SYNTHESIS OF SILVER NANOPARTICLES WITH GREEN TEA-EXTRACTED REDUCTANTS: A PRELIMINARY STUDY FOR OPTIMIZATION OF THE PREPARATION TECHNIQUE

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ABSTRACT

INTRODUCTION: The current research article is focused on the optimization of the preparation technique used for the "green" synthesis of silver nanoparticles.

AIM: A primary goal of the study was to define and adjust critical variables in the production process so that maximally small, pure, stable in solution, and eligible for future drug conjunction silver nanoparticles would form.

MATERIALS AND METHODS: Silver nanoparticles were obtained by reducing silver nitrate (1 mM and 10 mM solutions) with Camellia sinensis (green tea)-derived catechins or total green tea aqueous extract in varying concentrations and pH. The samples' visual appearance and short-term physical stability were observed within 7 days. The formation of nanosilver suspensions was established and semi-quantified by UV-Vis spectral analysis.

RESULTS AND DISCUSSION: The alkalization of the reducing agent (pH 8) before its utilization revealed potentiation in the silver nanoparticle synthesis and stability. Among all formulations, samples obtained with alkalized purified catechin solution in 1 mM of silver nitrate showed maximally sharped absorbance peaks at 417–424 nm, minimal excess of unattached bioactive compounds, and no signs of turbidity for the suggested test period.

CONCLUSION: These samples were considered the most promising and suitable for further investigation, processing, conjunction, and potential therapeutic application.

Keywords: *silver nanoparticles, green synthesis, green tea extract, green tea catechins*

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INTRODUCTION

When particular metals, such as silver, are in their nano-sized state, they acquire highly differential functionality owing to the critically increased active surface area (1). Nanosilver is a subject of growing interest in the fields of nanomedicine and drug delivery due to its antimicrobial, anticancer, and antioxidant properties (2–4). In recent years the focus has been directed to the so-called "green" meth-

ods of synthesis of silver nanoparticles (3–5). They are based on natural reducers (plant extracts, purified bioactive compounds derived from plants, yeast, bacteria, or fungi) and non-toxic solvents and satisfy the need for eco-friendly technology (6). Capping and stabilization of the silver nanoparticles in suspension often occurs during the process of reduction due to the lamination of the silver surface with natural high-molecular-weight substrates present in the extracts (such as polysaccharides, proteins, glycoproteins, and lipoproteins) (3). The thus achieved surface functionalization leads to changes in the optical, morphological, and pharmacological properties of the silver nanoparticles (7,8) and therefore is a subject of optimization for the intended purpose. The use of purified herbal preparations or pure bioactive compounds rather than total extracts might be preferred in particular cases when controlled, predictable, and assessable functionalization and conjunction with targeted molecules is aimed.

The surface plasmon resonance (SPR) is a characteristic optical property of the silver nanoparticles owed to a resonant oscillation of electrons on the surface upon irradiation with visible and near-infrared light (9). This electromagnetic phenomenon is routinely monitored by UV-Vis spectroscopy; the manifestation of an intense peak/s in the violet-blue sector of the visible spectrum is considered indicative of the presence of nanosilver (3). The geometry of the resultant absorbance bands (symmetry, width, intensity, multiplicity, etc.) is well-related to the silver nanoparticles' morphology and the tendency for aggregation (10,11).

Camellia sinensis, known commercially as the tea plant, is an evergreen shrub or a small tree of the *Theaceae* family, native to the Asian continent but widely cultivated worldwide (12). The leaves, being a main source for the production of different types of tea, are rich in polyphenols (flavan-3-ols, flavonols, phenolic acids, condensed tannins, hydrolyzable tannins), purine alkaloids, and saponins (13). Among them, the flavan-3-ols (catechins), namely epigallocatechin, epigallocatechin-3-gallate, epicatechin, and epicatechin-3-gallate, are of highest importance for the reduction (antioxidant) properties of the green tea herbal preparations (14,15).

AIM

The present work aims to establish and optimize a simple and reliable technology for the preparation of silver nanoparticles with the aid of green tea-derived reducers by varying the type, concentration, and pH of the herbal preparation. This paper presents a preliminary study that relies on UV-Vis spectral screening and visual observation. In prospect, the optimized formulations are intended for drug conjunction, characterization, pharmacological and microbiological studies.

MATERIALS AND METHODS

Materials

Sencha green tea was supplied from a local drug store. Silver nitrate >99.9% and sodium hydroxide pellets >98% were purchased from Thermo Fisher Scientific, United Kingdom; all organic solvents were purchased from Sigma-Aldrich (USA) in analytical grade.

Methods

1. *Isolation of green tea catechins*

Catechins from *Sencha* green tea were isolated by an established and adapted for the purpose protocol (16). *Sencha* green tea leaves were extracted with methanol, and the obtained filtrate was evaporated in a water bath. To the dried extract were added 10 mL of water and sodium chloride 10%. The mixture was placed in a separating funnel with 10 mL of ethyl acetate, and the combined ethyl acetate extracts were washed twice with water. After evaporation of the organic solvent, a mixture of catechins was obtained. Before use, aqueous solutions of catechins 0.6 mg/mL were prepared on a magnet stirrer.

2. *Preparation of green tea total aqueous extract*

A total of 10.0 g of *Sencha* green tea were added and stirred into 500.0 mL of pre-heated up to 90°C distilled water. The extraction was allowed at the same temperature for 20 minutes. The mixture, while hot, was filtered through a cotton and gauze filter and cooled down to room temperature. Thereafter, additional extract purification was carried out by filtrating through Whatman filter paper (medium-flow). The as-prepared extract was used within 24 hours after preparation.

3. *Synthesis of silver nanoparticles*

Silver nitrate ($AgNO_3$) solutions (1 mM and 10 mM) were prepared by dissolving the salt into distilled water under manual stirring at room temperature. Two types of reductants (R) were used for the synthesis—total aqueous extract (E) or purified catechin aqueous solution, 0.6 mg/mL (C). Each reductant was applied unadjusted or alkalized in advance to pH 8.0 with the aid of NaOH 10% solution (reductants E8 and C8, respectively). The reaction vials were filled with 3.0 mL of silver nitrate solution (1 mM or 10 mM). The reducer was droplet-wise added at varying volumes (0.1 mL, 0.5 mL or 1.0 mL) and at room temperature (detailed composition of the samples is provided in Table 1). The mixtures were sealed and briefly shaken to homogenize. The vials were left to rest under dark conditions and observed for visual changes at chosen time intervals.

4. Visual observation

The samples were macroscopically observed for change in color, the occurrence of opalescence, turbidity, or phase separation. Photographs were taken right after the reductant addition, then each hour for the first 3 hours, on the 24th hour, and on day 7 after preparation.

5. UV-Vis spectroscopy

UV-Vis scans of the samples were carried on T60 UV-Vis spectrophotometer (PG Instruments, United Kingdom), in the spectral range from 200 to 800 nm; the spectral analysis was performed on the 1st hour after preparation, on the 24th hour after preparation, and on day 7 after preparation. Appropriate dilution (1:5) was carried on all specimens in advance. Pure catechin solution and total green tea extract were also scanned for reference in the same spectral interval. UVWin 6.0 software was used for data curation and processing.

RESULTS

1. Macroscopic observation

A total of 24 formulations were prepared: silver nitrate solutions—1 mM or 10 mM concentration (x2), reduced by catechin solution or total green tea extract (x2), used in an unadjusted or alkalized state (pH8) (x2), each applied in increasing volumes at three levels—0.1 mL, 0.5 mL, and 1.0 mL (x3). The experimental models are presented with codes, which, for convenience, will be clarified in advance: 1) the code consist of 3 symbols; 2) the first symbol is a number and indicates the silver nitrate concentration—samples obtained in 1 mM solutions start with **2**, while samples obtained in 10 mM solutions—with **3**; 3) the second symbol denotes the type of reducer used: non-alkalized total green tea extract—**E**; alkalized total green tea extract—**E8**; non-alkalized catechin solution—**C**; alkalized catechin solution—**C8**; the last number indicates the volume of the reducer applied—**R1** corresponds to 0.1 mL, **R2**—to 0.5 mL; **R3**—to 1.0 mL.

Example: formulation 2C8R2 is prepared in 1 mM silver nitrate solution with 0.5 mL alkalized catechin solution.

An almost immediate change in color to the reddish-brown scale (within minutes to an hour) was observed for most samples obtained in 1 mM and 10 mM solutions and indicated nanosilver formation; an exception made the samples reduced with non-alkalized catechin solution (C). In the latter case, a visible color change was not observed until the 24th hour post-synthesis and even later on, further substantial progress was not recorded. With time, all samples obtained in 10 mM silver nitrate solution (again, with the exclusion of the ones reduced with non-alkalized catechin solution—C) showed a tendency for turbidity, followed by the formation of a slurry and phase separation. In addition, the samples obtained with non-alkalized total green tea extract (E) turned grey soon after preparation and resembled a coarse silver dispersion. Turbid samples with grey-brownish color were also formed at the lower silver nitrate concentration (1 mM) after 24 hours, but only when non-alkalized total green tea extract (E) was used as a reducer. The best and most endurable appearance (transparent or semi-transparent, orange-to-reddish-brown colored solutions) was recognized for samples obtained in 1 mM silver nitrate solutions and reduced with either alkalized total green tea extract (E8) or alkalized catechin solution (C8).

Each destructive process (e.g., turbidity, phase separation) demonstrated acceleration by increasing the reductant volume. For samples that remained stable (1 mM silver nitrate samples reduced with E8 or C8), the higher the volume of the reducer, the darker the coloration of the solution.

After showing signs of turbidity and/or phase separation, physically unstable samples were not fur-

ther subjected to UV-Vis spectroscopy. Samples that showed a negligent formation of silver nanoparticles within 24 hours (all samples obtained with non-alkalized catechin solution—C) were also not investigated further. Their visual appearance and change with time can be followed in Fig. 1. All other samples' photographs are shown along with their UV-Vis spectra in the next sub-section.

2. UV-Vis spectral analysis

The UV-Vis spectra of pure catechins and green tea total aqueous extract showed a similar absor-

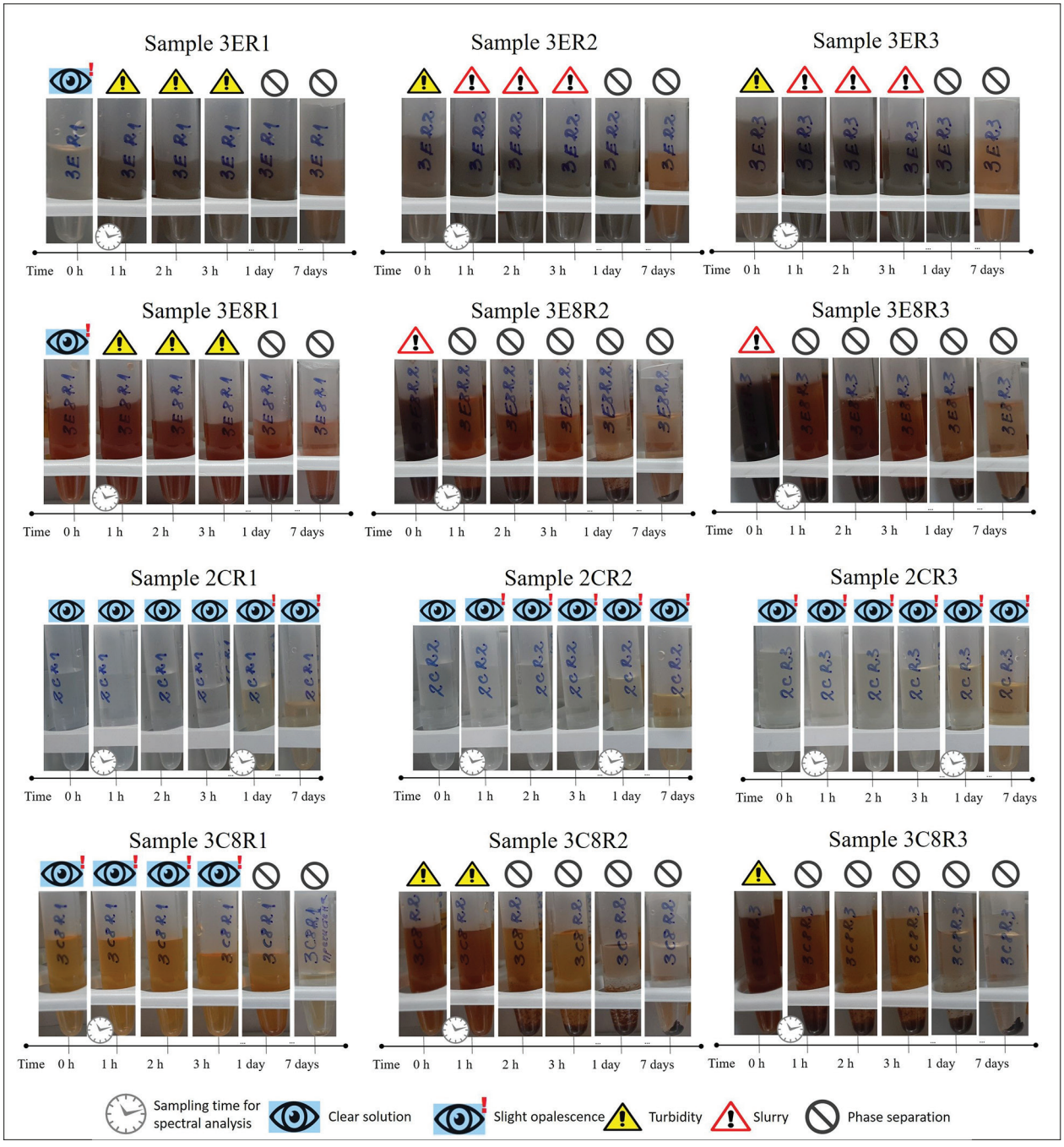


Fig. 1. Visual changes of samples that did not demonstrate substantial physical endurance or resulted in low concentrations of silver nanoparticles and were, therefore, not spectroscopically analyzed from the beginning to the end of the experiment

bance pattern with a maximum at around 273–277 nm (Fig. 2).

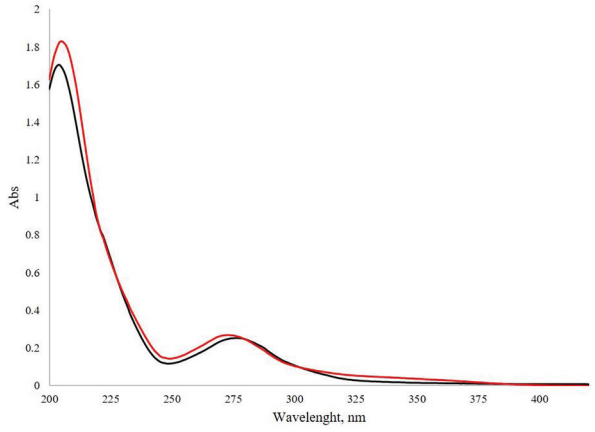


Fig. 2. UV-Vis spectra of total green tea aqueous extract (red line) and catechins in an aqueous solution (black line)

The spectral data obtained from all samples is summarized in Table 1. The intensity of the occurring absorption peaks, corresponding to the silver nanoparticles' plasmon resonance (Abs1), was related to the intensity of the intrinsic absorbance of the green tea-derived reductants (λ_{max} 273–277 nm; Abs2). An *Abs1/Abs2* value was calculated to help us identify which experimental conditions led to the formation of the highest concentration of silver nanoparticles with minimal excess of uninvolved reductant in suspension.

The effects of alkalization (pH 8) of the green tea herbal preparations prior to their utilization as reducers, the concentration of $AgNO_3$, and the time, on the spectral and macroscopic properties of the samples, are shown in Fig. 3, 4, and 5.

Table 1. Experimental design for the "green" synthesis of silver nanoparticles with green tea-extracted reducers and corresponding spectral data

EXPERIMENTAL DESIGN			RESULTS				
Code/ color[a]	**Reductant type**	**Reductant volume, mL (R)**	**Time of storage**	**λmax, nm (attributed to the silver nanoparticles formation)**	**Absorbance (Abs_1)**	**Absorbance at 273–277 nm (extremum, attributed to the green tea components) (Abs_2)**	**Abs_1/ Abs_2 ratio**
Samples obtained in $AgNO_3$ **1 mM** solution (lev. **2**[b])							
2ER1	Green tea total aqueous extract (**E**)	0.1 (**R1**)	Day 0	426	0.173	0.518	0.33
			Day 1	445	0.605	0.663	0.91
2ER2	Green tea total aqueous extract (**E**)	0.5 (**R2**)	Day 0	447	0.739	2.15	0.34
			Day 1	444	1.118	2.318	0.48
2ER3	Green tea total aqueous extract (**E**)	1.0 (**R3**)	Day 0	449	0.93	3.107	0.30
			Day 1	440	1.13	3.041	0.37
2E8R1	Alkalized (pH8) green tea total aqueous extract (**E8**)	0.1 (**R1**)	Day 0	417	0.828	0.641	1.29
			Day 1	419	1.142	0.72	1.59
			Day 7	422	1.398	0.807	1.73
2E8R2	Alkalized (pH8) green tea total aqueous extract (**E8**)	0.5 (**R2**)	Day 0	410	1.889	2.376	0.80
			Day 1	413	1.968	2.414	0.82
			Day 7	413	2.1061	2.425	0.87
2E8R3	Alkalized (pH8) green tea total aqueous extract (**E8**)	1.0 (**R3**)	Day 0	411	1.896	3.229	0.59
			Day 1	411	2.027	3.103	0.65
			Day 7	414	2.154	3.042	0.71
2CR1	Purified green tea catechin solution (**C**)	0.1 (**R1**)	Day 0	n/a	n/a	0.076	n/a
			Day 1	431	0.017	0.076	0.22

2CR2	Purified green tea catechins solution **(C)**	0.5 **(R2)**	Day 0	419	0.023	0.329	0.07
			Day 1	428	0.037	0.332	0.11
2CR3	Purified green tea catechin solution **(C)**	1.0 **(R3)**	Day 0	423	0.043	0.602	0.07
			Day 1	426	0.057	0.593	0.10
2C8R1	Alkalized (pH8) purified green tea catechins solution **(C8)**	0.1 (**R1**)	Day 0	420	0.256	0.119	**2.15**
			Day 1	422	0.34	0.127	**2.68**
			Day 7	424	0.421	0.144	**2.92**
2C8R2	Alkalized (pH8) purified green tea catechin solution **(C8)**	0.5 **(R2)**	Day 0	420	0.983	0.484	**2.03**
			Day 1	423	1.08	0.517	**2.09**
			Day 7	424	1.103	0.513	**2.15**
2C8R3	Alkalized (pH8) purified green tea catechin solution **(C8)**	1.0 **(R3)**	Day 0	417	1.611	0.845	1.91
			Day 1	420	1.729	0.869	1.99
			Day 7	420	1.741	0.874	1.99
Samples obtained in $AgNO_3$ **10 mM** solution (lev. **3**[b])							
3ER1	Green tea total aqueous extract (**E**)	0.1 (**R1**)	Day 0	462	0.191	0.556	0.34
	Green tea total aqueous extract (**E**)	0.5 **(R2)**	Day 0	495	0.526	2.264	0.23
3ER3	Green tea total aqueous extract (**E**)	1.0 **(R3)**	Day 0	509	0.713	3.303	0.22
3E8R1	Alkalized (pH8) green tea total aqueous extract (**E8**)	0.1 (**R1**)	Day 0	478	0.659	0.675	0.98
3E8R2	Alkalized (pH8) green tea total aqueous extract (**E8**)	0.5 **(R2)**	Day 0	464	1.496	2.45	0.61
3E8R3	Alkalized (pH8) green tea total aqueous extract (**E8**)	1.0 **(R3)**	Day 0	479	2.309	3.342	0.69
3CR1	Purified green tea catechin solution **(C)**	0.1 (**R1**)	Day 0	n/a	n/a	0.081	n/a
			Day 1	436	0.016	0.08	0.20
3CR2	Purified green tea catechin solution **(C)**	0.5 **(R2)**	Day 0	426	0.027	0.346	0.08
			Day 1	437	0.065	0.36	0.18
3CR3	Purified green tea catechin solution **(C)**	1.0 **(R3)**	Day 0	424	0.051	0.609	0.08
			Day 1	438	0.177	0.649	0.27
3C8R1	Alkalized (pH8) purified green tea catechin solution **(C8)**	0.1 (**R1**)	Day 0	454	0.243	0.15	1.62
3C8R2	Alkalized (pH8) purified green tea catechin solution **(C8)**	0.5 **(R2)**	Day 0	449	0.525	0.452	1.16
3C8R3	Alkalized (pH8) purified green tea catechin solution **(C8)**	1.0 **(R3)**	Day 0	444	0.883	0.765	1.15

[a] Each formulation is presented with the same color on graphics as here, in the table
[b]The codes for samples obtained in 1 mM silver nitrate solution start with 2, and those for samples obtained in 10 mM silver nitrate solution—with 3

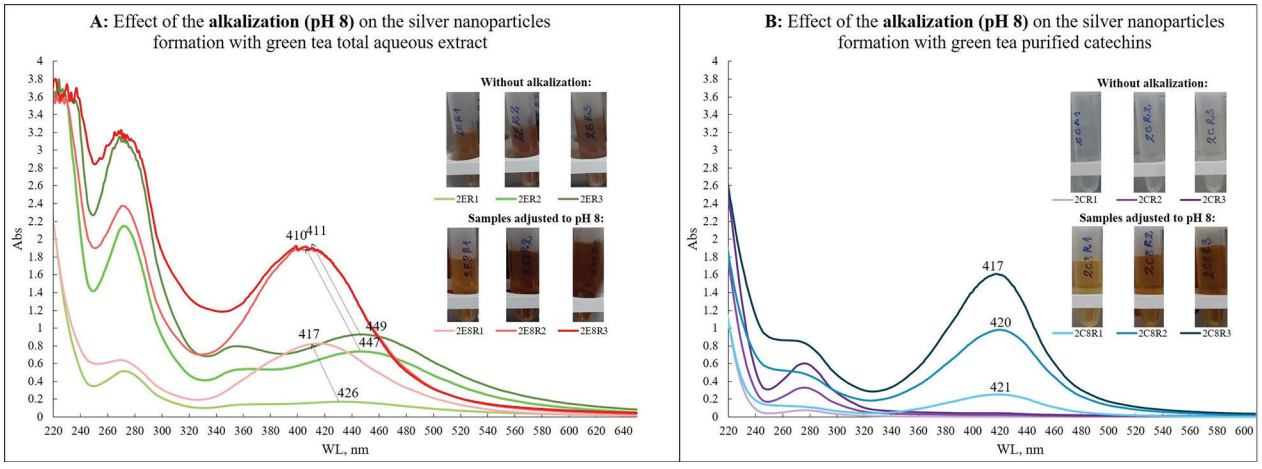


Fig. 3. Effect of the reductant alkalization (pH 8) on the silver nanoparticles' formation in the case of total green tea extract (A) and catechin solution (B)

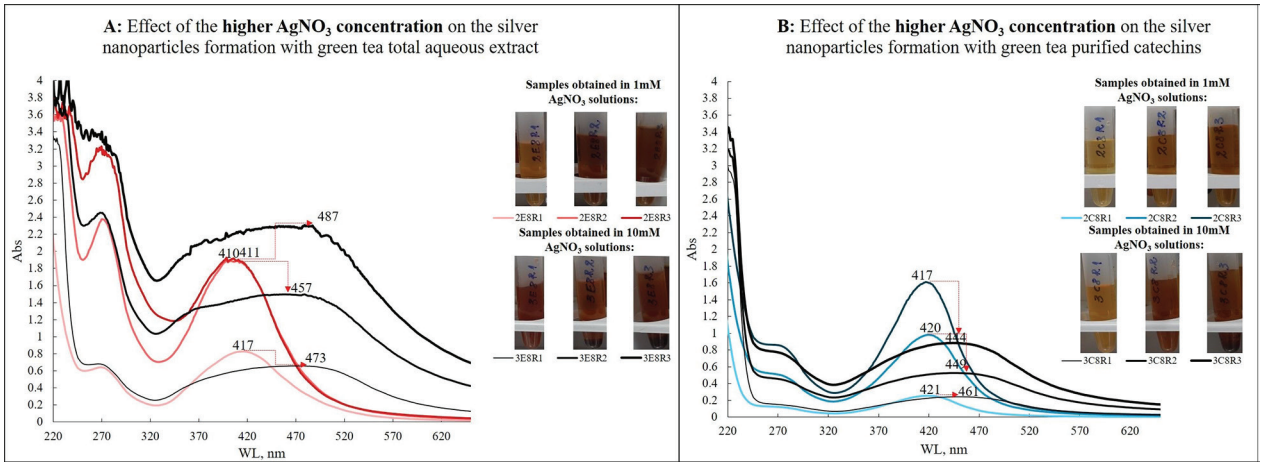


Fig. 4. Effect of the silver nitrate concentration on the silver nanoparticles' formation in the case of total green tea extract (A) and catechin solution (B)

DISCUSSION

As the results showed, the formation of silver nanoparticles was registered in the spectra of all 24 experimental samples. However, only samples obtained in 1 mM silver nitrate with alkalized green tea preparations (total extract "E8" or catechin solution "C8") demonstrated the desired colloidal stability and high concentration of silver nanoparticles. Therefore, these formulations will be discussed with priority. The single-belled, sharp, and intense peaks with a maximum at 410–424 nm in the spectra of the samples in question suggest the presence of separate (non-aggregated) and spherical in shape silver nanoparticles with an average diameter of 40–60 nm (10,17). A preference can be made for the C8 samples (namely, 2C8R1, 2C8R2, and 2C8R3), which were found to possess greater *Abs1/Abs2* value and thus "purer" active surface compared to the corresponding E8 samples (i.e., 2E8R1, 2E8R2, and 2E8R3). Although expected, the last observation confirms that the pure catechin solution (C8) retains the reduction (antioxidant) potency of the total green tea extract (E8) while it still possesses the necessary sufficient "organic load" to stabilize a silver nano-suspension. As the quantity of the reductant increased, so did the concentration of silver nanoparticles. However, a point of saturation was recognized for the E8 samples since samples obtained with 0.5 mL and 1.0 mL reductant solution (samples 2E8R2 and 2E8R3) presented with relatively the same absorbance in the

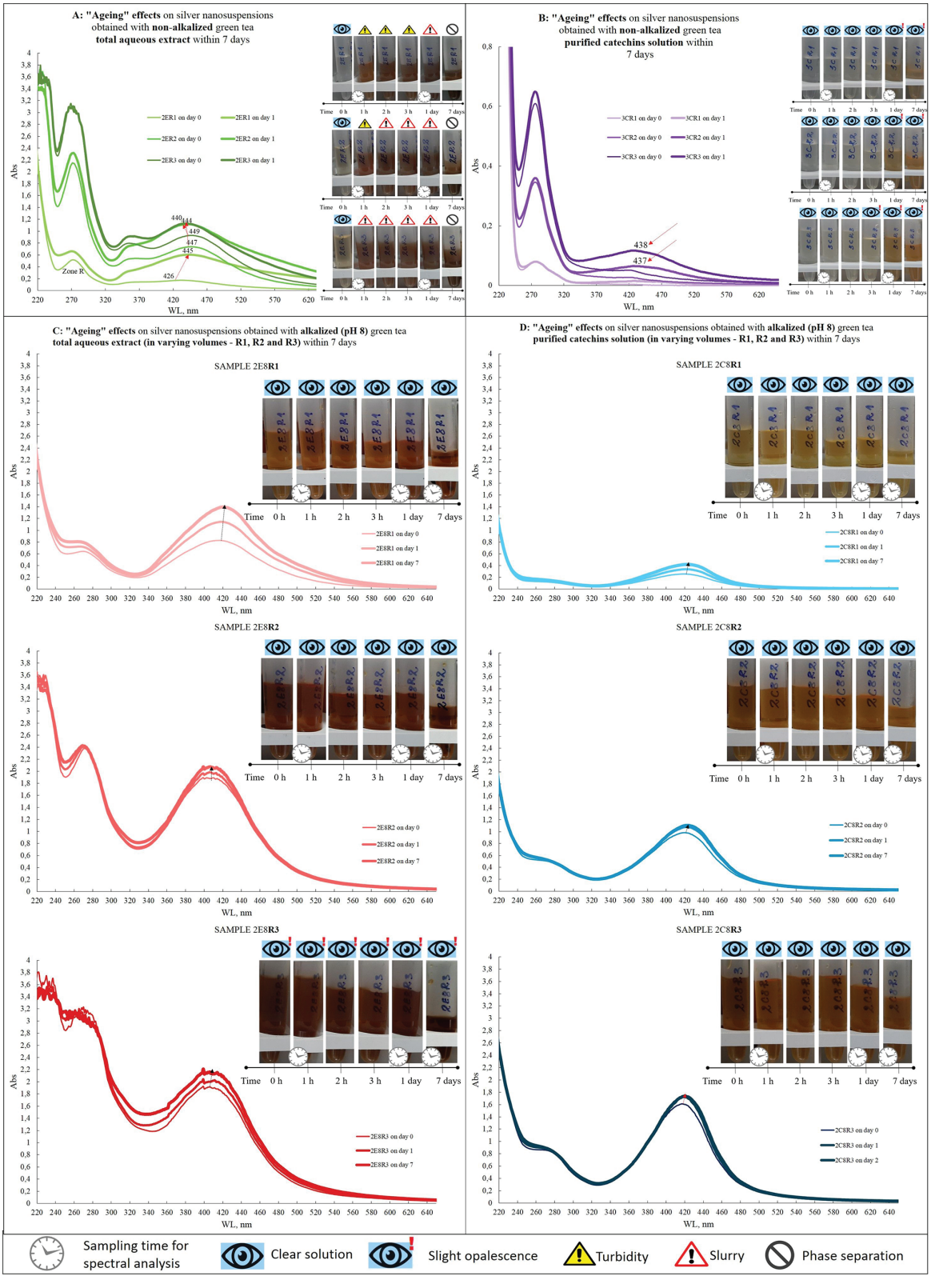


Fig. 5. Time-dependent changes in the silver nanoparticles suspensions in the case of non-alkalized total green tea extract (A), non-alkalized catechin solution (B), alkalized total green tea extract (C), and alkalized catechin solution (D)

silver nanoparticles' range. The storage time did not negatively influence the visual appearance or spectral profile of the 2E8R1÷3 and 2C8R1÷3 formulations. Only a slight increase in the absorbance, as a sign of maturation, was observed, whereas the absorption maximum did not shift by more than 3–5 nm to the red spectrum. The so-called red shift is a well-documented sign of the appearance of larger-sized particles and/or aggregation (18).

Unlike the 2E8R1÷3 and the 2C8R1÷3 samples (obtained in 1 mM silver nitrate), the same formulations obtained in a ten-fold higher concentration of silver ions (10 mM; 3E8R1÷3 and the 3C8R1÷3 samples) showed a very short-term physical stability (1–2 hours the longest) and quick-falling sediments after resuspension. These results led us to the understanding that the higher ionic strength of the 10 mM samples and the higher concentration of nanoparticles are likely potentiating the process of aggregation and the formation of sediments (19); meanwhile, the 1 mM [Ag^+] concentration appeared to be much closer to the desired stoichiometric reaction with the chosen type and quantity of reductant and increased the monodispersity of the silver nanosuspensions.

Alkalization appeared as a critical tool in the green tea-based synthesis of silver nanoparticles. When samples were obtained with non-alkalized reductants (E and C samples), broad and less intense peaks were registered with a maximum at substantially higher wavelengths (419–449 nm); according to other studies in this area, such findings indicate the formation of polydisperse fractions of nanoparticles with an average size of 50–80 nm and a great tendency for aggregation (17,18). Moreover, the intensity of these peaks was increased significantly in the first 24 hours, which revealed that a longer time is required for the complete reduction of the silver ions with non-alkalized green tea reductants. With time, prominent red shift of the absorption maxima was also recorded for some formulations (samples 2ER1, 2CR2, 2CR3, 3CR2, 3CR3). In accordance with the above said, all E-samples, regardless of the other synthesis variables, showed turbidity, which, later on, led to phase separation for most of them; the C-samples were opalescent, but, likely due to the very low concentration of silver nanoparticles, did not precipitate. The alkalization of the reductants with NaOH 10% solution to pH 8 promoted the reductant's solubility rate (ionization) and the formation of fold-higher concentration of single, smaller-sized and stable in aqueous media silver nanoparticles. This observation might be attributed to the increased antioxidant potential of the green tea catechins in alkaline media and in the presence of hydroxyl [OH^-] ions (20–22).

CONCLUSION

Silver nanoparticles were synthesized by a "green" approach using green tea herbal preparations as reductants. When alkalized to pH 8, the green tea-derived reducers demonstrated improved water solubility and antioxidant properties and led to a fast and complete formation of single, spherical, and stable in suspension silver nanoparticles according to the UV-Vis spectra interpretation. The application of pure catechin solution instead of total extract appeared to be advantageous with a view to higher surface purity of the particles and yet satisfactory stability. The highest concentration of silver nanoparticles with maximal stability and purity on the surface was achieved when 1 mM silver nitrate solution was reduced with 0.6 mg/mL alkalized catechin solution (pH 8) in a 3:1 volume ratio (3.0 mL silver nitrate solution plus 1.0 mL reductant; sample 2C8R3). The so optimized formula will be the subject of our further studies and investigation.

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