SYNTHESIS OF SOPHOROLIPIDS USING ORGANICALLY SYNTHESIZED CARBOXYLIC ACID AND THEIR APPLICATION IN SYNTHESIS OF SILVER NANOPARTICLES

Shabana K[1*] and B L V Prasad[2]

[1]*PG Department of Chemistry, KAHM Unity Women's College, Manjeri-676122, Kerala*

[2]*Physical & Material Chemistry Division, National Chemical Laboratory, Pune, Maharashtra*

[*]*E-mail: shabshabana@gmail.com*

Abstract: Sophorolipids as a body care products, therapeutic agents for cancer and immune disorders, oil recovery agents etc. In this study C14-COOH-SL prepared and checked its efficiency as a capping and reducing agent for silver nanoparticles. We found that this new sophorolipid is indeed capable of reducing Ag ions in to silver nanoparticles. Metal nanoparticles are known to be very important for many important applications. Preparing them and capping them with active molecules like SLs can enhance their utility and open new avenues for research and applications.

Introduction

Sophorolipids (SL) formerly named sophorosides is found and excreted into the culture medium by Candida or related yeast species and is a good surfactants. They were first described by Tulloch AP and found to be composed of a disaccharide moiety linked to one hydroxyl group of one ω or (ω -1)-hydroxy fatty acid (saturated or monounsaturated). The sugar (sophorose or 2-O-glucopyranosyl--D-glucopyranose) may further show mono- or diacetylation at the 6' and 6" positions. The nature of the hydroxy fatty acid is characteristic, with the hydroxyl group being located on the n or n-1 carbon atom. In general, fatty acids of carbon chain length of 16, 17 or 18 are used and subjected to modification to lead to different SLs by the composition of the growth medium. Sophorosides with unsaturated C18 fatty acids have been recognized in Candida bogoriensis. Sophorolipids exhibit surfactant activity because of their amphiphilic structure. Among the sophorolipid producers, Candida bombicola is the most studied species because it produces sophorolipid molecule in large quantities. Products yields up to 300 g of sophoroses per liter of culture medium have been reached. The composition of the hydroxylated fatty acid varies depending on the culture conditions. Furthermore, lactonization frequently occurs between the carboxyl group and the 4" OH group of the sophorose. Sophorolipids are used as bacteriocides in the formulation of skin and body-care products (deodorant, anti-acne ingredient, from Soliance) but these emerging biosurfactants may have many other application potentials such as use as a capping agent for providing stability to nanoparticles. Hydroxy fatty acids may be released from sophorolipids and lactonized into macrocyclic esters; they are used in the perfume and fragrance industry.

Materials and Methods

Chemicals used: ***Synthesis of sophorolipids*** [Malt extracts (Hi-media chemicals), Glucose extracts (Hi-media chemicals), Yeast extracts (Hi-media chemicals), Peptone (Hi-media

ISBN 978-93-5279-420-1

chemicals), Ethyl acetate, Sodium sulphate, Precursors: Oleic acid (Aldrich chemicals)], Pure culture of yeast; ***Synthesis of SL-Ag*** [Silver nitrate, Sophorolipids, Sodium borohydride, Potassium hydroxide].

Methods: ***MGYP broth (Composition of 100ml MGYP broth)*** Maltose extract: 0.3 g, Glucose extract: 2 g, Yeast extract: 0.3 g, Peptone: 0.5g, Millipore water: 100 ml

Organism: The fungal culture, Candida bombicola was used for the sophorolipid synthesis.

Synthesis of sophorolipids : Two to three weeks old culture of Candida bombicola grown on MGYP slant was used for the pre-inoculum preparation. Colonies were transferred to 20ml MGYP broth in a test tube and kept at room temp for 18 hrs, 2ml of this culture were added to each of 100 ml and 20 0ml MGYP broth in 500ml and 1000ml conical flask respectively and kept it on rotary shaker for 2 days. The biomass of culture grown for 2 days were separated by centrifugation at 5000 rpm for 10 minute at 20°C and these cells were redispersed in 100ml of 10% glucose solution and 10^{-3}M precursor (oleic acid, C14-1-OH, C12-1-OH) were added in each flask and kept on rotary shaker for 24hrs (All precursors are dissolved in 1ml ethanol). After 1day, 10^{-2}M of precursors in ethanol was again added to the respective flask and kept on rotary shaker for 3-4 days followed by addition of 50ml of 10% glucose in each flask and kept on shaker for 8-10 days till complete conversion of precursor to sophorolipid occurred.

Separation of sophorolipid: Sophorolipid formed was taken out and was collected in a test tube. Remaining sophorolipid along with biomass was collected as a pellet by centrifugation at 5000 rpm for 10 minute and supernatant was discarded. The pellet was redispersed in 100ml of 10% glucose and with 10^{-2}M respective precursor and kept on rotary shaker for 10-15 days until the formation of sophorolipid. The sophorolipid formed was then extracted by mixing with equal volume of ethyl acetate in a separating funnel, organic layer was dried over anhydrous sodium sulphate and the excess solvent was removed using rotary evaporation. A yellowish brown, honey like viscous liquid was obtained. This is the sophorolipid. The yield for a flask of 250ml of sophorolipid was approximately 1.045g. A minimum of three batches were required to make sufficient amount of sophorolipid enough for further analysis.

Synthesis of silver nanoparticles: The set of experiment performed are given in Table 1.

Table 1. ***Effect of capping and reducing agents on the formation of silver nanoparticles***

Sl. No.	*Capping agent*	*Substrate*	*Reducing agent*	*Result*	*Time taken for reduction*
1	C14-COOH (10^{-4}M)	$AgNO_3$ (10^{-4}M)	Nil	No colour	Nil
2	C14-COOH (10^{-4}M)	$AgNO_3$ (10^{-4}M)	NaBH4	Yellow	Instantaneous
3	C14-COOH (10^{-4}M)	$AgNO_3$ (10^{-4}M)	KOH	Yellow	After 10 minutes
4	C14-COOH-SL (10^{-4}M)	$AgNO_3$ (10^{-4}M)	Nil	No colour	Nil
5	C14-COOH-SL (10^{-4}M)	$AgNO_3$ (10^{-4}M)	$NaBH_4$	Yellow	Instantaneous
6	C14-COOH-SL (10^{-4}M)	$AgNO_3$ (10^{-4}M)	KOH	Yellow	After 5 hrs

ISBN 978-93-5279-420-1

Results and Discussion

1. ***UV-VISIBLE Analysis of Silver (Ag) nanoparticles and Sophorolipid capped Silver nanoparticles***

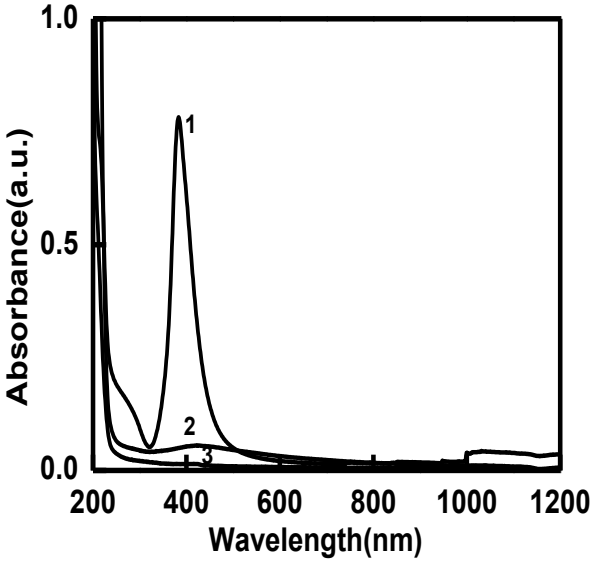

Fig. 1. UV-VISIBLE spectrum of Ag nanoparticle synthesized using C14-COOH of concentration 10^{-4}M with $NaBH_4$ (curve 1) and KOH (curve 2)

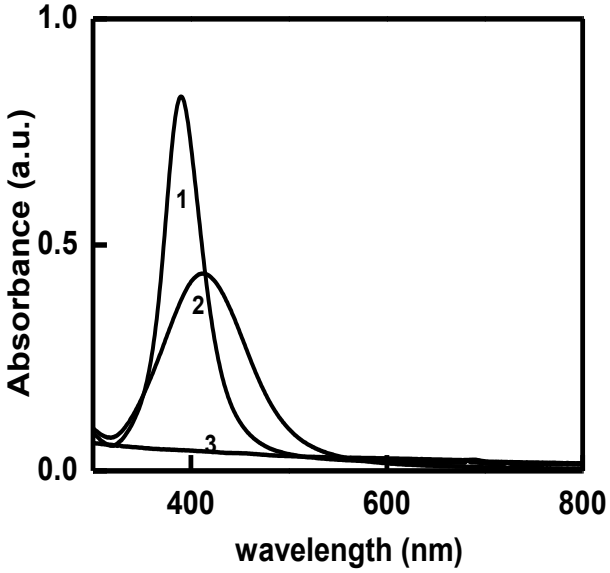

Fig. 2. UV-VISIBLE spectrum of Ag nanoparticles synthesized using C14-COOH-SL of concentration of 10^{-4}M with $NaBH_4$ (curve 1) and KOH (curve 2)

In Fig. 1,Curve 1 represent the silver nanoparticles synthesized using C14-COOH (10^{-4}M) as capping agent and $NaBH_4$ as reducing agent (C14-COOH+$AgNO_3$+$NaBH_4$), curve 2 represent the silver nanoparticles synthesized using C14-COOH (10^{-4}M) in presence of KOH (C14-COOH+$AgNO_3$+KOH) and curve 3 corresponds to C14-COOH (10^{-4}M) and $AgNO_3$ without any reducing agent (C14-COOH+$AgNO_3$). Curve 1 shows sharp peak at 380nm, which indicate the formation of Ag nanoparticles. Curve 2 shows peak positioned at 438nm, which indicate the formation of Ag nanoparticles and which also indicate the reduction can also occur with C14-COOH in presence of KOH. Curve 3 shows monotonous increase and no peak is observed, which implies that, when Ag^+ ions are exposed to C14-COOH alone there is no reduction.

In Fig. 2, curve 1corresponds to Ag nanoparticles synthesized using C14-COOH-SL (10^{-4}M) and $AgNO_3$ in presence of $NaBH_4$ (C14-COOH-SL+$AgNO_3$+$NaBH_4$), curve 2 represent the silver nanoparticles synthesized using C14-COOH-SL (10^{-4}M) and $AgNO_3$ in presence of KOH (C14-COOH-SL+$AgNO_3$+KOH) and curve 3 corresponds to C14-COOH-SL (10^{-4}M) and $AgNO_3$ in absence of reducing agent (C14-COOH-SL +$AgNO_3$). Curve 1 shows sharp peak at 390 nm, which indicate the formation of Ag nanoparticles. Curve 2 shows peak centered at 412nm, which indicate the formation of Ag nanoparticles and which also

ISBN 978-93-5279-420-1

indicate that the reduction can also occur with KOH. Curve 3 shows monotonous increase and no peak is observed, which implies that in absence of reducing agent no formation of silver nanoparticles take place.

2. *TEM Analysis*

The TEM images (Fig. 3) for sophorolipid capped Ag nanoparticles of a concentration of 10^{-4}M show that the particles were observed to be irregular in shape, polydisperse in nature and well separated from each other having an average particle size of 25nm.

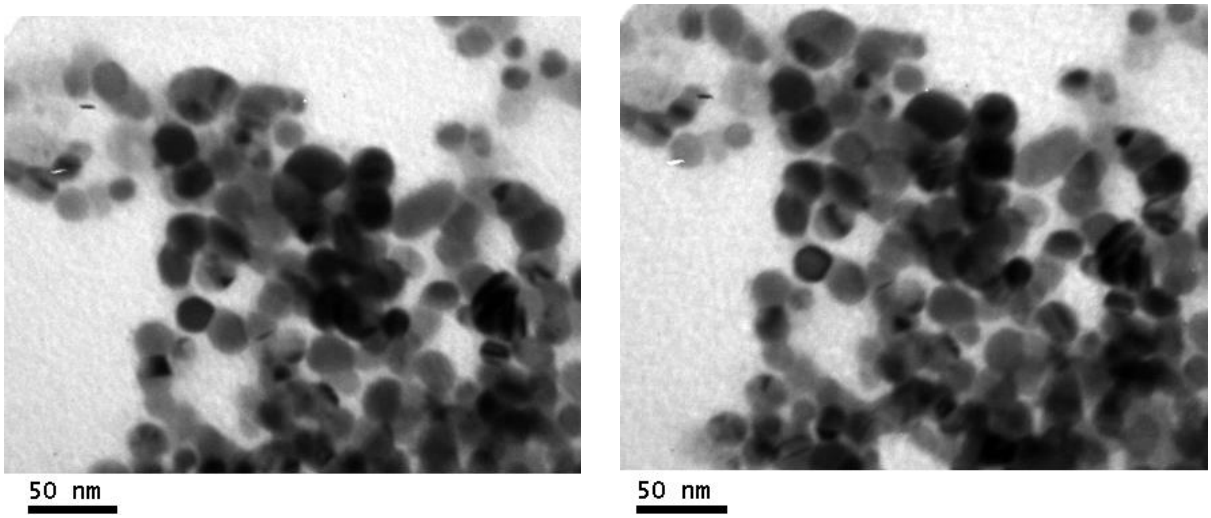

Fig. 3. TEM images of Ag nanoparticle synthesized using C14-COOH-SL with $NaBH_4$.

Conclusions

In this study, we have described the synthesis of C14-COOH-SL and its application as a capping and reducing agent on silver nanoparticles. It is characterized by using UV-VISIBLE Spectra. The TEM images indicate the presence of spherical, polydisperse silver nanoparticles capped with sophorolipids with average particle size of 25nm.

References

1. http://www.Nanoscience.com/education/overview.html.
2. www.nanotec.org.uk/
3. www.en.wikipedia.org/wiki/Nanotechnology.
4. New methods for the synthesis of magnetic nanoparticles- Thesis by Tanushree Bala University of Pune.
5. www.crnano.org/whatis.htm
6. Sophorolipid production by candida bombicola: Medium composition and culture methods- Journal of Bioscience and Bioengineering 88,No.5,488-494.1999.
7. Phadtare Sumant, Parekh Parag, Shah sachin, Tambe Amruta, Joshi Rohini, Sainkar S.R, Prabhune Asmita, and Sastry Murali Biotechnol. Prog. (2003) 19, 1659-1663.

ISBN 978-93-5279-420-1