Dairy Products Mediated Green Synthesis of Silver Nanoparticles: A Comparative Study

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ABSTRACT:

The success of the production of biologically synthesized metal nanoparticles has led many researchers to jump on the bandwagon. This paper presents an extremely simple approach to the synthesis of bulk quantities of highly stable silver nanoparticles (AgNPs) using different dairy products: milk, cream, mava, paneer, whey protein, curd, butter milk as reducing agents and the natural hydrocolloid guargum as a stabilizing agent. The synthesized AgNPs are found to be stable in aqueous solution over a period of two months at room temperature. Moreover, dairy products with a high fat content produce smaller nanoparticles, whereas those with a low-fat content yield larger nanoparticles. The size of these nanoparticles was found to be in the range of 3 to 255 nm as analyzed using Dynamic Light Scattering (DLS). This result was consistent with the SEM study. The SEM analysis revealed the cubic shape of AgNPs.

Keywords: *Silver nanoparticles, green synthesis, milk, guargum, fatty acids, UV-Visible spectroscopy, DLS, SEM.*

I. INTRODUCTION:

Nanotechnology has emerged as one of the most important research pursuits of the early twenty-first century, since every area of our lives has been impacted by nanotechnology. This branch of science includes the study of matters at the nanoscale (1-100 nm), i.e., synthesis and their employment in a variety of applications. Nanomaterials can be made in a number of sizes and forms, as well as with a variety of coatings, giving them unique qualities that allow them to be used in a wide range of commercial applications. Hence, the chances of human beings encountering nanomaterials from various sources are rapidly increasing [1]. Metallic nanoparticles have piqued the curiosity of chemists, physicists, biologists, and engineers interested in developing new nanodevices. The intriguing properties of metal nanoparticles have sparked a boom of scientific interest in nanosensors [2,3], catalysis [4,5], transistors [6] and biomedical applications [7,8] in recent decades. Among all the metallic nanoparticles, silver nanoparticles (AgNPs) are one of the most prestigious entities that have found numerous promising applications in science and technology. Over the last few years, AgNPs have become one of the most researched and scrutinized nanotechnology-derived nanostructures, considering that nano silver-based materials have been shown to have intriguing, demanding, and exciting properties that make them suited for a variety of biomedical applications [8]. The Caldeans knew about metallic silver as early as 4,000 B.C.E., and it was the third most common metal among the ancients, after gold and copper. Metallic silver has been used for a variety of medical ailments for centuries, even before bacteria were identified to be the agents of infection [9]. Because of its antibacterial activities and minimal toxicity to human cells, silver was possibly the most important antimicrobial substance until the discovery of antibiotics in the 1940s, and it is still utilized in a wide range of medicinal applications today [10]. The importance of drinking water in silver cups by Persian monarchs was also emphasized by Herodotus, the father of history, because water was kept fresh for years in silver containers [11].

Silver nanoparticles are a cluster of silver atoms in the size range of between 1-100 nm. The extremely tiny size offers various advantages due to the huge surface area. The small size of nano silver brings biocompatibility by attaching firmly to membranes and cell wall proteins, probably because of its interaction with thiol groups on enzymes, making it a promising antibiotic. Silver's antibacterial activity is determined by particle size; 10-nm particles interact completely with bacteria, but larger particles do not [12]. Silver (Ag) and Ag-based compounds have long been used to inhibit microbial growth in various applications [13-15]. Silver nanoparticle coatings could be employed as an alternative anti-mycobacterial strategy. Colloidal AgNPs are more adherent to conventional filter materials than ionic silver [16]. Besides the medical field, they are increasingly being used in a variety of consumer products [17], such as fabrics, toothpastes, sprays, toys and much more, to prevent the growth of harmful bacteria. It's also becoming more popular in cosmetics. The absolute advantages of using silver nanoparticles in cosmetics involve improved stability of cosmetic ingredients (e.g., vitamins, antioxidants, and unsaturated fatty acids) by encapsulating them into nanoparticles [18]. Additionally, their small size with large surface area allows actives to quickly reach the skin and protect it from ultraviolet (UV) rays.

The vast range of applications demands a smart nanoparticle production strategy. Over the past few decades, researchers have developed a variety of methods for the synthesis of silver nanoparticles, such as sol-gel synthesis [19], hydrothermal methods [20], electrochemical techniques [21], photochemical reduction [22], sonochemical [23], microwave [24], γ-radiation [25], and laser ablation [26]. Unfortunately, several of the reagents employed in silver nanoparticle synthesis may harm the environment during large-scale production. The negative consequences of utilizing chemicals in nanoparticle synthesis have motivated researchers to focus their efforts on green synthesis methods. Greener nanoparticle synthesis is a step forward from earlier approaches, since it is simpler, more cost-effective and, most importantly, environmentally benign. Green nanoparticle synthesis strives to reduce waste by employing chemical-free agents while also developing environmentally friendly methods. Three major aspects that must be assessed from a green chemistry perspective; selection of ecofriendly solvent medium, use of environmentally benign reducing agent, selection of non-toxic stabilizer for nanoparticles [27]. For instance, many researchers have synthesized AgNPs using protein from Capsicum annuum L. extract as a reducing agent [28], polyphenols and gallic acid from green tea (Camellia sinensis) extract as a reducing agent and capping agent respectively [29], an aqueous solution of Ficus benghalensis leaf extract as a reducing agent [30], β-D glucose as a reducing agent and starch as a capping agent [31], and Pleurotus sajor caju fungi as a reducing agent [32]. The use of microorganisms and green plants in the production of AgNPs has been extensively studied. However, the use of microbes and green plants in nanoparticle manufacturing has a number of drawbacks. For example, mass microbe cultivation, high costs, maintaining an aseptic environment, plant extract preparation and purification [33]. To overcome these constraints, low-cost, readily available, environmentally friendly dairy products are employed for silver nanoparticle synthesis in this article, a further advancement in the eco-friendly approach.

Another factor worth exploring further is the distinctive trait of plants that reduce metals. The key premise of using green plants in nanofabrication is founded on our understanding of the reducing capacities of green plants, which also specifies that the protein content is responsible for the reduction of metals into metal nanoparticles. Accordingly, protein is abundant in many other natural products, such as milk, paving the way for their use in nanoparticle fabrication. The principal constituents of milk are proteins, fat, carbohydrates, and mineral salts [34]. Table 1.1 shows the amounts of major chemical constituents reported in various packages of Amul dairy products used in the current study.

Table 1.1: Chemical composition of Amul dairy products:

Milk products	Protein (%)	Fat (%)	Carbohydrates (%)
Amul Gold Milk (100 ml)	3.5	4.5	5.0
Amul Fresh Cream (100 g)	2.0	25	3.2
Mava (Amul Mithai mate) (100g)	8	9	55.5
Amul Fresh Paneer (100 g)	20	22	4.5
Whey Protein (100 ml)	0.3	0.13	5.03
Curd (Amul Masti Dahi) (100 g)	4.0	3.1	4.4
Amul Butter Milk (100 ml)	0.2	0.1	0.3

The biomolecules with various functional groups present in dairy products are capable of synthesizing nanoparticles. A very small quantity of dairy products is needed both to reduce ionic silver and to stabilize the silver nanoparticle. Furthermore, using dairy products to synthesize metal nanoparticles could make downstream processing considerably simpler than using plants or microorganisms. Exploring various milk products for silver nanoparticle production is therefore of great significance. Furthermore, it may aid in a better understanding of the silver nanoparticle reduction mechanism employing milk proteins.

Herein, we investigated the synthesis of AgNPs using seven different dairy products, including milk, cream, mava/khoa (a concentrated whole milk OR milk that has been boiled and reduced until it reaches a semi-solid stage), paneer (made by curdling hot milk using lemon juice, vinegar, or citric acid), whey protein (the remaining liquid after separating paneer from milk), curd and butter milk, as well as their underlying action. Highly stable AgNPs were prepared by reduction of $AgNO_3$ using dairy products in the presence of a hydrocolloid guargum. Hydrocolloids are used as a stabilizing agent, preventing nanoparticle aggregation and thus increasing additional stability. This work suggests an attainable procedure for the production of AgNPs without the involvement of toxic chemicals.

II. MATERIAL AND METHOD:

2.1 Collection of materials:

In the comparative study, we used seven different dairy products, including milk, cream, mava, paneer, whey protein, curd and butter milk. The fresh dairy products of the Amul brand were collected from the local market of Jodhpur (Rajasthan), India. $AgNO_3$ was purchased from Ases chemical works, Jodhpur.

2.2 Preparation of samples and synthesis of AgNPs:

The stock solution of silver nitrate (1 mM) was prepared in an aqueous medium. To make stock solutions for dairy products, 1 gm of each dairy product was weighed with a weighing balance and then mixed separately with 100 ml of distilled water. The detailed description of the procedure for the synthesis of AgNPs is given below.

The most accessible reaction conditions for the synthesis of AgNPs, such as temperature, time and concentration of dairy products, are as follow:

- 5 ml of each stock solution of dairy products was mixed with 15 ml of $AgNO_3$ solution separately and prepared into a series of 7 reaction mixtures (series A).
- All reactions were carried out at room temperature.
- Reduction of silver ions in the reaction mixture was monitored by physical inspection arising out of a gradual change in color from opaque white to yellow and then turned into a brown color in almost 2 hours, see Figure 1, indicating the appearance of AgNPs. λ_{max} was subsequently determined as part of their characterization.

The synthesized AgNPs have been perceived as unstable, hence they can't be stored at room temperature for long. To solve this issue, we prepared a fresh batch of seven reaction combinations (series B). To improve the stability of AgNPs in the mixture, a natural hydrocolloid called guargum was added. The addition of 10 ml of 1% guargum solution to each reaction mixture at the beginning provides excellent stability for the AgNPs. Furthermore, guargum is observed as highly effective in terminating particle growth by adsorbing on the solid-liquid interface and reducing the surface tension of nanoparticles.

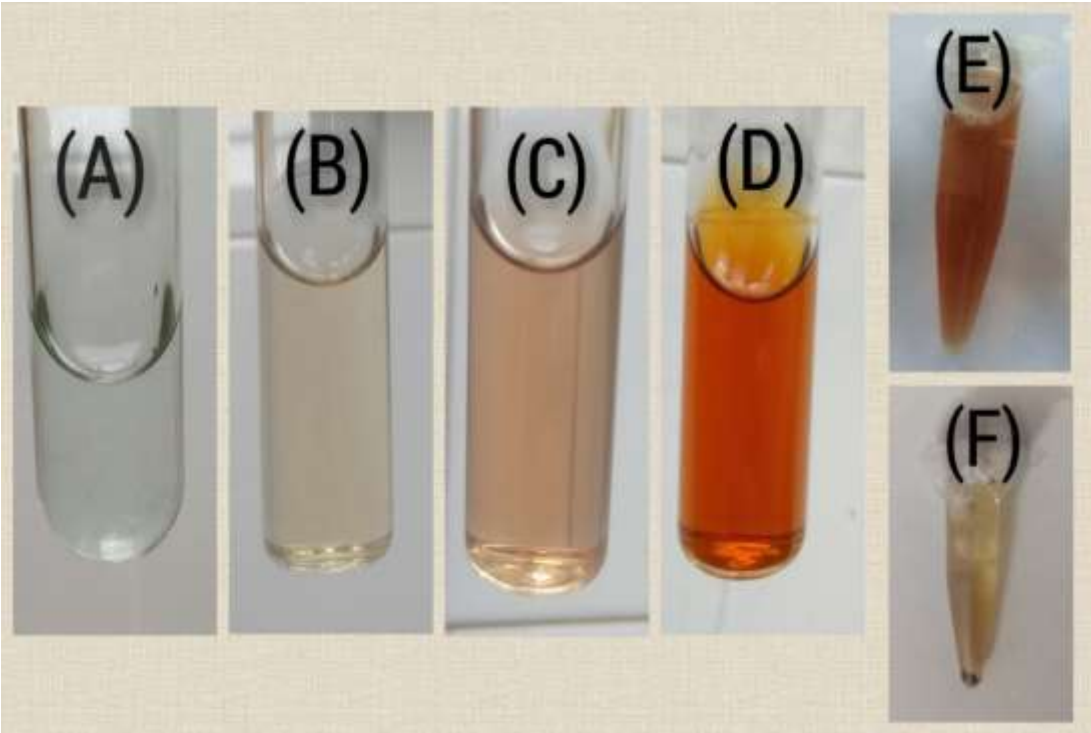

Figure 1: Physical inspection of silver nanoparticle synthesis: (A) $AgNO_3$ solution, (B) AgNPs formed using milk with guargum after half an hour, (C) after 2 h, (D) after 24 h, (E) after 2 months, (F) AgNPs formed using milk without guargum after 1 week.

III. Characterization, results and discussion:

The synthesis of AgNPs was primarily investigated using UV-visible spectroscopy. Absorption maxima of different samples were observed between 420-435 nm, using a UV-Visible Spectrophotometer (ELICO® SL 210 Double Beam UV-Visible Spectrophotometer), as shown in Figure 2.

Following confirmation, nanoparticles are stored in the dark for future analysis. The UV-Vis spectra recorded implied that, in series (A), the most rapid bioreduction among all the dairy products was achieved using milk as a reducing agent, followed by mava and then cream, whereas in series (B), whey protein showed fast bioreduction of Ag^+ ions followed by curd and then milk. The UV-Vis spectra of nanoparticle samples of series (B), revealed that the AgNPs in the solution remained stable even after two months of synthesis. The λ_{max} values of all the samples at different times are given in table 3.1, whereas the absorbance is given in table 3.2.

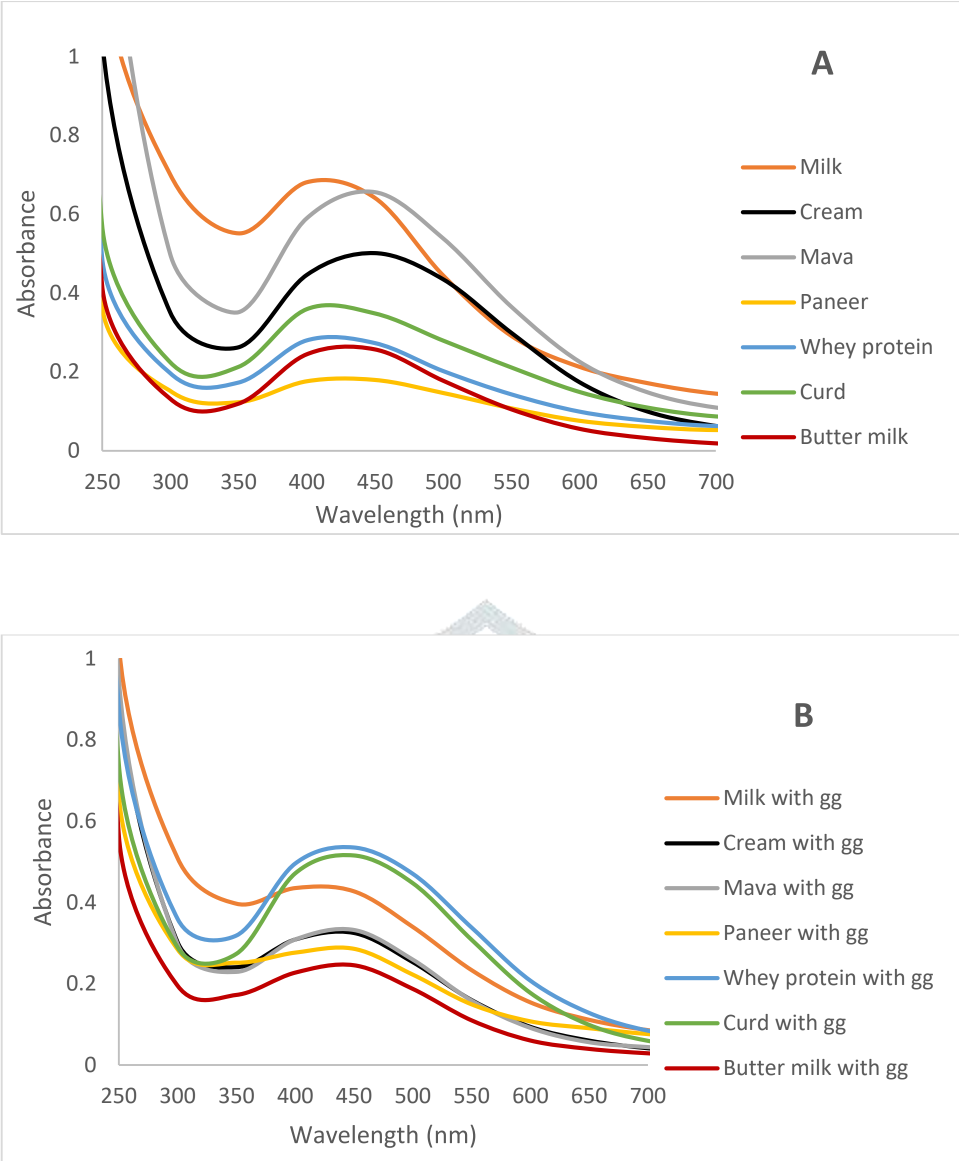

Figure 2: UV-Vis absorption spectrum of AgNPs, (A) without guargum, (B) with guargum(gg).

Table 3.1: λ_{max} values of silver nanoparticles prepared using seven different dairy products.

Ag NPs formed using	λ_{max}(nm) after 2 hours		λ_{max}(nm) after 24 hours		λ_{max}(nm) after 1 month		λ_{max}(nm) after 2 months	
	Without guargum	With guargum	Without guargum	With guargum	Without guargum	With guargum	Without guargum	With guargum
Milk	415	423	420	423	settled	425	settled	426
Cream	450	433	450	433	settled	433	settled	434
Mava	443	438	449	438	settled	439	settled	441
Paneer	425	433	430	434	settled	436	settled	439
Whey Protein	425	452	435	455	settled	457	settled	459
Curd	420	453	428	454	settled	457	settled	460
Butter Milk	425	443	432	444	settled	444	settled	446

Table 3.2: Absorbance values of silver nanoparticles prepared using seven different dairy products.

AgNPs formed using	Absorbance after 2 hours		Absorbance after 24 hours		Absorbance after 1 month		Absorbance after 2 months	
	Without guargum	With guargum	Without guargum	With guargum	Without guargum	With guargum	Without guargum	With guargum
Milk	0.6862	0.4402	0.7125	0.4829	0.7534	0.5359	0.7545	0.5361
Cream	0.5012	0.3294	0.5586	0.3749	0.5792	0.4357	0.5799	0.4358
Mava	0.6562	0.3357	0.6792	0.3756	0.7145	0.4167	0.7172	0.4165
Paneer	0.1849	0.2909	0.2094	0.3284	0.2263	0.3546	0.2265	0.3568
Whey Protein	0.2943	0.5345	0.3243	0.5582	0.3485	0.5629	0.3491	0.5668
Curd	0.3674	0.5157	0.3954	0.5429	0.4157	0.5683	0.4155	0.5684
Butter Milk	0.2706	0.2482	0.3104	0.2794	0.3573	0.2965	0.3586	0.2998

The size range of nanoparticles and their distribution in the samples were ascertained using Dynamic Light Scattering (DLS) (Malvern, Zetasizer Nano ZS90), as shown in figure 3A and 3B. The smallest-sized AgNPs were formed using cream and milk, followed by paneer and then mava. The reason for this could be because these dairy products have the highest fat content of the rest of the dairy products. Fat acts as a capping agent, preventing nanoparticles from interacting with one another. As a result, particles are able to maintain their smaller size. Hence, dairy products having a high fat content showed good results, whereas those dairy products which had a low-fat content showed average results. For instance, whey protein is completely free of fat content, so the particles formed using whey protein are larger than the rest of all other dairy products [35].

Fatty acids adsorb on the surface of nanoparticles and provide a particle surface charge to stabilize the nanoparticle. The majority of fatty acids have a unit negative charge. As shown in Figure 4, the tail component can consist of carbon ring structures and additional group branches that generate a hydrophobic effect. On a molar and weight basis, the fatty acid synthesizing mechanism in the mammary gland of the cow produces fatty acids with an even number of carbons and a length of 4-16 carbons, accounting for approximately 60 and 45 percent of the fatty acids, respectively. The fatty acid present in milk products is palmitic acid (C_{16}). It has an unbranched saturated long carbon chain. Palmitic acid comprises approximately 30% by weight of the total fatty acids. Other fatty acids present in milk products are butyric acid (C_4) and caproic acid (C_6) [36].

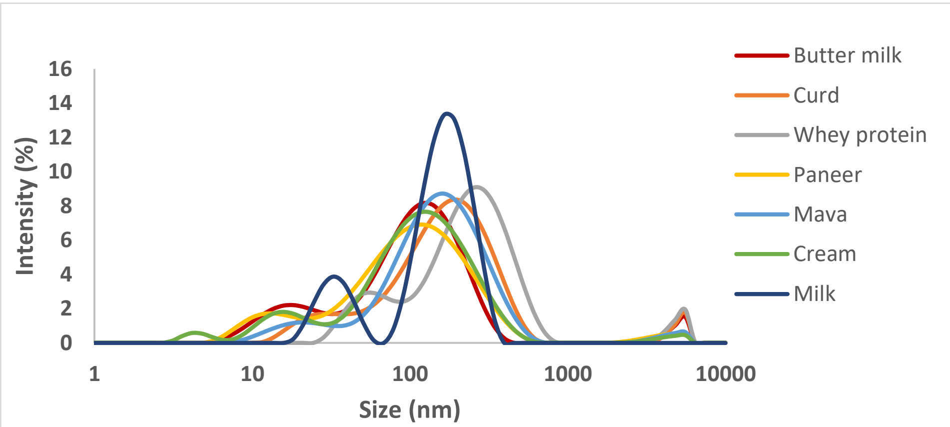

Figure 3A: Size distribution of AgNPs from DLS analysis: graphical representation of the intensity and size of AgNPs formed using different dairy products.

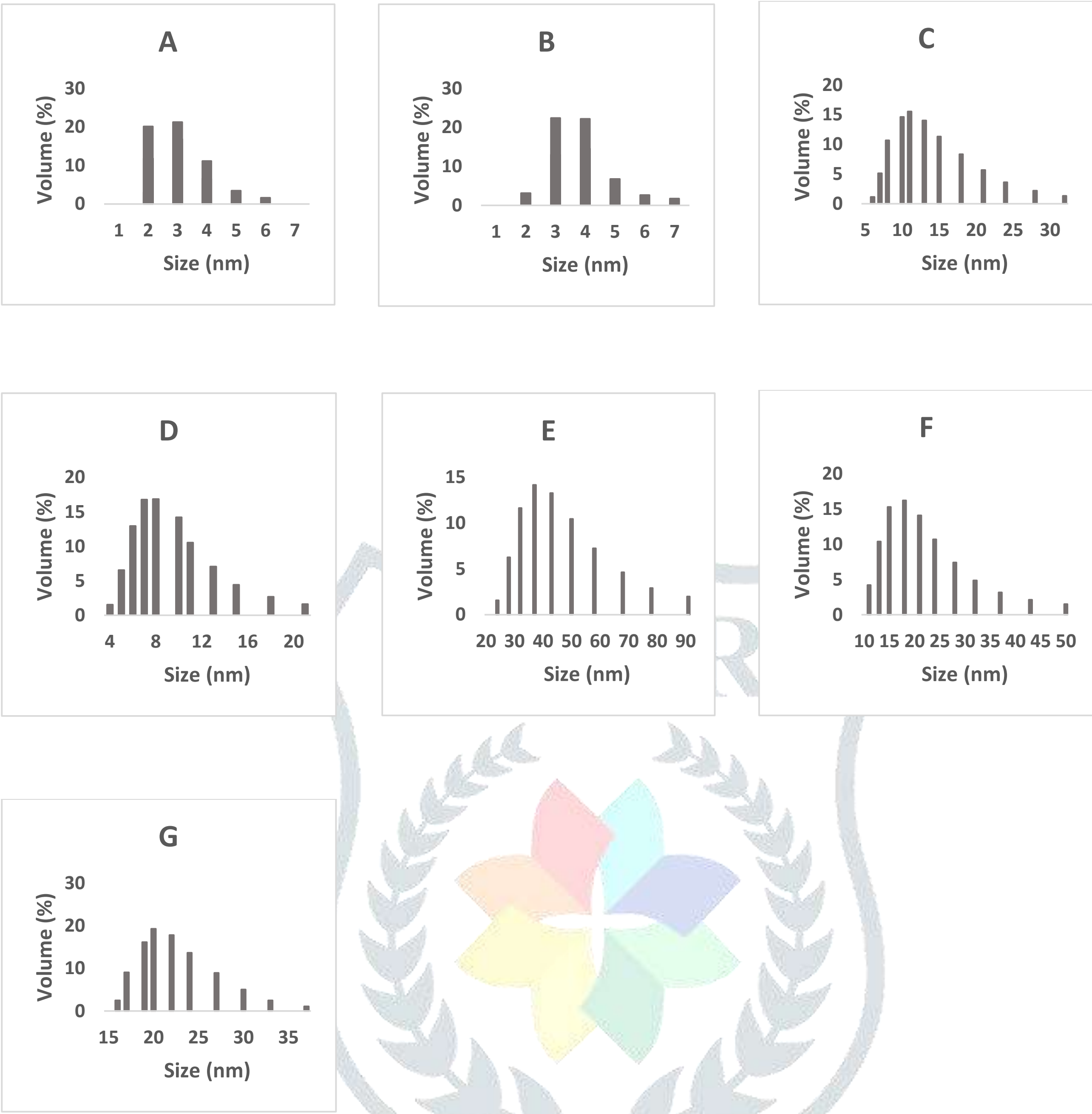

Figure 3B: Size distribution of AgNPs from DLS analysis: graphical representation between volume and particle size of AgNPs formed using different dairy products (A) Milk, (B) Cream, (C) Mava, (D) Paneer, (E) Whey protein, (F) Curd, (G) Butter milk.

Depending on the varying properties of different dairy products, the order of size of AgNPs recorded using DLS was as follows:

(Cream ≈ Milk) < Paneer < Mava < Curd < Butter milk < Whey protein

This study revealed the effect of fat content present in dairy products on the size distribution of metallic NPs. The presence of well-dispersed AgNPs with an average diameter of 50 nm was further confirmed by the Scanning Electron Microscope (SEM) (Bruker XFlash® 6-30). The SEM image is shown in Figure 5.

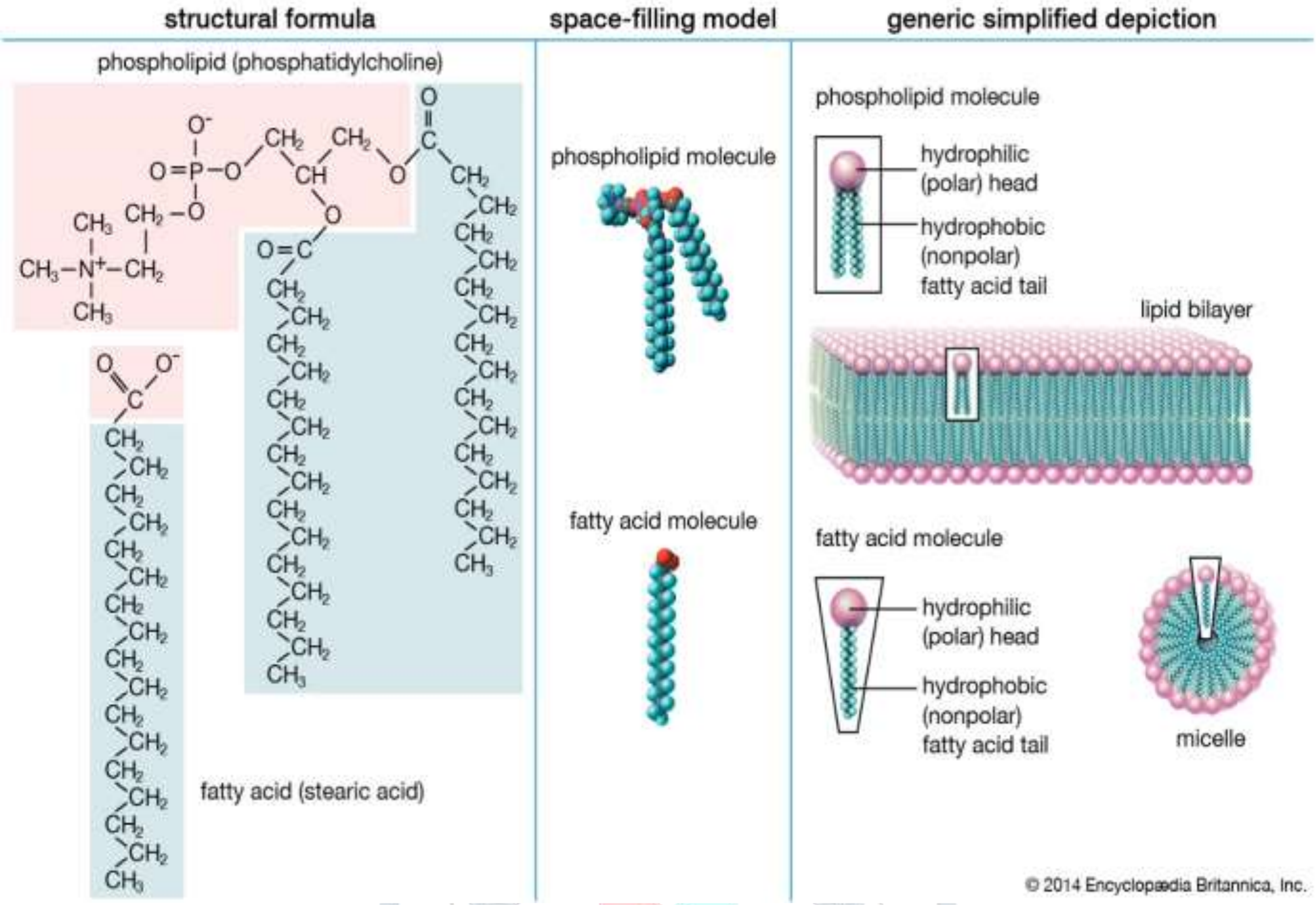

Figure 4: General structure of fatty acid. (Britannica, T. Editors of Encyclopaedia (2020, May 12). Fatty acid. Encyclopedia Britannica. https://www.britannica.com/science/fatty-acid)

Figure 5. (A) Figure 5. (B)

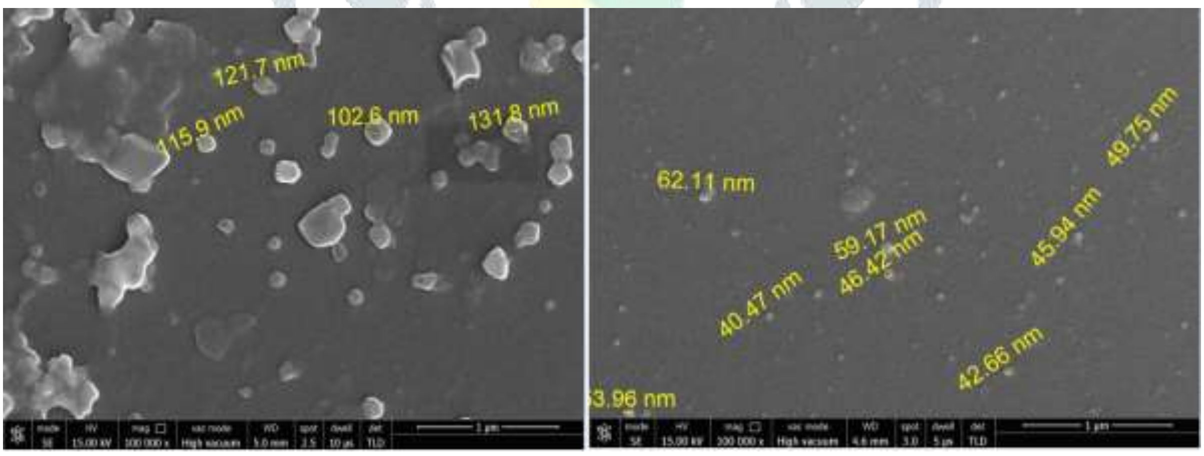

Figure 5: Scanning electron microscopy images of AgNPs formed using milk (A) without guargum, (B) with guargum.

According to the SEM analysis, all dairy products yielded approximately spherical and cubic AgNPs. Silver nanoparticles obtained using guargum were comparatively more stable. The highly stable AgNPs can be utilized for research purposes due to their stability and antimicrobial efficacy against microbes. Further studies are needed to fully examine the toxicity and the antimicrobial activity of these particles.

IV. CONCLUSION:

This study reveals a feasible method for producing AgNPs without the use of harmful chemicals. The key factors in dairy products that govern the formation and stability of AgNPs were investigated in this study. Interestingly, the smallest AgNPs were formed using milk and cream without adding guargum, whereas other samples required the addition of guargum to produce a small size for the nanoparticles. The active compounds found in milk fat were discovered to have a considerable impact on the stability of nanoparticles. The addition of guargum provides extra stability to silver nanoparticles. After adding guargum, the nanoparticles managed to stabilize for two months at room temperature. Nanoparticles must be capable of remaining stable for more than two months. Furthermore, the size difference could be due to the different types of proteins found in milk. However, more research is needed to understand how different proteins influence the size distribution of nanoparticles.

One of the most important aspects of the study presented here is that the reducing agents used are not only environmentally friendly, but all of them are also well-known edible products, and so is the stabilizer. Hence, AgNPs, so formed, are expected to be absolutely safe for all kinds of medical applications. These findings may provide a great idea for developing a low-cost, environmentally friendly method of producing nanoparticles on a large scale using dairy products.

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