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Proceeding Paper

pH Responsive Tunable Plasmonic Resonators Based on Gold-Polysaccharide Nanocomposites †

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Abstract: The development of advanced compositions that combine high stability and tunable activity is a forefront trend in modern interdisciplinary science. This important scientific problem is solved by the formation of plasmonic nanostructures embedded in the structure of the adaptive organic matrix of natural polysaccharides. This makes it possible to obtain unique materials with the possibility of optimization for specific tasks both at the stage of synthesis and when used due to the excitation of plasmon resonance of inorganic architecture by external light. We report the results of the study of physicochemical and structural features of composites depending on trigger factors (i.e., pH), the change of which leads to conformational transformations of macromolecules. Considering a nanocomposite as molecular nanobot, "…autonomic preprogrammed structure of atomic level…", opens the way to design an adaptive natural nanomachine, the properties of which can be controlled by external influences.

Keywords: polysaccharide; nanocomposite; nanoparticle; local plasmon resonance; pH-responsive structure

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1. Introduction

Gold nanoparticles (AuNP) are among the most widely used noble metal nanobodies due to their numerous and well-characterized surface functionalities, as well as the manifestation of tunable by local environment Local Surface Plasmon Resonance (LSPR) effects [1]. AuNPs can be combined with various biomolecules to form nano-biological assemblies, such as oligonucleotides, antibodies, enzymes, and other proteins, to extend or enhance their functionality [2]. The immobilization of biomolecules on the AuNP surface changes the LSPR excitation conditions, electrical, optical, and chemical (such as the occurrence of redox reactions) properties of the system as a whole. Biomolecules can also act as active components in the process of nanoparticle synthesis: they can be both reducing and stabilizing agents for AuNP synthesis. In other words, biomolecules are capable of both reducing Au(III) ions to zero-valent gold and stabilizing the emerging metal phase by forming an organic coat on the outer surface of NPs [3]. One class of such multifunctional biomolecules are polysaccharides, - the monosaccharides that are linked together by glycosidic bonds. It has been shown that polysaccharides can serve as reagents in the reduction of Au(III) ions to the metallic state [4].

AuNPs have the ability to bind to other materials through bonds that are responsive to external conditions or triggers. These factors, which may affect binding affinity, electrostatic or hydrophobic interactions, may control the strength of non-covalent attachment of specific functional entities to AuNPs. This allows modifications to AuNPs that are suitable for drug delivery because they require easy drug release upon reaching the target.

For example, it was shown that the rate of release of drugs depends on the pH of the medium - it increased with a decrease in the pH level [5]. This study demonstrated the possibility of developing smart carriers, where the amount of drug release is minimal during delivery (pH 7.2), and when the tumor area is reached (pH 4.5–6.5), the drug release rate increases dramatically. It is interesting to note that pH dependent processes are characteristic not only for complexes of nanostructures with organic structures [6], but are also observed for nonorganic polymer matrices, namely Carbon Nanotubes (CNTs) and Halloysite Nanotubes (HNTs) [7-9]. Those studies demonstrate a large class of pH-dependent processes involving nano-biological assemblies, which stimulate further research in this area.

In addition to practical interest in obtaining new materials with unique properties, the combination of nanostructures with macromolecules opens the way for the development of new methodological approaches for analyzing the structure and properties of such complex objects. Indeed, the dissolution of a biopolymer with ionogenic groups in an aqueous medium leads to the formation of various conformations of the macromolecule, which significantly depend, in particular, on the pH value [10]. Due to the large size, complex and labile structure of such macro-objects, instrumental tools for studying their spatial conformation are extremely limited. The use of LSPR of a gold nanoparticles embedded in a macromolecular structure can be an extremely useful tool for studying and, in fact, monitoring the conformational changes of a macromolecule in an aqueous solution.

The features of the manifestation of the effects of LSPR of gold nanoparticles embedded in labile organic matrices has not been studied in detail due to the complexity of the problem and the ambiguity in the interpretation of the results obtained. In this work, we studied the simultaneous influence of several factors accompanying the procedure of changing the pH level on the features of the manifestation of LSPR effects in such systems. We consider how the characteristics of LSPR change during successive cycles of changing the pH level (from 2 to 11 and vice versa) for the same sample (i.e. acid, then alkali, etc. are added to the initial sample without replacing it with a new one). A feature of this process is that with an increase in the number of cycles, the ionic strength of the solution increases, since the concentration of ions formed during the dissociation of hydrochloric acid (used to lower the pH level) and sodium hydroxide (used to increase the pH level) increases with each cycle. Thus, the aim of this work is to elucidate the role of ionic strength and the presence of sodium and chlorine ions potentially interacting with the components of the bio-nano-assembly on the nature of the pH dependence of the LSPR band. Establishing the features of this process will make it possible, in particular, to develop the basis for adequate procedures for the analysis of conformational changes in macromolecules using built-in probes based on plasmonic nanoparticles of noble metals.

In this work, we studied biocomposites of gold nanoparticles grown inside a polysaccharide matrix; polysaccharide glucuronoxylomannan (GXM), known by its' antiviral activity [11] extracted from the yellow brain fungus *Tremella mesenterica*, was used both as a reducing and stabilizing agent in the formation of Au NPs [12].

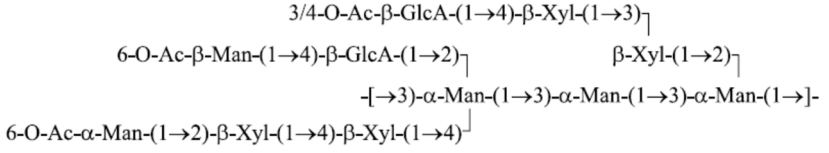

Figure 1. Structure of GXM.

GXM consists of a linear backbone (1 → 3)-linked α-D-mannose with mainly xylose and glucuronic acid in the side chains (Fig.1). Glucuronic acid contains a functional carboxylic acid, which at certain pH levels can stimulate the agglomeration of various polysaccharide assemblies due to the formation of intermolecular hydrogen bonds. The carboxyl group

in the sugar backbone affects the intramolecular structure of the biocomposite, i.e. determines the conformation of individual polysaccharide macromolecules in an aqueous solution.

2. Materials and Methods

2.1. Reagents

Tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$) was purchased from Sigma-Aldrich. Glucuronoxylomannan (GXM) was separated from the culture liquid of the *Tremella mesenterica Ritz. Fr.* (*Heterobasidiaceae*) *fungus* using the published procedure [13]. The water used in all the experiments was double distilled and deionized. All reagents were used as purchased without further purification.

2.2. Synthesis of AuNPs

First, GXM water solution was prepared by dissolution of 3 mg GXM in 2.9 ml H_2O, and then aqueous solution of $HAuCl_4$ (0.1 mL, 30 mM) was added at violent stirring. The mixture was stirred during 1 minute at room temperature, heated to 100 °C with boiling during 10 min.

2.3. Instrumentation and Measurements

The morphological property was characterized by transmission electron microscopy (TEM, JEM JEOL-1011, Japan, accelerated voltage 100kV). The optical measurements were performed using UV-Vis spectrometer (Umico UV-Vis). The spectra were collected over a range of 200–1100 nm.

3. Results

Morphological features of freshly prepared AuNP nanoparticles in a biopolymer polysaccharide matrix, obtained using a transmission electron microscope, are shown in Fig. 2. Individual nanoparticles are characterized by a spherical geometry with a typical size ranging from 10 to 20 nm; most of the nanoparticles are separated from each other. The images are characterized by isolated groups of NPs with a size of about 100 nm × 500 nm, which may correspond to nanoparticles located inside the organic matrix of one or more polysaccharides - however, this observation requires additional special studies.

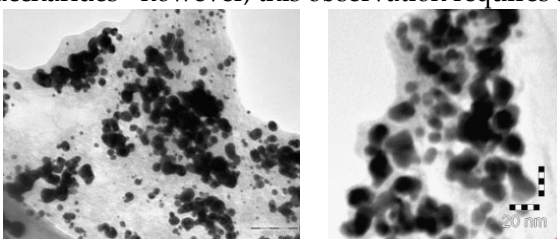

Figure 2. TEM image of Au nanoparticles embedded in glycan matrix.

The absorption spectrum of the biocomposite in water is characterized by the LSPR band typical for individual gold nanoparticles in solutions (Figure 3). The position of the absorption band with a maximum at about 550 nm indicates a dense organic environment of the metal core, which shifts the resonance conditions for gold nanoparticles 10–20 nm in size to lower energies. This fact confirms the assumption that gold NPs are formed inside the biopolymer matrix of the polysaccharide.

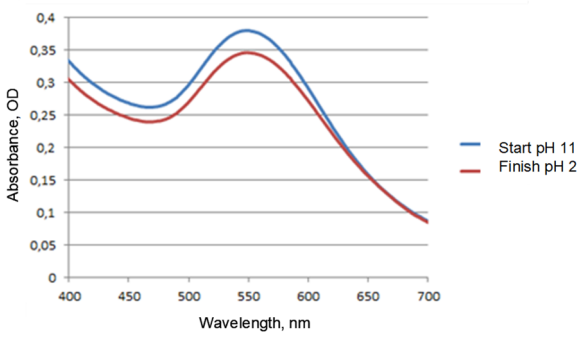

Figure 3. The LSPR absorption bands at ***the acidification cycle 1.***

The 1 mL of the stock solution was subjected to successive acidification-alkalinization processes (from acidification 1, alkalization 1, acidification 2, alkalization 2 to acidification 3) by sequentially adding a certain volume of 1 M HCl solution (acidification) and 1 M NaOH solution (alkalinization), respectively. Since the observed changes in the absorption spectra are characterized by irreversible changes, let us consider the changes typical for each of the cycles carried out.

Figure 3 shows the absorption spectra of the biocomposite at the first decrease in pH to a value of about 1, when all groups in the macromolecule are protonated and electrostatic interactions do not prevent the formation of a dense globule. Taking into account the dilution effect caused by the addition of 100 μL of 1M HCl to 1 ml of the initial biocomposite solution, it can be concluded that a decrease in pH does not actually lead to any significant changes in the relative position of gold nanoparticles inside the polysaccharide matrix. Moreover, lowering the pH level below 5–6 also has no effect on the aggregation of biocomposite macromolecules.

In contrast to acidification, alkalization of the solution to a pH level of 10–11 leads to a significant change in the shape of the spectrum - the LSPR band shifts to the region of

high energies (up to 535 nm), its half-width decreases, significantly lowering the absorption in the region of 600–700 nm, which is typical for absorption by ensembles of nanoparticles (Fig.4). This behavior may be associated with the unfolding of polysaccharide molecules, as a result of which the thickness of the organic coating decreases and the solvent can come closer to the metal surface, and the nanoparticles, respectively, disperse over long distances relative to each other.

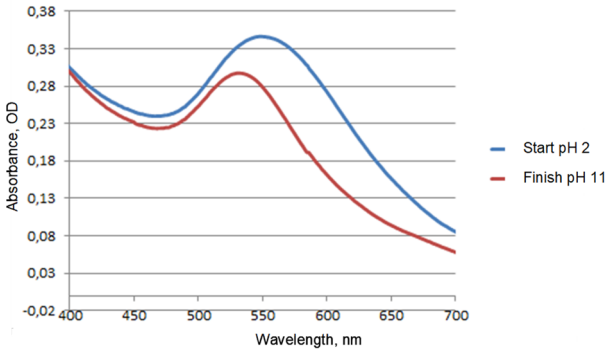

Figure 4. The LSPR absorption band at ***the alkalization cycle 1.***

Subsequent acidification leads to the appearance of a very weak band in the region of 600 nm observed during the first cycle, despite the fact that the main LSPR band does not change its position (535 nm) (Figure 5). This behavior indicates that, with a decrease in pH in the system, some densification is observed in the arrangement of nanoparticles, probably inside individual polysaccharide macromolecules, which manifests itself in an increase in the absorption of the band at 650 nm.

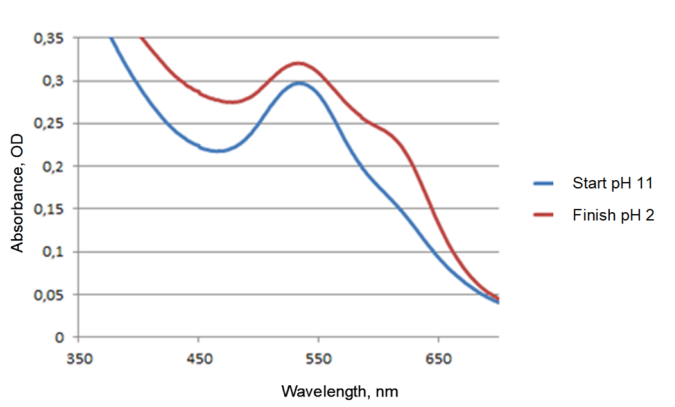

Figure 5. The LSPR absorption band at ***the acidification cycle 2.***

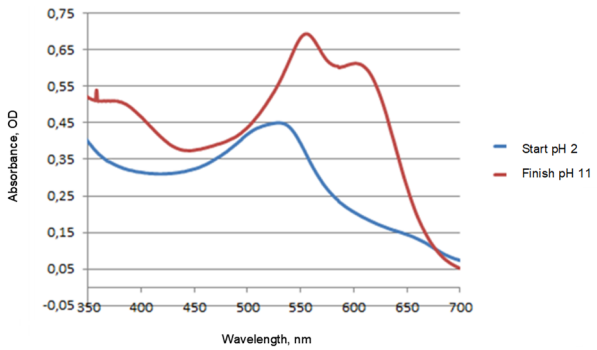

Figure 6. The LSPR absorption band at ***the alkalization cycle 2.***

A qualitatively new picture is observed during subsequent alkalization in the second cycle. Indeed, when pH~9 is reached, the previously observed absorption spectrum changes abruptly: the total absorption sharply increases, the main LSPR band shifts again to 550 nm (like initial solution in water), and the intensity of the band at 600 nm almost equals in intensity with the LSPR band of single gold nanoparticles (Fig.6). This pattern is qualitatively preserved with a further increase in pH up to 11. Moreover, a similar pattern with a sharp change in the spectrum at pH around 3 is also observed with subsequent acidification (cycle 3) of the solution (Figure 7).

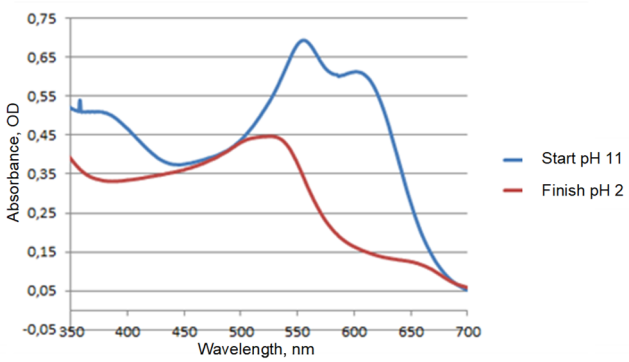

Figure 7. The LPR absorption band at ***the acidification cycle 3.***

4. Discussion and Conclusions

An analysis of the experimental results presented above on the nature of the change in the optical characteristics of the local plasmon resonance bands of gold nanoparticles embedded in a labile organic polysaccharide matrix allows us to draw the following main conclusions.

1) The synthesized biocomposite is a pH-sensitive structure, changes in the features of the local plasmon resonance spectrum of which are associated both with a change in the internal structure of the polysaccharide and with its ability to form multimolecular aggregates.

2) The initial solution of the biocomposite synthesized in water (at pH close to normal) is partially aggregated; this aggregation can be destroyed by increasing the pH above 8-9 when the linkage of polysaccharide chains of different macromolecules is broken and the macromolecule tends to realize a linear conformation.

3) Changes in the optical spectra of LSPR, and, accordingly, the conformation and nature of the intermolecular association of the biocomposite change during the first cycles of acidification-alkalinization. This is due to the process of self-organization of the internal structure of the polysaccharide under conditions of repeated cycles of folding-unfolding of the macromolecular globule. An increase in the ionic strength of the solution stimulates the process of achieving the optimal three-dimensional packing of chains by suppressing electrostatic interactions that prevent the implementation of the optimal conformation specified by the primary structure of the macromolecule.

4) After several cycles of acidification-alkalinization, a stable spatial configuration is achieved in which a reversible process of folding and unfolding of the biocomposite occurs when the pH level changes.

The results observed in this work are typical for polysaccharides isolated by alkaline extraction from extracts of *Heterobasidiaceae* mushrooms. It should be noted that the reason for the observed regularities can be not only the properties of the polysaccharide itself, but also other biocomponents released together with the main macromolecule in the process of isolation. The probable mechanisms of such processes will be considered by us in further studies.

Finally, gold nanoparticles embedded in polysaccharide chains are a useful tool for observing and studying the conformational features of a polysaccharide in aqueous solution. In addition, AuNP makes it possible to determine transition points from one conformation to another. These considerations open the way to the creation of an adaptive natural nanomachine whose properties can be controlled by external influences.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

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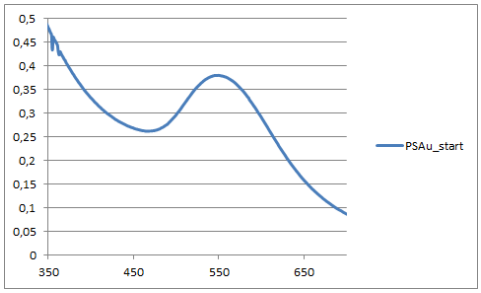
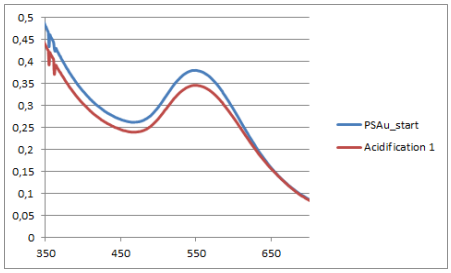
Support Information

SI 1

Starting Position	Acidification 1
	
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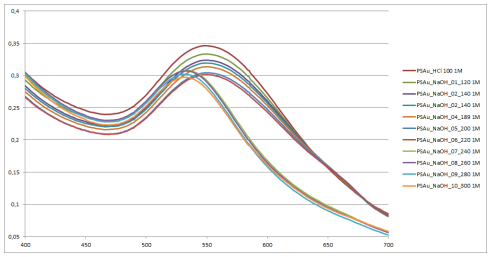	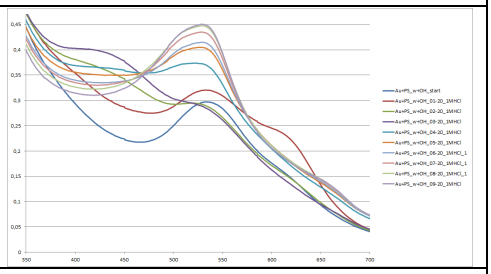
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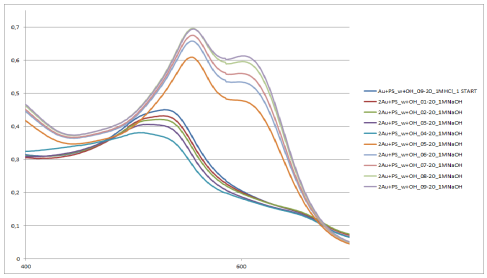	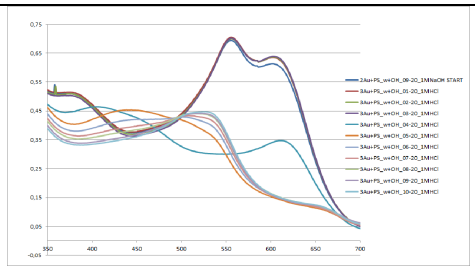

SI 2

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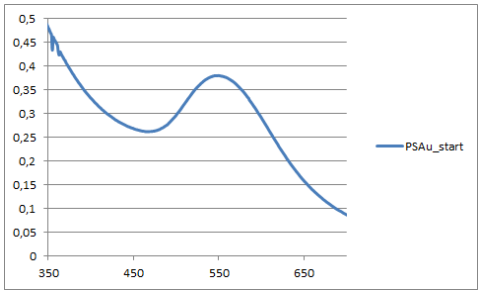	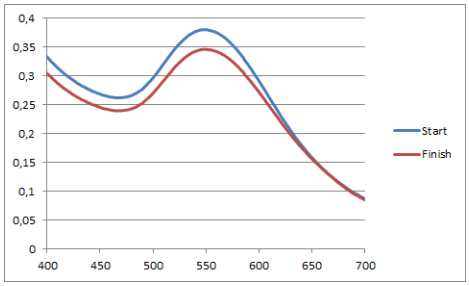
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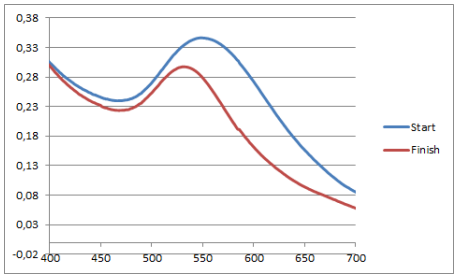	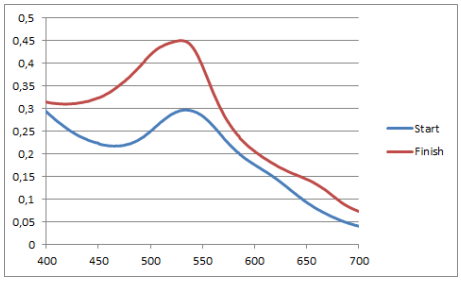
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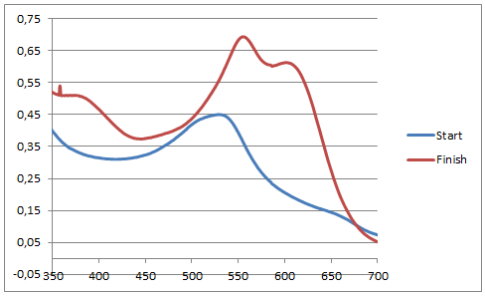	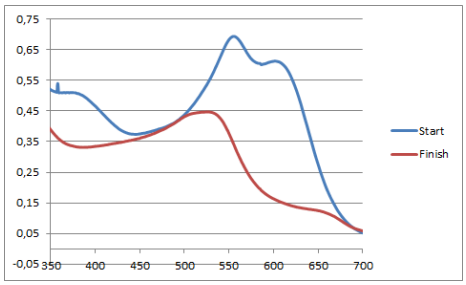

SI 3

Acidification 1-3	Alkalization 1-2