laser (633 nm, 4 mW) using U-shaped capillary cuvettes. Zeta-potential distributions were calculated using the Henry equation: $UE = 2ezf(Ka)/3Z$, where UE – electrophoretic mobility, z – zeta potential, e – dielectric constant, Z – viscosity, and $f(Ka)$ – Henry's function, $f(Ka)$= 1,5 for aqueous media.

3. Results and their discussion

As shown in fig. 1, a gradual change in the acidity of the medium in the corresponding sols leads to a transition from a homogeneous solution to a turbid system and then back to a homogeneous state, irrespective of the chemical nature of the silver salt. This effect is particularly pronounced in the system prepared with silver nitrite (see fig. 1 c). It should also be noted that the color gradually disappears in all systems, which is probably associated with changes in the electronic structure of the nanoparticles [24]. This issue requires further analysis and is not considered in the present work. The observed turbidity of the systems can be attributed to a loss of sol stability caused by neutralization of the nanoparticle surface charge.

Indeed, the turbidimetric titration results showed that, at a certain medium acidity, the systems reach a maximum turbidity value (see fig. 2). The chemical nature of the silver salt used for hydrosol preparation directly affects the position of this maximum on the pH axis. In all cases, a dome-shaped dependence of sol

turbidity on pH is observed.

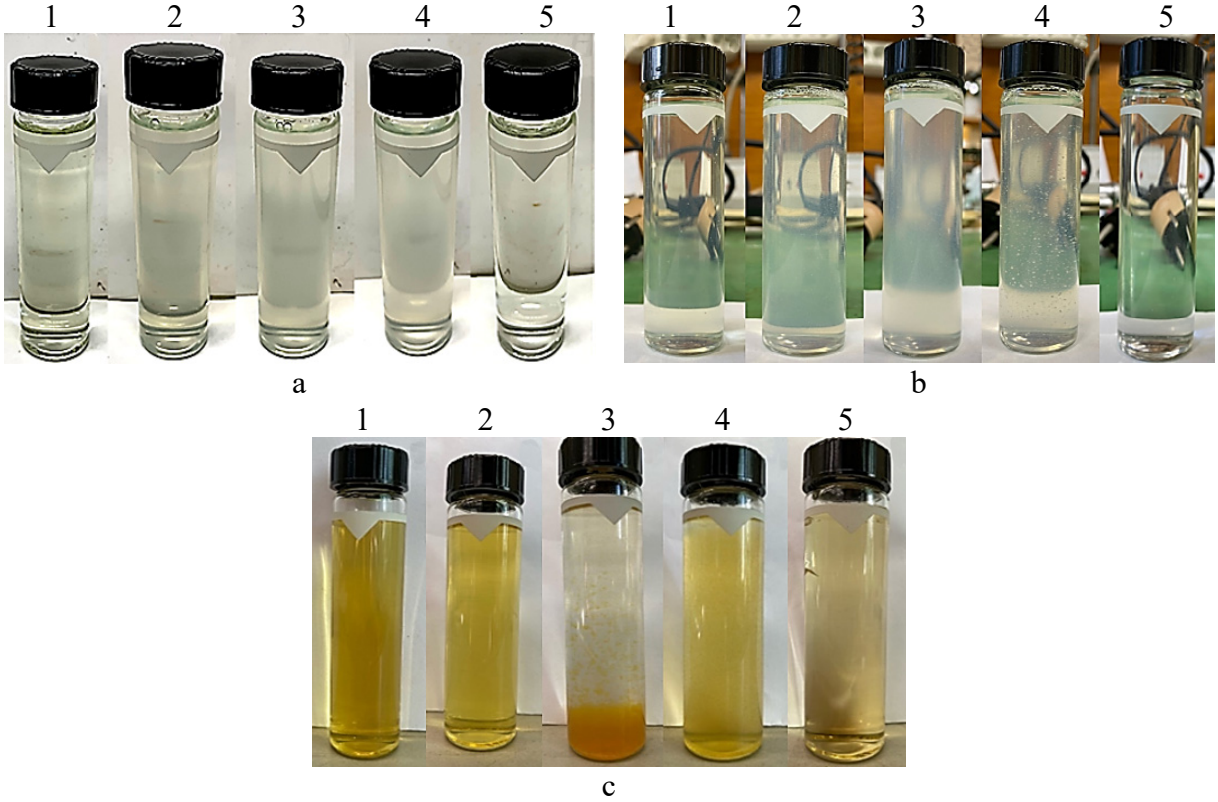

Fig. 1. The behavior of hydrosols (obtained based on different silver salts) at various pH . a – $AgNO_3$ -based CSS at pH : 1 – 2,57; 2 – 3,96; 3 – 5,61; 4 – 6,10; 5 – 7,26; b – $AgOOCCH_3$ -based CSS at pH : 1 – 3,96; 2 – 5,32; 3 – 6,36; 4 – 7,09; 5 – 9,01; c – $AgNO_2$ -based CSS at pH : 1 – 2,9; 2 – 3,18; 3 – 4,98; 4 – 5,91; 5 – 8,86.

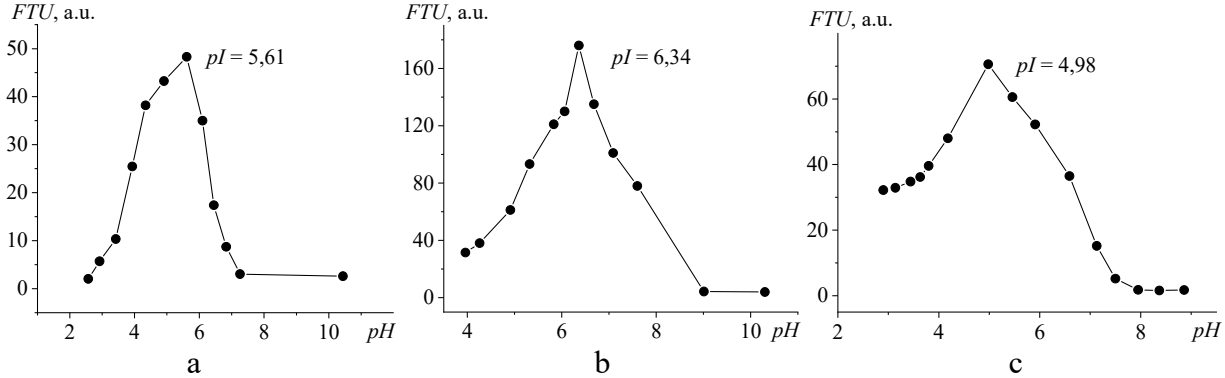

Fig. 2. Dependence of sols turbidity on pH : a – $AgNO_3$ -based CSS, b – $AgOOCCH_3$ -based CSS, c – $AgNO_2$ -based CSS.

The behavior of the systems, together with preliminary experimental data and computer simulation results [18, 23, 24], supports our assumptions regarding the nanoparticle structure: amino and carboxyl groups are located on the particle surface. In other words, the system behaves as a protein-like polyampholyte and should therefore have an isoelectric point at which the total particle charge is zero. In the native state, under acidic conditions ($pH < pI$), the

colloidal stability of the systems is governed by fully protonated amino groups of the cysteine residues. Electroneutrality is then reached at $pH = pI$, whereas at $pH > pI$ both amino and carboxyl groups become deprotonated.

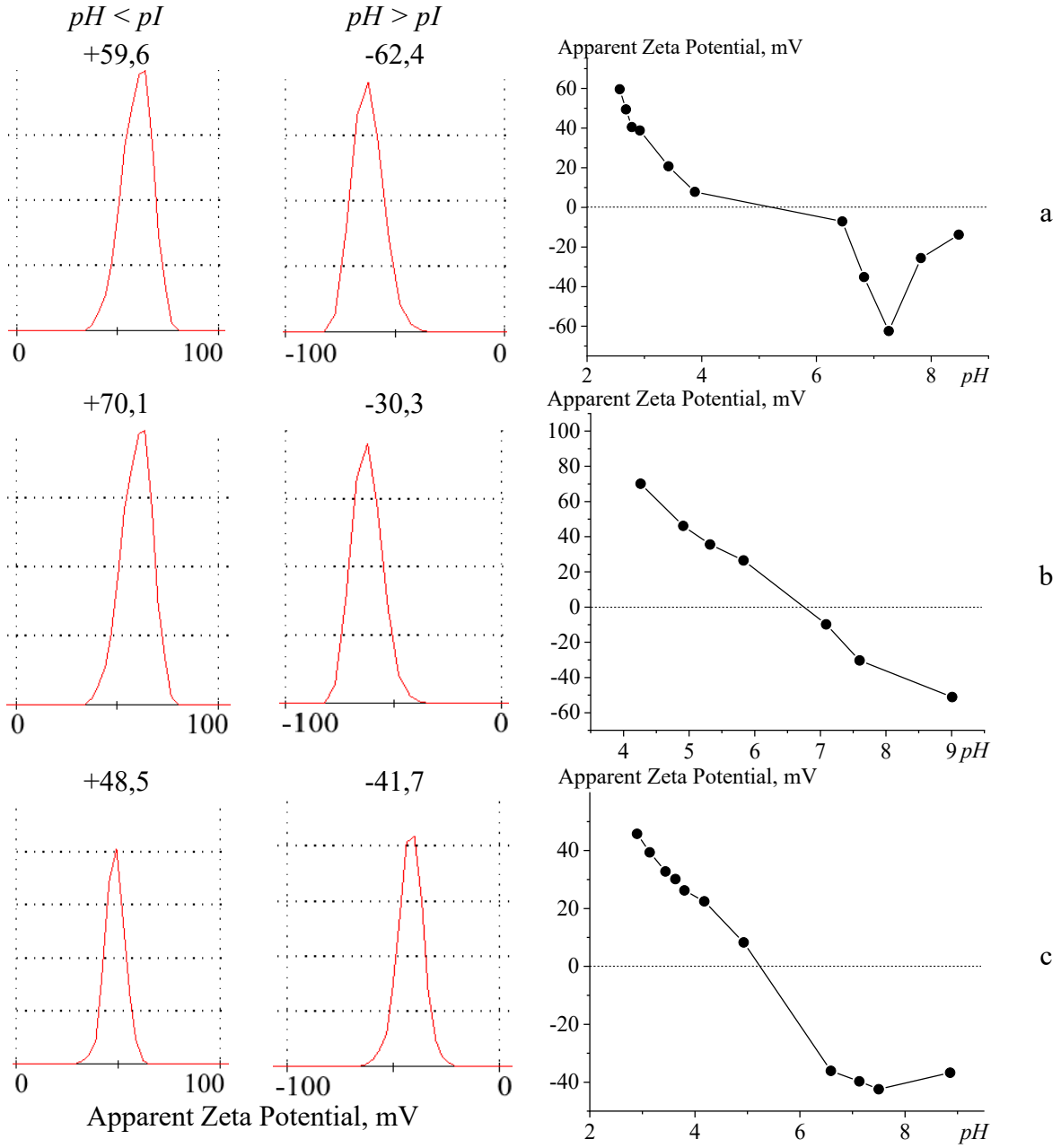

Fig. 3. Zeta-potential distributions (left) and its dependence on pH (right) for hydrosols: a – $AgNO_3$-based CSS, b – $AgOOCCH_3$-based CSS, c – $AgNO_2$-based CSS.

Electrophoretic light scattering data (see fig. 3) confirmed the turbidimetry results: in the native state, the nanoparticles have a positive surface charge. The system undergoes charge reversal upon passing through the isoelectric point. The unimodal zeta-potential distribution of the nanoparticles indicates their aggregative stability. The obtained results are fully consistent with the visual observations shown in fig. 1.

Taken together, the above data suggest the following surface structures of CSS nanoparticles, depending on the chemical nature of the initial silver salt:

– $AgNO_2$-based CSS has the lowest pI value. This can be explained by the synthesis history: as shown previously [25], diazotization occurs in this case. As a result, some of the protonated amino groups are converted into neutral hydroxyl groups (see fig. 4 c).

– In $AgNO_3$-based CSS, all surface groups remain unchanged; consequently, the pI of this system is shifted toward the more alkaline region (see fig. 4 a).

– $AgOOCCH_3$-based CSS has the highest pI value. This is probably due to the strong interaction between the protonated form of the carboxylate ion and the nanoparticle surface. As a result, the hydroxide ions added to the system are partially consumed in breaking these bonds and neutralizing this form (see fig. 4 b).

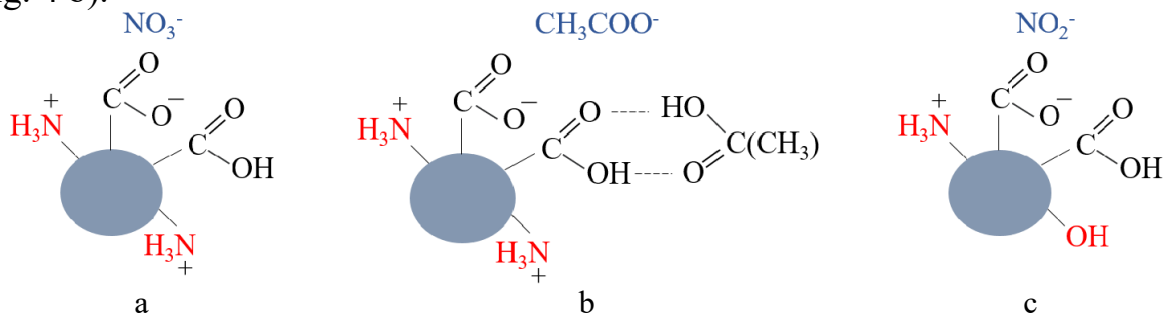

Fig. 4. The proposed surface structure of nanoparticles of hydrosols: a – $AgNO_3$-based CSS, b – $AgOOCCH_3$-based CSS, c – $AgNO_2$-based CSS.

5. Conclusions

In this work, we have shown that, regardless of the chemical nature of silver salts used as one of the components for cysteine–silver sol synthesis, the resulting systems behave as protein-like polyampholytes, as evidenced by the presence of an isoelectric point. Visual observations, supported by turbidimetric titration and zeta-potential measurements, confirm this conclusion. Based on the isoelectric point values, the studied systems can be arranged in order of increasing shift toward the alkaline region as follows: $AgNO_2$-based CSS ($pI = 4{,}98$), $AgNO_3$-based CSS ($pI = 5{,}61$), and $AgOOCCH_3$-based CSS ($pI = 6{,}34$). By combining previously obtained results with the data from the present study, we propose surface structures for the nanoparticles forming the corresponding sols.

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