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Research Article

Synthesis and characterization of nanosilver fluoride particles from two different precursors

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Abstract
Nanosilver Fluoride (NSF) is an emerging anticaries material with demonstrated antimicrobial properties that do not cause staining on carious lesions. However, its availability for research and development remains limited, particularly regarding variations in its synthesis methods, which can affect its physicochemical properties. This study focused on the physicochemical characteristics of NSF synthesized through two distinct methods: chemical synthesis and the use of commercially available colloidal nanosilver. Both versions of NSF were characterized using Ultraviolet-Visible (UV-Vis) spectrophotometry and Transmission Electron Microscopy (TEM). The chemically synthesized NSF exhibited an absorption band at 400-410 nm, while the colloidal nanosilver-based NSF demonstrated no peak from the UV-Vis absorption. TEM analysis revealed that the Silver Nanoparticles (AgNPs) in the chemically synthesized NSF had a mean diameter of 4.99±0.83 nm, compared to the 3.50±0.74 nm diameter observed in the colloidal silver-based NSF. These findings highlight that different synthesis methods yield significant differences in nanoparticle size and absorption characteristics. In conclusion, while both methods are viable for NSF production, researchers should carefully consider the synthesis approach, especially when using commercially available colloidal silver, as it may result in varying properties that could impact the material's efficacy.

Keywords: nanosilver, fluoride, colloidal silver, anticaries, optical properties

Introduction

Minimally invasive dentistry for preventing and arresting caries in children provides cost-effective options to manage caries in deprived communities where the limited availability of advanced dental equipment is of concern. With the advancement of nanotechnology, Nanosilver Fluoride (NSF) was introduced in dentistry by Targino et al. [1] to stop the progression of dentinal caries topically. It was developed as an anticaries agent that can halt the progression of dentinal caries in primary and permanent dentition. Notably, it has a better antimicrobial effect and less toxicity than Silver Diamine Fluoride (SDF) [2]. Unlike SDF, NSF does not cause blackish staining upon its application on the carious lesion [2]. As a new anticaries agent, NSF is not widely available in the market for research and development purposes. Due to the increasing interest in this nanomaterial, different researchers synthesized NSF using variable methods with different precursors and stabilizers. Nanosilver solution can be defined as tiny silver particles dispersed throughout another liquid with the particle size ranging from 2-500 nm [3]. The phrases "colloidal silver" and "nanosilver" have been used by researchers interchangeably. When colloidal silver is made, it is a combination of water and nanoparticles of silver. It is composed of silver ions (Ag^+) and particles. These particles are so small that they would not settle out and stay suspended. Numerous websites and companies claim that they sell colloidal silver, yet somehow, it is only ionic silver [4].

Ionic silver is a silver salt dissolved in water and has somewhat different chemical properties from colloidal silver. Unlike colloidal silver, which contains silver particles, ionic silver does not contain solid particles. Instead, it is composed of Ag^+, which are individual silver atoms or molecules carrying a positive charge [5]. Notably, Ag^+ is highly reactive and often binds

with other molecules present in the liquid. Ionic silver solutions are typically clear or transparent since they do not contain solid particles. Generally, colloidal silver is yellow to brown, and ionic silver is clear color. Thus, the darker the color of the colloidal silver, the more concentrated it is. The normal color of a high-quality colloidal silver solution changes from light golden at low concentrations to yellow-brown, then dark brown, almost black at higher concentrations [6].

Ionic and colloidal silver have been widely used in healthcare settings due to their potent antibacterial properties. In preventive dentistry, the most commonly used method in synthesizing nanosilver is the chemical method [7]. As such, Dos Santos et al. [2] invented NSF synthesis via this method. However, as nanosilver is one of the most marketable nanomaterials in the world [8], it provides an avenue for researchers to prepare an NSF solution using this simple method directly. Several researchers [9–11] used commercial nanosilver in synthesizing NSF, with promising results in NSF as an anticaries agent. The size, morphology, stability, and chemical and physical properties of the Silver Nanoparticles (AgNPs) are influenced strongly by the experimental conditions, the kinetics of interaction of Ag^+ with reducing agents, and the adsorption processes of stabilizing agents with AgNPs [12]. For example, smaller particles, especially those below 10 nm, exhibit greater antimicrobial activity due to their increased surface area, allowing better interaction with bacterial cells [13]. Hence, the design of a synthesis method of NSF in which the size, morphology, stability, and properties are controlled has become a major field of interest, and later on, this might affect its effectiveness as an anticaries agent.

For the preparation of stable AgNPs by chemical reduction, it is crucial to select the appropriate reducing agent. Reducing agents generally reduce silver salts into pure metals, while stabilizer or capping agents are polymers used in wet chemical synthesis to stabilize nanoparticles against aggregation by steric repulsion. Since the AgNPs were in their particle form, they were expected to have zero-net charge. However, the lack of charge on the particle's surfaces will lead to agglomeration. Hence, a well-established method to counter this issue is employing a capping agent such as chitosan. The chitosan-long polymeric chains in the reaction solution inhibit the overgrowth of the coalescing silver atoms from agglomerating thus maintaining their nanoscale size [14]. In synthesizing NSF, fluoride was added to AgNPs to enhance its antimicrobial and anticaries properties [1].

The variety of approaches in synthesizing nanosilver would affect the physicochemical properties of the material. NSF is currently not commercially available for dental use in Malaysia. There is a need to ascertain the chemical properties of commercially available colloidal silver, which will be used to produce NSF. Later on, this NSF will be used as a topical anticaries agent to arrest caries progression in primary and permanent teeth. Hence, this research aims to evaluate the physicochemical properties of NSF derived from the chemical method and NSF derived from the colloidal silver available from the manufacturer. In this study, NSFs were produced using two different methods, and the characterization of each NSF was evaluated and discussed to understand the varying physicochemical properties of each NSF produced using the two different methods.

Materials and Methods

Materials

Silver nitrate ($AgNO_3$) (4.0 mL, 0.012 mol/L) and acetic acid (20%) were purchased from Merck (Gamma Scientific Research, Malaysia). Sodium borohydride ($NaBH_4$) (0.3 mL), Sodium fluoride (0.5% NaF) and chitosan medium molecular weight were bought from Sigma-Aldrich (Gamma Scientific Research, Malaysia). The colloidal silver (300 ppm) (Colloidal Silver No. 1) was obtained from the manufacturer (Colloidal Silver No. 1, Wealth Galleon Ent, Malaysia). All chemicals were used immediately upon receiving them without any further purification.

Preparation of NSF solution

NSF was synthesized using two different methods. In the first method, NSF was prepared via the chemical method, while $AgNO_3$ was used as a precursor, chitosan as a capping agent, and $NaBH_4$. This method was adopted by Targino et al. [1] using $AgNO_3$ as the precursor and $NaBH_4$ as the reducing agent. Briefly, 1.0 g of chitosan (28.7 mL, 2.5 mg/mL) was dissolved in 1% (200 mL) of acetic acid. The solution was stirred overnight and filtered under a vacuum. The chitosan solution (60 mL) was then added to an $AgNO_3$ solution (4 mL, 0.012 M). Consequently, the freshly prepared $NaBH_4$ (0.3 mL, 0.8 M) was added dropwise until changes of color from clear to light yellow to a brownish hue were noticeable. $NaBH_4$ solution must be freshly prepared as it easily reacts with the ambient air. Subsequently, 5% NaF (22,600 ppm of fluorine) was added at the end of the experiment and stirred overnight using a magnetic stirrer. The suspension was then transferred into a brown bottle and was kept at 2-4°C in a refrigerator. In the second method, 5% of NaF (22,600 ppm of fluorine) was directly added to 300 ppm of colloidal silver No. 1, and the solution was stirred overnight and stored in the way mentioned above.

Characterization method

An Ultraviolet-Visible (UV-Vis) absorption is a very useful and reliable technique for the primary characterization of synthesized nanoparticles, and it is also used to monitor the synthesis and stability of AgNPs. The absorbance of the solution was measured using UV-Vis spectroscopy. The absorbance spectrum or the Surface Plasmon Resonance (SPR) produced by the colloidal sample was obtained using a visible spectrophotometer (PRIM SECOMAM) with distilled water as a reference.

The morphologies of the AgNPs were examined using a high-resolution FEI TECNAI F30 Transmission Electron Microscopy (TEM) at 200 keV. TEM is mainly used to examine the surface morphology and size of the synthesized nanosilver. It provides the most accurate and high-resolution imaging information about the size, shape, morphology, state of aggregation, and distribution of nanoparticles at nanometer resolution. Furthermore, TEM utilizes a beam of electrons to interact with the ultrathin sample prepared on the grid [15]. As such, the TEM samples were prepared via the drop-casting method onto carbon-coated copper grids in a dark room using a micropipette. The samples were then allowed to dry under ambient conditions for 24 hours, and the grid was then transferred to the TEM chamber for analysis. The TEM images were recorded with two different magnifications, 20 nm and 50 nm. Average nanosilver size, distributions and standard deviations were calculated and analyzed for each sample by averaging 60-80 particles from the TEM images using ImageJ software (developed at the National Institutes of Health) and OriginPro 2024b software [16].

Results and Discussion

Nanosilver preparation

AgNPs are a product of the chemical reduction of a silver salt with a reducing agent in the presence of a stabilizer. The most common reducing agents in preparing AgNPs involve reducing agents such as $NaBH_4$, citrate, or ascorbic acid [17]. On the other hand, stabilizers such as citrate, Polyvinylpyrrolidone (PVP), surfactant and chitosan are commonly used to prevent nanoparticles from agglomerating during synthesis by providing electrostatic charges or steric functions on the particles' surfaces [17].

In this work, the reduction of $AgNO_3$ was performed using $NaBH_4$. It is a very strong reducing agent, and the expected reaction was as follows [18]:

$$AgNO_3 + NaBH_4 \rightarrow Ag^0 + 0.5H_2 + 0.5B_2H_6 + NaNO_3 \quad \text{(Eq.1)}$$

The reduction of $AgNO_3$ with $NaBH_4$ reducing agent occurs very quickly, characterized by the appearance of color changes in the color of the solution. Apart from being a good capping agent, it also has excellent biocompatibility, antimicrobial activity and good heat resistance with the presence of the amino and hydroxyl groups [19].

AgNPs were reacted with NaF to form NSF. As mentioned above, the AgNPs were at a zero-net charge; therefore, no chemical interactions were expected between them and the capping agent. On the other hand, NaF is known to dissociate completely in water, as in the following:

$$NaF \xrightarrow{water} Na^+(aq) + F^-(aq). \quad \text{(Eq. 2)}$$

Meanwhile, the addition of acetic acid to solubilize chitosan also protonated the amine moieties on the capping agent polymeric chains. The positive charges then acted as binding sites to the free fluorine ion, F^-, illustrate in **Figure 1**:

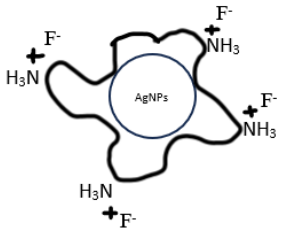

Figure 1. Interaction between free fluoride ion and AgNPs

On the other hand, Kumar and Goia [4] extensively evaluated 14 commercial silver colloidal products available from the market, and they reported that out of 14 products, only four were truly colloidal. This is due to the absence of the plasmon band characteristic of AgNPs, and it should not be labeled as "colloidal silver." This proved that most manufacturers are unaware that stable "colloidal silver" dispersions should not be colorless even at a very low level of silver concentration [4]. In addition, the lack of the yellow color characteristic caused by the plasmon absorption band was a strong indication that they contain ionic silver and, therefore, could not be labeled "colloidal silver." In this study, we discovered that the chemically synthesis nanosilver produced a dark yellowish to brown color of the solution while the commercially available nanosilver is colorless. Kumar and Goia [4] used pure nanosilver/colloidal nanosilver without adding any other chemical substance, while in our study, we added fluoride to evaluate the material's properties. It is revealed that no changes in colour could be observed by adding the fluoride into the silver solution. Due to limited budget and time constraints, we only managed to evaluate colloidal silver from this one manufacturer.

UV-Vis spectral analysis

The appearance of the typical SPR peak at 410 nm (**Figure 2**) for the chemically synthesized NSF indicates that the nanoparticles were successfully synthesized. However, no SPR peak was detected from the commercial grade NSF, indicating no formation of nanoparticles and that the reactants stayed in their ionic state.

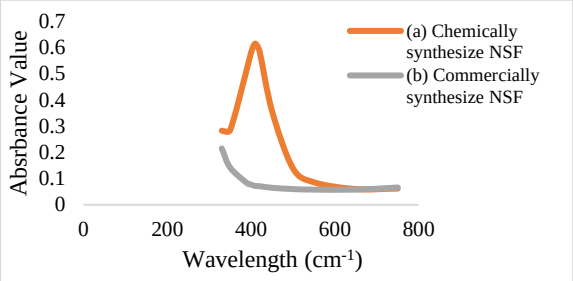

Figure 2. UV-Vis spectral analysis of a) Chemically synthesized NSF and b) Commercially synthesized NSF

SPR refers to the collective oscillation of free electrons in response to an external electromagnetic field, usually light [20]. When light hits the surface of AgNPs, the conduction electrons on the nanoparticle surface resonate with the light's frequency, causing an absorption of light at a specific wavelength [20]. AgNPs have free electrons, which yield the SPR absorption band due to the mutual vibration of electrons of metal nanoparticles in resonance with the light wave. The appearances of the peaks indicate the characteristics of the SPR of AgNPs. Meanwhile, the peak in the SPR spectrum represents the wavelength at which the maximum light absorption occurs due to this resonance effect. At the same time, the absence of an SPR peak suggests that no AgNPs were formed. The AgNPs absorbed light within the wavelength range of 400-430 nm [18]. However, the NSF from the commercial method did not exhibit any SPR peak in the wavelength range. This proved that the commercial grade silver was in the ionic form.

As reported by Mikac et al. [18], the AgNPS feature SPR band extended in the range of 350-500 nm with a peak position at around 410 nm. It is known as a specific band of nanoparticle formation. This result confirms the availability of the chemically synthesized NSF particles, which are comparable to the peak obtained in this study. The UV-Vis absorption spectra provide a deeper insight into the optical properties of the AgNPs as it is highly dependent on the distribution and the surface properties of such particles [21]. The color changes of the solutions from colorless to golden yellow, further indicating SPR phenomena induced by the chemically synthesized NSF.

TEM analysis

The TEM analysis presented that the chemically synthesized NSF were spherical with a mean diameter of 4.99±0.83nm, while the commercial synthesized grade NSF revealed a slightly lower mean value of 3.50±0.74 nm in diameter (**Figure 3**). Both samples demonstrated good dispersion and less aggregation of nanoparticles.

TEM image of chemically synthesized NSF demonstrated distinct spherical-shaped nanoparticles with good dispersion and less agglomeration.The main signal for TEM analysis comes from the electron density of the sample, which provides imaging contrast. Suitable samples include solid, electron-dense materials, such as metallic particles or crystalline structures [22]. Ionic silver is individual silver ions (Ag^+) dispersed in a solution; they lack the criteria to yield morphological data for TEM analysis. In this study, we can observe that the TEM image forms from an ionic silver sample. In that case, there is a possibility that Ag^+ interacts with the carbon grid or organic molecules on the grid surface or during the drying process, leading to their oxidization and subsequent formation of silver oxide [23]. The Ag^+ is in precipitated form due to drop casting sample during the preparation prior to TEM imaging. In this dry-form condition, silver normally bonds with oxygen from the air to form silver oxide. This explains the formation of hazy particles, typically appearing as dark spots or clusters in the image due to ionic silver interaction with oxygen.

The differences in NSF sizes could affect their antibacterial properties and cytotoxicity. Smaller size particles have better bacteria inhibition activities [24]. This could be due to the different surface properties of the AgNPs, which can influence their interaction with the bacterial membrane. Aside from the antibacterial activity, size control also helps researchers understand the cytotoxicity of the materials. It was proven that 10 nm nanoparticles were more cytotoxic than 75 nm nanoparticles and that 10 nm nanoparticles were also immunosuppressive [25]. Moreover, smaller nanoparticles release higher amounts of Ag^+ in the cellular medium than the larger ones [25].

NSF from the manufacturers has more free ions or charged particles than the chemically synthesized NSF. Charged groups within the NSF particles also affect the cytotoxicity of the NSF. Positively charged surface groups of AgNPs (poly-vinylpyrrolidone) have higher cytotoxicity compared to negatively charged bis(2-ethylhexyl) sulfosuccinate sodium as a

stabilizer [26]. The physical and chemical properties of AgNPs have been proven to influence their cytotoxicity. Thus, the preparation of AgNPs needs to consider the balance between the antibacterial and any

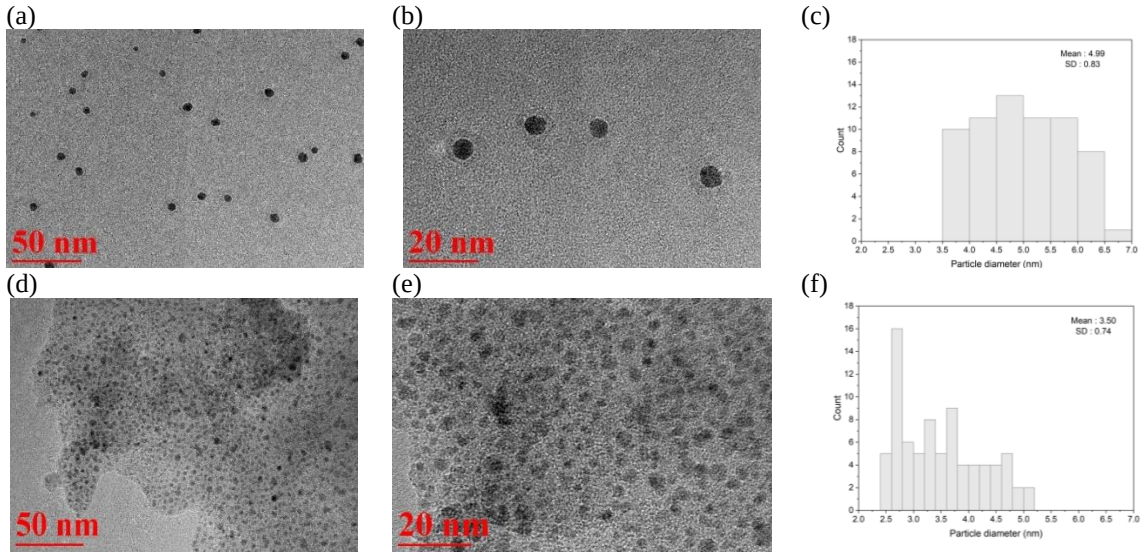

Figure 3. TEM image; (a) chemically synthesized NSF in 50 nm magnification, (b) chemically synthesized NSF in 20 nm magnification, (c) size distribution of chemically synthesized NSF, (d) commercially synthesized NSF in 50 nm magnification, (e) commercially synthesized NSF in 20 nm magnification, and (f) size distribution of commercially synthesized NSF

possible toxicity effects to avoid risks to human health.

Ag^+ is the species responsible for the antimicrobial properties. Hence, it can be deduced that pure ionic silver solution is more effective than nanosilver as the concentration of free Ag^+ ion is significantly higher for the same total silver concentration. However, the antimicrobial activity of AgNPs at nanomolar concentration was reported to be comparable to the micromolar level recorded for Ag^+ [24]. The higher efficacy of nanosilver was due to its capability to attack bacteria by physically altering the properties of the cell wall and entering the intracellular space. This capability of nanosilver "internalization" makes them efficient transporters of Ag^+ into the bacterial cell wall, where the critical transcriptional processes need to be inhibited [4].

AgNPs were proven to be a better antibacterial agent than ionic silver due to its significantly lower toxicity. The nanosilvers are comparable to the ionic silver; they suppress bacterial and yeast growth at concentrations equal to approximately 1-3 mg/L [27]. However, with the same concentrations, ionic silver is also toxic against eukaryotic organisms, including human cells. Nevertheless, nanosilver can sufficiently suppress bacterial and yeast growth at these concentrations without being toxic to human fibroblasts [27]. Thus, nanosilver represents less risk for humans when used in medical applications and commercially available products, but only when the silver concentration is retained at units of mg/L, sufficiently suppressing bacterial and yeast growth [27].

Conclusion

This study demonstrates that NSF can be synthesized using chemical methods and commercially available colloidal silver, with each method producing significant differences in the physicochemical properties of the resulting NSF. The chemically synthesized NSF, which utilized $AgNO_3$, $NaBH_4$, and chitosan, exhibited distinct optical and morphological characteristics compared to NSF derived from colloidal silver. Specifically, the chemically synthesized NSF displayed a larger average nanoparticle size and a more defined SPR band. These findings underscore the importance of carefully selecting the synthesis method, as particle size, morphology, and chemical composition variations can significantly impact the antimicrobial efficacy and potential applications of NSF in dental treatments. Considering the discrepancies between colloidal and ionic silver products in the market, researchers and manufacturers should implement stricter quality controls and clearer labeling to ensure that the desired nanosilver properties are consistently achieved.

Nevertheless, further research into the long-term stability and biological effects of NSF produced by different methods will enhance its application as a reliable anticaries agent.

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