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Behavior and bioactive properties of aqueous L-cysteine–$AgNO_3$ solution at different pH

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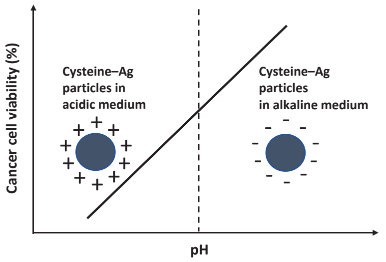
Polypeptide-like supramolecular aggregates are formed in aqueous cysteine–$AgNO_3$ solution. At low pH, their positively charged shell consists of several layers of cysteine–Ag^+ complexes held by hydrogen bonds; with an increase in pH, the particles aggregate at isoelectric point; while at higher pH, the shell acquires negative charge and starts being destroyed. The pH control has been demonstrated for cytotoxic effect of the solution against MCF-7 breast cancer cells.



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Self-assembly of low molecular weight gelators (LMWGs) has been explored in its fundamental and applied aspects.[1] Bioactivity and biocompatibility of amino acids and their derivatives influence their LMWG properties.[2–11] Supramolecular network gels originate from sulfur-containing amino acids, such as glutathione (GSH), *N*-acetyl-L-cysteine (NAC) or L-cysteine (CYS), and salts of metals like Cu, Au or Ag due to reactivity of the thiol group. GSH- and NAC-based gels were investigated by the team of I. Odriozola,[12–15] while our group examined the gels of this type formed from aqueous solutions of CYS and various Ag salts.[16–19]

In this work, we used the CYS–silver (CSS) aqueous solution as a gel precursor to clarify structure of the aggregates formed and their effect on MCF-7 cancer cells. For this purpose, we added various amounts of KOH to the initial CSS solution (Figure 1) and analyzed the system by physicochemical methods (for details, see Online Supplementary Materials). In our works[17–19] we demonstrated that the aggregates of CSS contained a core of cystine-stabilized Ag nanoparticles (AgNPs) and a shell of CYS–Ag^+ complexes [Figure 1(*g*)]. We managed to prove and verify the structure of the core, but there remained questions about the shell. The aggregates represent zwitterions formed by carboxyl and amino groups, which are responsible for solubility of the particles. Thus, one can expect that the change in pH should influence the aggregate structure, the system colloidal stability and properties of CSS.

Addition of KOH to the initial CSS solution (pH 2.57) led to a progressive change in its appearance from a transparent light yellowish green solution to a transparent colorless one [Figure 1(*a*)–(*c*)]. Note that the AgOH/Ag_2O precipitate formation was observed after the same manipulations with an aqueous solution of $AgNO_3$ [Figure 1(*d*)–(*f*)]. One can propose that the carboxyl and amino groups protect Ag atoms from interaction with hydroxyl ions, while the color change may originate from transformation of the CYS–Ag^+ complexes and AgNPs.

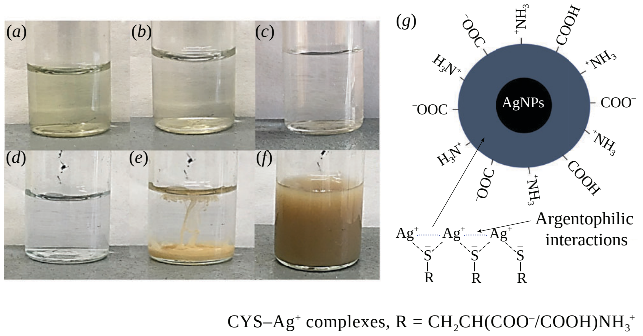

Figure 1 (*a*) Initial aq. CSS solution; (*b*),(*c*) result of gradual addition of KOH to CSS; (*d*) aq. $AgNO_3$ solution; (*e*),(*f*) result of gradual addition of KOH to $AgNO_3$; and (*g*) structure proposed for the aggregates in CSS solution according to our work.[19]

According to the proposed structure of aggregates [Figure 1(*g*)] and the visual observations above, the system behaves as a polypeptide and, therefore, like a polyelectrolyte should possess an isoelectric point. Indeed, turbidimetric titration of the initial CSS solution (Figure 2) demonstrated an initial increase in turbidity with growth of pH as well as a maximum at pH 5.5 followed by a decline. The ζ-potential measurement verified these data (Figure S1, Online Supplementary Materials). Aggregates in the CSS solution are positively charged in acidic medium and acquire a negative charge in the alkaline region. Their ζ-potential tends to zero at isoelectric point and after pH 7.26 with a minimum value of –62.4 mV it starts growing towards zero.

The UV spectra of the systems (Figure 3) reveal two characteristic absorption bands at ~310 and ~390 nm corresponding to Ag–Ag interactions[20] in CYS–Ag^+ complexes and the local plasmon resonance of AgNPs, respectively.[17–19] Their intensity gradually diminishes with an increase in pH. Near the isoelectric

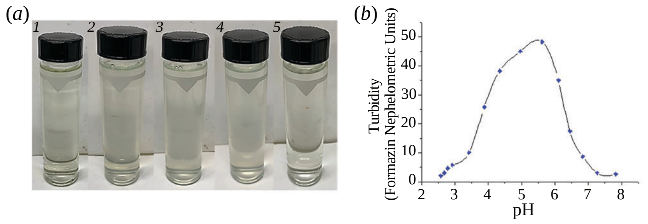

Figure 2 (*a*) CSS solution at pH (*1*) 2.57, (*2*) 3.96, (*3*) 5.61, (*4*) 6.10 and (*5*) 7.26. (*b*) The CSS solution turbidity *vs.* pH.

point the intensities are minimal as expected, while in alkaline region the bands are completely smoothed out, probably due to (i) suppression of the Ag–Ag interactions and further destruction of the shell as well as (ii) the AgNPs plasmon resonance band disappearance originating from the nanoparticles core aggregation.[21] In the particle size distribution (Figure S2), a significant drop in the size occurs near the isoelectric point corresponding to more negative values of ζ-potential. Presumably, at the minimal aggregates size the linear charge density on their surface increases. Besides, there is a sharp growth of the particle size in alkaline region, which explains a decrease in the absolute negative values of ζ-potential and its tending to zero.

AFM investigation of the system (Figure S3) reveals uniform aggregates with spherical/elliptical shape, the particles start to associate with an increase in pH from acidic to alkaline.

Thus, the following mechanism of CSS aggregates transformations with the change in pH can be proposed. In acidic medium the particles have colloidal stability since their surface has a positive charge due to protonation of amino groups and partially the carboxyl ones, while hydrogen bonds formation between these groups keeps the stability of CYS–Ag^+ complexes. Addition of an alkali shifts pH of the system to its isoelectric point where the particles aggregate, after that their surface acquires a negative charge due to deprotonation of the amino and carboxyl groups. At this point the aggregate shell starts being destroyed due to repulsion of the negatively charged carboxyl groups. The shell in initial CSS constructs has several layers of CYS–Ag^+ complexes, which interact through hydrogen bonds between positively charged amino groups and negatively charged/protonated carboxyl groups, according to our hypothesis.[19]

Cytotoxicity of the CSS solution was explored using the MTT test. CSS has pronounced toxic effect against MCF-7 breast carcinoma cells and no toxicity to normal fibroblasts (Figure 4). MCF-7 cell death rate decreases with an increase in pH. It is known that the membrane charge of cancer cells is more negative compared with the normal ones. Aggregates of CSS at low pH have a positive charge, therefore, their interaction with the membrane during cellular capture is stronger than for the aggregates bearing negative charge at alkaline pH. The particle size has a weak influence on the cancer cell inhibition. The CSS system can also be effective against A549 lung carcinoma cells.[22]

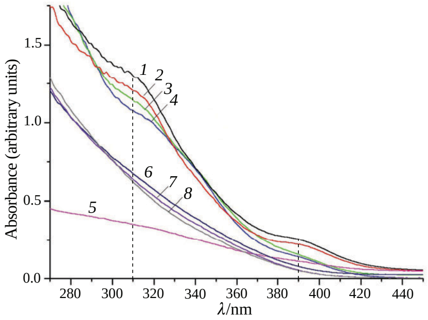

Figure 3 UV spectra of CSS solution at pH (*1*) 2.57, (*2*) 2.92, (*3*) 3.48, (*4*) 3.96, (*5*) 4.34–6.10, (*6*) 6.83, (*7*) 7.26 and (*8*) 8.48.

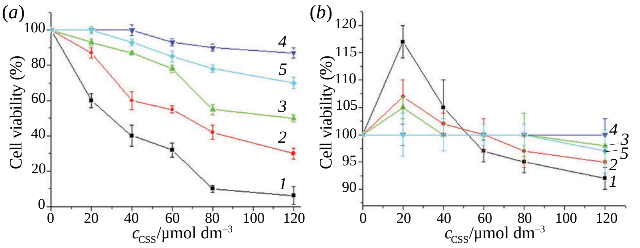

Figure 4 Cytotoxicity of CSS solution to (*a*) MCF-7 cancer cells as well as (*b*) WI-38 normal fibroblasts at pH (*1*) 2.57, (*2*) 3.48, (*3*) 6.83, (*4*) 7.26 and (*5*) 8.48, results of the MTT test.

In summary, mixing an aqueous solution of CYS with silver nitrate led to the formation of polypeptide-like aggregates consisting of an AgNPs core and a CYS–Ag^+ complexes shell. At low pH, the positively charged shell consists of several layers of CYS–Ag^+ complexes held by hydrogen bonds, under these conditions the CSS solution has colloidal stability. At the isoelectric point the particles aggregate, while at higher pH the shell acquires negative charge and starts being destroyed. This behavior allows one to control toxic effect of the CSS solution to cancer cells.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.04.042.

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