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Research Article

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Bio-Efficacy of Synthesized Silver Nanoparticles against Food Spoilage Fungi by using Different Food Packaging Sheets

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ABSTRACT

The aim of present study was to develop food packaging sheets. In present experiment Blotting and Whatman paper sheets (No. 1) used as food packing material, initially these paper sheets soaked in aqueous solution of $AgNO_3$ and reacted with aqueous solution of $NaBH_4$ to generate Ag-nanoparticles in paper matrix. It was observed that generation of Ag-nanoparticles, XRD showed peak at 2θ value 38.14 with a size of 44nm. After 5 days of incubation, the untreated polythene showed maximum fungal colony (360) while minimum (4) in treated paper. The Ag-nanoparticles embedded Blotting paper sheets showed maximum percentage-inhibition against A. fumigatus, M. canis, A. niger.

Keywords*: Ag-nanoparticles, Silver nitrate, Food packaging, Aspergillus niger, Microsporum canis.*

INTRODUCTION

Nanotechnology, shortened to "nanotech", is the study of the controlling of matter on an atomic and molecular scale. Generally nanotechnology deals with structures sized between 1 to 100 nanometer in at least one dimension, and involve developing materials or devices within that size[1]. Nowadays, most materials used for food packaging are practically undegradable, representing a serious global environmental problem. New bio-based materials have been exploited to develop edible and biodegradable films as a big effort to extend shelf life and improve quality of food while reducing packaging waste[2].

Food packaging suggests is packaging of all types of food plays a vital role in the packaging industry. Food packaging is needed to provide protection as well as to market the food product. Some of the most exciting developments in food packaging involve nanotechnology; the science about very small materials is poised to have a big impact in food packaging materials. Nanoparticles can be applied as reactive particles in packaging materials. The nanosensors are able to respond to environmental changes (for example temperature or humidity in storage rooms, levels of oxygen exposure), degradation products or microbial contamination[3]. A novel microbial route to synthesize silver nanoparticles using fungus *Hormoconis resinae*[4]. Mycosynthesis of Silver Nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria[5].

Nano-sized innovation could produce remarkable new packaging concepts for barrier and mechanical properties, pathogen detection and active and intelligent packaging. At the forefront of nano-sized development in food packaging are nanocomposites[6]. Cellulose, the building material of long fibrous cells, is a highly strong natural polymer. Cellulose nanofibers are inherently a low cost and widely available material. Moreover, they are environmentally friendly and easy of recycling by combustion, and require low energy consumption in manufacturing. All of these makes cellulose nanofibers an attractive class of nanomaterials for elaboration of low cost, lightweight, and high-strength nanocomposites[7,8].

There are several wet chemical methods for creating silver nanoparticles. Typically, they involve the reduction of a silver salt such as silver nitrate ($AgNO_3$) with a reducing agent like Sodium borohydride ($NaBH_4$) in the presence of a colloidal stabilizer[9]. Polymer – Clay Nanocomposites (PCN) is also a novel food packaging substance. These are used now days for packaging because of their reduced weight, high tensile strength, heat resistant property, better barrier property against Carbon Dioxide, Oxygen, moisture and Ultraviolet along with the property of preserving the flavours in food and beverages[10-14].

Nanoparticles characterization is necessary to establish understanding and control of nanoparticles synthesis and applications. Characterization is done by using a variety of different techniques, common techniques are electron microscopy (TEM, SEM), atomic force microscopy (AFM)), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD) and so XRD characterization is made in present study insert after so on[15].

MATERIALS AND METHODS

The main purpose of the study was to prepare Ag-nanoparticles embedded paper sheets used, to examine the antifungal property of Ag-nanoparticles embedded paper sheets and explore their possible role in developing alternate, low-cost and more efficient food packaging products.

Preparation of Ag-nanoparticles embedded paper sheets

From the literature, it is evident that Ag-nanoparticles were generated from silver nitrate ($AgNO_3$) when it was reacted with sodium borohydride ($NaBH_4$). In this reaction not only the nature of reluctant but even its conc., with respect to metal conc. in solution, is also important. By regulating the conc., metal nanoparticles of desired dimension were generated. Nanoparticles were created in the system within the pores of paper matrix. In this experiment, Blotting paper sheets and Whatman (No.1) filter paper sheets were immersed in aqueous solution of silver nitrate in different conc. (0.01 M, 0.03 M, 0.1 M & control) for one min, followed by rinsing with Ethanol for 30 second. After which, these sheets were soaked in aqueous solution of Sodium borohydride ($NaBH_4$) (200 mM) and then rinsed in pure and sterilized water for 1 min. For control, Blotting and Whatman sheets were not treated with silver nitrate and sodium borohydride. The obtained specimens were dried overnight at room temperature.

Characterization:

X-ray diffraction analysis

To validate the hypothesis that silver nanoparticles are generated by the reaction employed, in a experiment, aqueous solution phase reaction of sodium borohydride with silver nitrate were performed and the resulting precipitate were collected through filtration. Then XRD analysis of this residue was attempted.

Efficacy of silver nanoparticles embedded paper sheets for food packaging

Ag-nanoparticles embedded paper sheets were used as packing alternative for white bread. Freshly procured fresh bread slices (2 gm) were packed in Ag- nanoparticles embedded sheets. For the sake of comparison, the experiment was repeated with other more conventionally used packaging sheets also such as, untreated blotting paper sheets, Polythene sheets and untreated Whatman filter paper sheets. The packed pieces of bread were stored under room condition for 10 day. After 10 day, the bread pieces were taken out and weighed to assess moisture loss and the fungal growth in the samples was estimated by colony counter method as follows:-

Colony counter method

1 gm of each food sample was dispersed in 50 ml of distilled and sterilized water. The content was stirred vigorously for 10 min to get uniform suspension. After stirring, the content was filtered to get the fungal suspension; fungal conc. was determined by incubating one ml of fungal suspension in SDA (Sabouraud's Dextrose Agar) medium for 24-42 h at 26-28 °C for fungal growth the number of fungal colony per gram of food sample was counted after several days (1^{st}, 2^{nd}, 3^{rd}, 4^{th}& 5^{th} day) of incubation period[16].

Identification of Fungi

For the identification of fungus present in food sample (bread) after 10 day of self-keeping, Lacto phenol cotton blue was a stain commonly used for making semi- permanent microscopic preparation of fungi[17].

Assessment of antifungal effects via zone inhibition-paper disc method

In order to further asses the efficacy of Ag-nanoparticles as antifungal agent, with the prospect of their use as performance enhancing agent in food packaging material, zone inhibition study was also performed[18]. SDA medium was spread uniformly in petridishes and allowed to solidify. With the help of spreader, spread fungus culture evenly over it. Further, circular disc of 1 cm dia of Ag-nanoparticles embedded Blotting and Whatman papersheets were placed over the top. This was performed in aseptic conditions. These petridishes were incubated for 24 h at 28 °C temperature. After the desired incubation, the petridishes were examined to observe the effect of Ag-nanoparticles on growth of fungi. The circular zones of reduced or no fungal activity were expected around the areas where such discs were placed.

RESULT AND DISCUSSION

X-ray diffraction analysis

In order to confirm the generation of silver nanoparticles (SNPs) in the treated paper matrix, it was clear from the observation of XRD (x-ray powder diffraction) (Figure 1), the pattern of peak that the reaction of silver nitrate with sodium borohydride ($NaBH_4$) showed the Ag-nanoparticles (The peak obtained at 2θ value 38.14) and the size of Ag-nanoparticles was 44 nm (De bye Scherrer formula) in 0.1 M conc. of $AgNO_3$ which is reduced with $NaBH_4$.

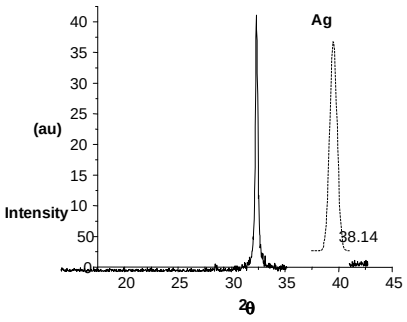

Figure 1-Observed XRD pattern of the Powered Residue obtained on reaction of $AgNO_3$ with $NaBH_4$

Scherrer's formula to determine mean particle size. Its formula:-

$d = 0.9 \lambda / \beta \cos \theta$, Where, d= mean diameter of the nanoparticles

λ = wavelength of x-ray radiation source, β = the angular FWHM of the XRD peak at the diffraction angle θ

Silver nanoparticles embedded paper sheets for food packaging

The untreated paper sheets showed more fungal colony followed by silver nanoparticles embedded paper sheets. The maximum fungal colony showed by polythene sheets (360) followed by untreated Blotting paper sheets (350), treated Whatman paper sheets in Table 1 and the minimum fungal colony showed by silver nanoparticles embedded Blotting paper sheets in Table 1 at 0.1M conc. of $AgNO_3$ as compare to untreated paper sheets, after 5 day of incubation period. After 10 days of self keeping, change in weight of bread pieces packed with silver nanoparticles embedded Blotting paper, Whatman paper, polythene sheet, untreated Blotting and untreated Whatman paper sheets. The maximum weight was 1.92 gm in silver nanoparticles embedded Whatman paper sheet whereas, minimum was 1.78 gm in untreated blotting paper sheet. The microscopic examination of fungi obtained in this study revealed the presence of most of the *Aspergillus* species including *Aspergillus flavus, Aspergillus sulphurious, Aspergillus niger, Aspergillus fumigatus* and *Saccharomyces* sps.

Maximum number of colony indicates the presence of food poisoning fungi in Table 1.

Table 1: Effect of silver nanoparticles (SNPs) on the fungal growth in white bread packed at ambient condition with different packaging material. (-) denotes absence of fungal colony

Packaging Material used	Incubation period (d)	Untreated Whatman paper sheets (No. 1)	Untreated Blotting Paper	Transparent polythene sheets	SNPs embedded Blotting paper sheets(Conc. of $AgNO_3$)			SNPs embedded Whatman paper sheets (No. 1) (Conc. of $AgNO_3$)		
					0.01M	0. 03M	0.1 M	0.01M	0. 03M	0.1 M
Number of fungal colony per gram of food sample	1	-	-	-	-	-	-	-	-	-
	2	1	1	4	1	-	-	1	3	-
	3	1	6	8	5	4	2	3	6	1
	4	1	8	14	5	4	5	10	9	2
	5	6	350	360	6	5	10	20	20	4

Zone inhibition and colony counting

The silver nanoparticles embedded Blotting as well as Whatman paper showed maximum percentage inhibition at 0.1 M conc. followed by 0.03 M and 0.01 M conc. of $AgNO_3$ in Table 2. Silver nanoparticles embedded Blotting paper sheets showed maximum percentage inhibition (35.48%) at 0.1 M conc. and minimum (24.81%) at 0.01 M conc. against *Aspergillus fumigatus*. Silver nanoparticles embedded whatman (No.1) paper sheets showed maximum percentage inhibition (30.07%) at 0.1 M conc. and minimum (16.67%) at 0.01 M conc. against *Aspergillus fumigatus* (Figure 2) in Table 2. Silver nanoparticles embedded Blotting paper sheets showed maximum percentage inhibition (35.48%) at 0.1 M conc. and minimum (04.76%) at 0.01 M conc. against *Microsporum canis* in Table 2. Grevenstuk[19] found a *Drosera intermedium* is the source of an interesting compound for the food industry as an alternative to preservatives.

Silver nanoparticles embedded Whatman paper sheets (No.1) showed maximum percentage inhibition (27.54%) at 0.1 M conc. and minimum (13.04%) at 0.01 M conc. against *Microsporum canis* in Table 2. Silver nanoparticles embedded Blotting papersheets showed maximum percentage inhibition (27.54%) at 0.1M conc. and minimum (4.76%) at 0.01M conc. against *Aspergillus niger* (figure 3). SNPs embedded Whatman paper sheets (no.1) showed maximum percentage inhibition (20.00%) at 0.1M conc. and minimum (4.76%) at 0.01M conc. against *Aspergillus niger* in Table 2. In case of SNPs embedded papersheets both Blotting as well as in Whatman Paper sheets at lower conc., the antifungal effect was less and as the conc. of $AgNO_3$ solution increases, the antifungal effect was also increases. This was probably suggestive of the fact that it was not the conc. but the size of particle also, which was more crucial for antifungal effect. Another two antimicrobial mechanisms were proposed by Rabea[20] *et al.*, chelation of trace metals by chitosan, inhibiting enzyme activities and in fungal cells, penetration through the cell wall.

Table 2: Antifungal activity of silver nanoparticles embedded Blotting and Whatman (No.1) paper sheets against food spoilage fungi, with different conc. (conc.) of silver nitrate

Ag-nanoparticles embedded paper sheets	Name of fungi	$AgNO_3$ Conc. (M)	Paper disc (cm)	Zone of Inhibitin (cm)	Percentage zone of inhibition (%)	Ag-Loaded Paper sheets	Dia of zone (cm)	Zone of Inhibition (cm)	Percent-age zone of Inhibition (%)
Blotting paper sheets	*A.flavus*	0.01M	1.33	0.33	24.81%	Whatman	1.20	0.20	16.67%
		0.03M	1.53	0.53	34.64%	(No.1)	1.35	0.35	25.92%
		0.10M	1.55	0.55	35.48%	paper sheet	1.43	0.43	30.07%
Blotting paper sheet	*M.canis*	0.01M	1.05	0.05	04.76%	Whatman	1.15	0.15	13.04%
		0.03M	1.10	0.10	09.09%	(No.1)	1.20	0.20	16.67%
		0.10M	1.55	0.55	35.48%	paper sheet	1.38	0.38	27.54%
Blotting paper sheets	*A.niger*	0.01M	1.05	0.05	04.76%	Whatman	1.05	0.05	04.76%
		0.03M	1.10	0.10	09.09%	(No.1)	1.20	0.20	16.67%
		0.10M	1.38	0.38	27.54%	Paper sheet	1.25	0.25	20.00%
Control			0.00	0.00	0.00%	Control	0.00	0.00	0.00%

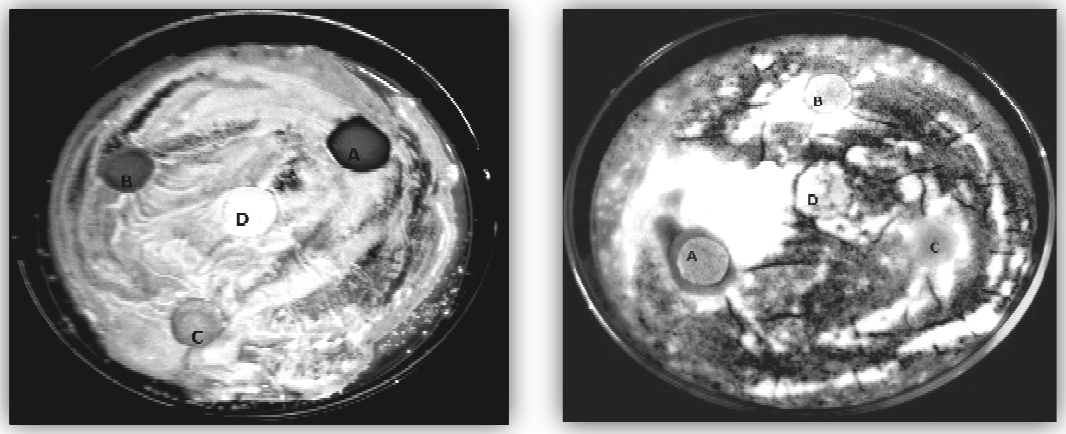

Figure 2- Antifungal activity of AgNPs embedded Whatman papersheets against *A. fumigatus* with different conc. (A = 0.1 M, B = 0.03 M, C = 0.01 M, D = control) of $AgNO_3$. Figure 3- Antifungal activity of AgNPs embedded Blotting papersheets against *A. niger* with different conc. (A = 0.1 M, B=0.3 M, C=0.01 M.

CONCLUSION

This study was prompted from the fact that antifungal behavior of silver nanoparticles can be exploited to generate low cost and more efficient alternatives to conventional packaging product. Silver nanoparticles (SNPs) embedded paper sheets were developed by aqueous solution phase reaction of Silver nitrate with Sodium borohydride. The result clearly highlight that silver nanoparticles have the high antifungal activity and the size of particle also more crucial for antifungal effect. Nanoparticles of suitable dimension (44 nm) can pave the way to produce more efficient packaging alternative for food items.

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REFERENCES

1. Buzea, C. Pacheco, II. Robbie, K. Biointerphases., **2(4)**: MR17-71. (2007)
2. Tharanathan, R. N., Biodegradable films and composite coatings: past, present and future. *Trends in Food Sci & Tech* **14(3)**: 71–78 (2003)
3. Bouwmeester, H. Dekkers, Noordam S. M. Y, W. I Hagens, A. S. Bulder, C. de. Heer, S. E.Voorde, S. W. Wijnhoven, H. J. Marvin, A. *J. Sips,* **53(1)**: 52–62 (2009)
4. Varshney, R., Mishra, A.N. Bhadauria S. Gaur, M.S. *Digest J of Nano and Biost.* **4**: 349 – 355 (2009)
5. Ingle, A. Gade, A. Pierrat, S. Sonnichsen, C. Rai, M. *Curr Nanosci.* **4(4)**: 141-144 (2008)
6. Aaron, B.L. Bugusu, B. Jung, H. Claire Koelsch Sand, Tara, H. mchugh, H. **73(8)**: R107-116 (2008)
7. Helbert, W. C. Y. Cavaille, A. Dufresn. Polymer Composites **17(4)**: 604–611 (1996)
8. Podsiadlo, P. Choi, S. Shim, Y. B. Lee, J. Cuddihy, M. Kotov, N. A. **6**: 2914–2918 (2005)
9. Sureshkumar, M. Yovita, D. S. Lee, C. K. J. *Mater Chem.,* **20**: 6948-6955 (2010)
10. Moore, S. Modern Plastics **76**: 31 – 32 (1999)
11. Quarmley, J. Rossi, A. Industrial Minerals, **400**: 47 – 49 (2001)
12. Moraru, C.I. Panchapakesan, C.P. Huang, Q. Takhistov, P. Liu, S. Kokini, J.L., Nanotechnology: a new frontier in food science. Food Technol. **57**: 24–9 (2003)
13. Lauge, J. Wyser, Y. *Packaging Technol. and Sci.***16**: 149 – 158 (2003)
14. Ranade, A. D'souza, Gnade, N.A. B. Dharia, *A. J. Plastic film and Sheeting*, **19**: 271 (2003)

15. Fahlman, B. D. Materials Chemistry, Springer: Mount Pleasant, Michigan, 1st ed. 282–283,2007.
16. R. Sabouraud Sur la radiotherapie des teignes. Annales de dermatologie et desyphiligraphie, Paris, **5**: 577-587
17. Aneja, K. R. India, **64**: 351-356 p (1993)
18. Okigbo, R.N. Mbajiuka, C.S. Njoku, C.O.J. Mole. *Adv. Sci.***1(4)**: 392-397 (2005)
19. Rabea, E. I. Badawy, M. E. Stevens, C. Smagghe, V. G. Steurbaut, W. Biomacro. **4(6)**: 1457–1465 (2003)
20. Grevenstuk, T. Gonçalves, S. Domingos, T. Quintas, C. J. J. van der Hooft, Vervoort, J. Romano, *A. J Sci Food Agric*, **92(8)**: 1638-42 (2012)