European Journal of Biotechnology and Bioscience

Volume: 3, Issue: 6, 01-05
June 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

Meignanalakshmi Sundaram
Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamilnadu, India.

Legadevi R
Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamilnadu, India.

Afrin Banu N
Department of Biotechnology, St.Joseph's College of Engineering, Old Mamallapuram, Chennai, Tamilnadu, India.

Gayathri V
Department of Biotechnology, St.Joseph's College of Engineering, Old Mamallapuram, Chennai, Tamilnadu, India.

Palanisammy A
Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamilnadu, India.

Correspondence:
Meignanalakshmi Sundaram
Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamilnadu, India.

A Study on Anti Bacterial Activity of Keratin Nanoparticles from Chicken Feather Waste Against *Staphylococcus aureus* (Bovine Mastitis Bacteria) and its Anti Oxidant Activity

Meignanalakshmi Sundaram, Legadevi R, Afrin Banu N, Gayathri V, Palanisammy A

Abstract
In the present study Keratin nanoparticles were synthesised from chicken feathers. The structure and morphological characteristic of keratin nanoparticles were investigated by using SEM and XRD. X-ray crystallography showed the crystalline peak at 37.5 degree, which indicates the presence of keratin nanoparticle. Crystalline size of the keratin nanoparticle was found to be 79.6 nm. Anti oxidant activity of keratin nanoparticles was found to be 78 % and 88% by DPPH and Superoxide method respectively. Antibacterial activity of keratin nanoparticle was identified by well diffusion method against bovine mastitis, *Staphylococcus aureus* isolated from milk and *E.coli*. The zone of inhibition was found to be 11 mm in diameter against *Staphylococcus aureus* and 9.5 mm against *E.coli* at 100µg/ml concentration.

Keywords: Keratin Nanoparticles, Chicken Feather, XRD, SEM, Antibacterial Activity, Antioxidant Activity

Introduction
Chicken feathers are the waste products from the poultry farms. Billions of feathers are generated each year by poultry processing plants; creating a serious solid waste problem [4, 21]. Five percent of the body weight of chicken is feathers. Poultry feathers are discharged to the environment which leads to pollutions and also cause human ailments such as chlorosis, fowl cholera [10, 3]. Even though, feathers are considered as waste products it contains large amount of proteins. So it is converted in to an animal feed such as feather meal [5, 13]. Chicken feather approximately contains 91% of keratin, 1% of lipid and 8% of water. The presence of keratin in chicken feather is very inconvenient and troublesome waste product of the poultry farming industry [9, 1]. Therefore it is presently objective of intensive investigation in many research centres. Keratin from the chicken feathers show elevated content of amino acid such as glycine, alanine, serine, cysteine and valine, but lower amount of lysine, methionine and tryptophan [11, 28].
Bovine mastitis is an inflammatory condition that affects the mammary glands of cows [40, 26]. It has a significant impact on milk quality, animal welfare and animal production [2, 7]. Several Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* are responsible for this pathology. Bovine mastitis is considered as one of the main causes of economic losses in the dairy industry [17]. The successful control of this condition generally relies on treatment with antibiotics [18]. Therefore, it is necessary to discover novel and safe compounds for the control of this disease.
Nanotechnology is an emerging field that can be used in drug delivery, food and cosmetics [24, 25]. Nanoscale materials have received attention as novel antimicrobial agents due to their high surface area to volume ratio and the unique chemical and physical properties [12, 15]. It is also used for targeted drug Delivery. Recently, the research on the preparation of nanoparticles and characterization is given much importance [41, 19]. Keratin is a fibrous structural protein that has been used in cosmetics such as shampoo, anti-aging cream and hair conditioners [30]. In the present study, keratin nano particles were synthesized from chicken feather waste, antibacterial and antioxidant property of keratin nanoparticles were studied.

Experiment Details

Keratin Extraction

Chicken feathers were collected from poultry shop. They were washed with detergent solution and distilled water followed by 70% ethanol. They were cut into small pieces and then air dried. Dried chicken feathers were dissolved in 5% NaOH solution and it was incubated for 4hrs at 40 ºC. Then it was filtered by using Whatman filter paper. Filtrate was lyophilized and stored.

Protein Estimation

To confirm the presence of protein in the extract, it was estimated by using Biuret method [22]. Bovine serum albumin was used as standard solution.

Preparation of Keratin Nanoparticles

100 mg of isolated keratin was suspended in 2ml of deionised water. 8ml of absolute ethanol was added to it under constant stirring.1 µl of 8% glutaraldehyde was added to it for the formation of keratin nanoparticles and it was stirred for 24hours. Nanoparticles were collected by centrifugation at 20,000rpm for 20 min. The prepared keratin nanoparticle was lyophilized and stored [23].

Characterization of Keratin Nanoparticle

The Keratin nanoparticles were characterized on X-per PRO diffractometer (PAN analytical) Philips 1710 using Cu Kα radiation [21].The crystalline size of the keratin nanoparticle was calculated using Debay Scherrers formula [20].

$$D = \frac{0.94\lambda}{\beta COS\beta}$$

The micro structure and the morphological studies of the lyophilized keratin powder were carried out using a scanning electron microscope (HITACHI model S-3000H) [31]

Antioxidant activity of Keratin Nanoparticle using DPPH method

Anti oxidant activity of keratin extract and keratin nanoparticles at different concentration were examined by using DPPH free radical scavenging activity. Ascorbic acid was used as a standard solution. The absorbance was read at 517 nm [35]. The percent activity was calculated using the following formula

$$\%\ of\ free\ radical\ scavenging\ activity = \frac{control\ OD - sample\ OD}{control\ OD} * 100$$

Antioxidant activity of Keratin Nanoparticles using superoxide method

Super oxide radical scavenging activity is another method for the determination of antioxidant activity [33, 29]. Control was prepared using riboflavin. Absorbance was noted at 590nm [32, 37]. Anti oxidant activity was studied for keratin extract, keratin nanoparticle and the standard riboflavin.

Anti Bacterial Activity of Keratin Nanoparticle

The anti bacterial activity of keratin nanoparticles and keratin extract was tested against *Staphylococcus aureus*, isolated from bovine mastitis milk and *Escherichia coli* by well diffusion method [34, 36]. 100µg/ml of synthesized keratin nanoparticles were used for antibacterial activity studies.

Results and Discussion

Keratin Extraction

The concentration of keratin extracted by using NaOH was found to be 11 mg/ml `by biuret method [39].

SEM Analysis

The keratin nanoparticles was characterized by SEM analysis (Fig.1). The size of the keratin nanoparticle was 78 nm.

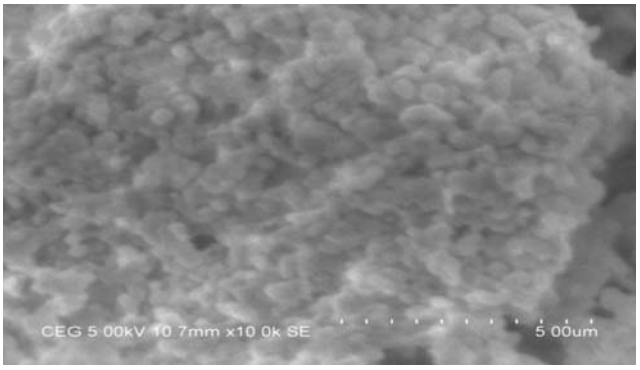

Fig 1: Scanning electron microscopic analysis of keratin Nanoparticles

X-Ray Diffraction:

The X-ray diffraction studies of keratin nanoparticles showed a strong peak at 37.5 degree (Fig.2), which indicated the presence of keratin nanoparticles. The crystalline size of the keratin nanoparticle was 79.6 nm.

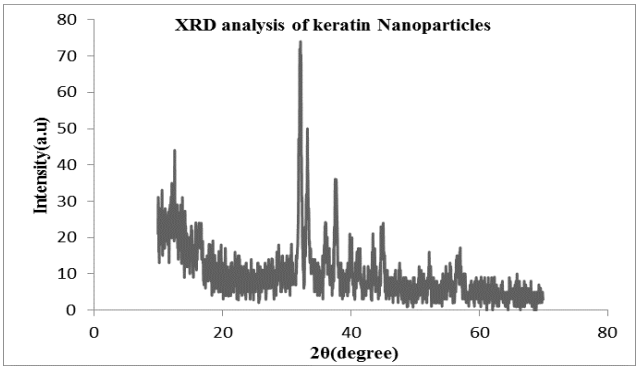

Fig 2: XRD analysis of Keratin nanoparticles.

Antioxidant Activity of Keratin nanoparticles using DPPH assay

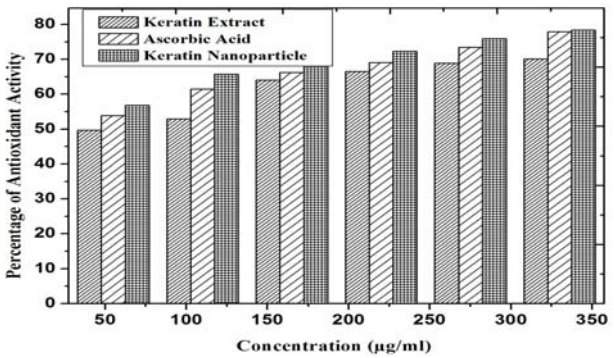

Fig 3: Antioxidant activity of keratin nanoparticles using DPPH assay

DPPH is a stable free radical that shows maximum absorbance at 517 nm [6, 7]. The DPPH free radical-scavenging activity was investigated at various concentrations such as 50,100, 150, 200,250 300 and 350 µg/ml. The maximum percentage of radical scavenging activity for keratin extract and ascorbic acid [9] was 70.13% and 77.9% at 350 µg/ml (Fig.3). The percentage of antioxidant activity increased with respect to the concentration of keratin extract, keratin nanoparticle. The percentage of free radical scavenging activity for keratin nanoparticles is 78.37 % at 350 µg/ml which is higher than the antioxidant activity of keratin extract. In the previous studies, Bunghez et.al [8] reported that Nanoparticles smaller in size, then having a larger total surface area, were more efficient in the antioxidant activity tests as compared with nanoparticles with bigger size. Thus, red carnation-AgNPs, the smallest AgNPs, possess the lower antioxidant activity: AA = 88.3%).

Antioxidant Activity of Keratin Nanoparticles by Super Oxide Method

Super oxide radical scavenging activity of keratin extract and keratin nanoparticles were measured by the riboflavin-NBT-light system invitro [37, 38]. Antioxidant activity was investigated at various concentrations such as 50,100, 150 200,250 300 and 350 µg/ml (Fig. 4) showed the maximum percentage of radical scavenging activity of keratin extract and riboflavin as 83.13% and 76.2% at 350 µg/ml concentration respectively. The increase in concentration of keratin nanoparticle increases the anti oxidant activity. Antioxidant activity of keratin nanoparticle was maximum 88.99% at 350 µg/ml concentration.

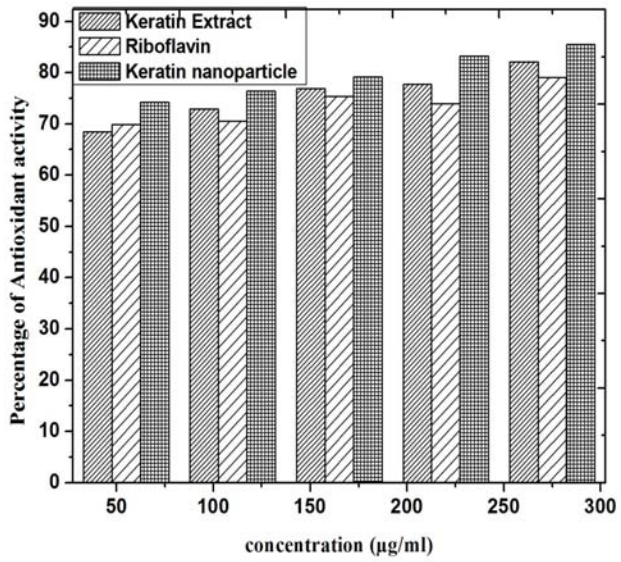

Fig 5: Antioxidant activity of keratin nanoparticles using superoxide assay

Anti Bacterial Activity of Keratin Nanoparticle against *Staphylococcus aureus* and *E.coli*

Antibacterial activity of keratin nanoparticles was studied against *Staphylococcus aureus* (Bovine mastitis pathogen) and *E.coli*. Zone of inhibition formed for keratin nanoparticles was higher than the keratin extract (Table.1). The radius of zone of inhibition for keratin nanoparticles against *Staphylococcus aureus* and *E.coli*. was 9.5 mm and 11mm at 100 µg/ml.

Table.1 Antibacterial activity of Keratin nanoparticle and keratin extract against *Staphylococcus aureus* and *E.coli*.

Organism	Concentration (µg/ml)	Zone of inhibition(mm)	
		Staphylococcus aureus	E.coli
Keratin Extract	25	4.5	5
	50	5	6
	75	6	6.5
	100	7.5	9
Keratin Nanoparticles	25	6	6.5
	50	7	8
	75	8.5	9
	100	9.5	11

Kush boo *et al.*, [16] studied the antibacterial activity for silver Nanoparticles's against multi drug resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. They reported that inhibition zone was 11mm for 10µg dose of silver nanoparticles. Kamyar *et al.*, [14] studied different stirring times on anti bacterial activity of silver nanoparticles in poly ethylene glycol (PEG) suspension. The anti bacterial activity of different sizes of silver nanoparticles against Gram positive (*Staphylococcus aureus)* and Gram negative (*Salmonella typhimurium)*. They reported that average inhibition zone observed against S.*aureus* & S.*typhimurium* was found to be 11.56 and 10.64 mm. Mercy *et al.*, [21] carried out the antimicrobial activity of biosynthesised silver nanoparticle for four human pathogens such *as klebsiella Pneumonia, Escheria coli, Staphylococus Saprophyticus and Bacillus cereus.K. Pneumonia and E.coli* and the zone of inhibition was 12, 10 and 8 mm

Conclusions

Utilization of chicken feather leads to the waste management in poultry industry. Keratin nanoparticles synthesized from chicken feathers were found to be having good antibacterial and antioxidant activity

References

1. Adriano Brandelli, Alessandro Riffel (2005), Production of an extracellular keratinase from *Chryseobacterium* sp. growing on raw feathers, *Electronic Journal of Biotechnology*, vol(8),no.1,pp.0717-3458.
2. Almeida, L.S.B.; Murata, R.M.; Yatsuda, R.; Dos Santos, M.H.; Nagem, T.J.; Alencar, S.M.; Koo, H.; Rosalen, P.L. Antimicrobial activity of *Rheedia brasiliensis* and 7-epiclusianone against *Streptococcus mutans*. *Phytomedicine* 2008, *15*, 886–891.
3. Andrew J.poole, Jeffrey S.church (2015), the effects of physical and chemical treatments on Na2S produced feather keratin films, *International journal of Biological macromolecules*, vol 73, pp. 99-108.
4. Arun Gupta,ramanan Perumal, Rosli Bin Mohd Yunus & Nuruldiyamah Bintu Kamarudin (2011), Extraction of keratin from chicken feather, Inchemeca.
5. Ayako Kurimoto, Toshizumi Tanabe, Akira Tachibana, Kiyoshi Yamauchi(2003),Keratin sponge Immobilization of lysozyme, *Journal of bioscience and bioengineering*, vol(96),issues 3,pp.307-309.
6. Barone JR, Schmidt WF(2006),Effect of formic acid exposure on keratin fiber derived from poultry feather biomass. *Bioresour Technol.*, vol(97),pp.233–42.
7. Baskaran, S.A. Kazmer, G.W. Hinckley, L. Andrew,

S.M. Venkitanarayanan, K. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. *J. Dairy Sci.*2009, *92*, 1423–1429.
8. Bunghez. I. R., 2012.Antioxidant silver nanoparticles green synthesized using ornamental plants *Journal of Optoelectronics and Advanced Materials* Vol 14 (11-12), pp.1016 - 1022
9. Dr.Sundaram Meignanalakshm, Huldah J, Palanisammi A and Dhinakar Raj G Antioxidant Activity of Papain Hydrolysates of Oyster Mushroom (Pleurotus Ostreatus) Protein. *World journal of pharmaceutical* research, vol 3(4), pp.641-648.
10. Endo R, Kamei K, Iida I, Kawahara Y(2008),Dimensional stability of waterlogged wood treated with hydrolyzed feather keratin, J *Archaeol Sci*, vol(35),pp.1240–46.
11. Heide, H. W, Werner E, and Franke, W. (1986). The complement of native alphakeratin polypeptides of hair-forming cells: a subset of eight polypeptides that differ from epithelial cytokeratins, Differentiation, vol(32),pp 101-119.
12. Hillerton, J.E. Berry, E.A. Treating mastitis in the cow—A tradition or an archaism. *J. Appl.Microbiol.* **2005**, *98*, 1250–1255.
13. Jillian G. Rouse, Mark E. Van Dyke (2010), A Review of Keratin- Based Biomaterials for Biomedical Applications,*Materials,* vol(3),pp. 999-1014.
14. Justin J.Martin, Jeanette M.Cardamone, Peter L.Irwin, Eleanor M.Brown (2011),Keratin capped silver nanoparticles-synthesis and characterization of a nanomaterial with desirable handling properties, *colloids and surfaces B: Biointerfaces*, vol(88),pp 354-361.
15. Kamyar Shameli1, Mansor Bin Ahmad1, Seyed Davoud Jazayeri, Parvaneh Shabanzadeh, Parvanh Sangpour,Hossein Jahangirian and Yadollah Gharayebi (2012),Investigation of antibacterial properties silver nanoparticles prepared via green method, *Chemistry Central Journal*, vol(6),pp.73.
16. Katoh, K, Tanabe, T, and Yamauchi, K(2004) Novel approach to fabricate keratin sponge scaffolds with controlled pore size and porosity, *Biomaterials*, vol(25),pp.4255-4262.
17. Khushboo Singh, Manju Panghal, Sangeeta Kadyan, Uma Chaudhary and Jaya Parkash Yadav(2014), Antibacterial Activity of Synthesized Silver Nanoparticles from Tinospora cordifolia against Multi Drug Resistant Strains of Pseudomonas aeruginosa Isolated from Burn Patients,*Nanomedicine & Nanotechnology,* vol(5),pp.2**.**
18. Krystyna Wrzesniewska-tosik, Janisz Adamiec (2007) Biocomposites with a content of keratin from chicken feathers, *the institute of biopolymers and chemical fibres*, vol.15, No.1 (60).
19. Kuzma, L, Rozalski, M. Walencka, E. Rozalska, B. Wysokinska, H. Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclarea* L.: Salvipisone as a potential anti-biofilm agent active against antibiotic resistant *Staphylococci*. *Phytomedicine* 2007, *14*, 31–35.
20. Laport, M.S. Marinho, P.R. da Silva Santos, O.C. de Almeida, P. Villela Romanos, M.T. Muricy, G. Vasconcelos Paiva Brito, M.A.; Giambiagi-deMarval, M. Antimicrobial activity of marine sponges against coagulase-negative staphylococci isolated from bovine mastitis *Vet.Microbiol.* 2012, *155*, 362–368.
21. Lynch, M. H., O'Guin, W. M., Hardy, C., Mak, L., and Sun, T. T.:(1982) Acidic and basic hair/nail ("hard") keratins: their co localization in upper cortical and cuticle cells of the human hair follicle and their relationship to "soft" keratins, *J. Cell. Biol.,* vol(103), pp.2593-2606.
22. Mercy ranjitham. 2013 Invirto evaluation of anti oxidant, antimicrobial, anticancer activities and characterization of *Brassica oliracea.* var. borteytis. synthesised silver nanoparticles. International journal of pharmaceutical and pharmaceutical sciences Vol 5(4), pp(1-13)
23. Menandro N.Aeda (2011), Waste chicken feathers as reinforcement in cement bonded composites, *Philippine Journal of Science*, vol.139, pp.161-166.
24. Newman, D.J. Natural products as leads to potential drugs: An old process or the new hope for drug discovery? *J Med. Chem.* 2008, *51*, 2589–2599.
25. R.D Bruce Frase, David A.D. Parry (2008), Molecular packing in the feather keratin filament, *International journal of Biological macromolecules*,vol 162,pp.1-13.
26. R.Karthikeyan, S.Balaji and P K Shegal, Industral applications of keratin- A review, *journal of scientific & industrial Research,* vol(66), pp. 710-715.
27. Roberta Fontoura, Daniel J. Daroit, Ana P.F. Correa, Stela M.M. Meira, Mauricio Mosquera3 and Adriano Brandelli (2014) Production of feather hydrolysates with antioxidant, angiotensin-I converting enzyme- and dipeptidyl peptidase-IV- inhibitory activities, *New biotechnology*, vol(31).
28. Saravanan s, D.K.Sameera, A.Moorthi, N.Selva Murugan (2013), Chitosan scaffolds containing chicken feathers keratin nanoparticles for bone tissue engineering, *International journal of Biological macromolecules*, vol.62, pp.481-486.
29. Salminen, E & Rintala J(2002),Anaerobic digestion of organic slaughter house waste, *Bioresource technology*, vol(83),pp 13-26.
30. Saravanan S, Sricharan nethala, Soumiri pattanaik, Anjali tripathi,Ambigapathi moorthi,N.selvamurugan(2011),Preparation, characterization and anti microbial activity of a biocomposite scaffold containing chitosan/nano hydroxypatite/nano silver for bone tissue engineering, *International journal of Biological macromolecules*, vol.49,pp.188-193.
31. Stephan Reichi(2009),Films based on human hair keratin as substrate for cell tissue and bone tissue engineering,*Biomaterials*,vol(30),pp 6854-6866.
32. Stephan Reichi(2009),Films based on human hair keratin as substrates for cell tissue and tissue engineering, *Biomaterials*, vol(30),pp 6854-6866.
33. Tachibana, A., Furuta, Y., Takeshima, H., Tanabe, T., and Yamauchi, K (2002),Fabrication of wool keratin sponge scaffolds for long-term cell cultivation, *J. Biotechnol.,* vol(93), pp.165-170.
34. Thannhauser TW, Konishi Y, Scherega HA(1984). Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Anal Biochem* ;vol(8),pp.138-181
35. Vasant Naidu, Ahamed Kandu Sahib S.K.A, Sivabharathy M, Legadevi R, Senthil Kumar A Synthesis and Characterization of Novel Nano Ceramic Magnesium Ferrite Material Doped with Samarium and

Dysprosium for Designing–Microstrip Patch Antenna, *Defect and Diffusion Forum, Trans Tech Publications*, Vol. 332 (2012) pp 35-50.

36. Wang, Q., Yu, H., Zhong, L., Liu, J., Sun, J., Shen, J., 2006. Incorporation of silver ions into ultrathin titanium phosphate films: in situ reduction to prepare silver nanoparticles and their antibacterial activity. *Chem. Mater*. Vol(18),pp. 1988–1994.
37. Xu, S.Q., Zhang, J.G., Paquet, C., Lin, Y.K., Kumacheva, E., 2003. From hybrid microgels to photonic crystals. *Adv. Funct. Mater.*vol(13), pp.468–472.
38. Yamauchi, K., Yamauchi, A., Kusunoki, T., Kohda, A., Konishi, Y., 1996. Preparation of stable aqueous solution of keratins, and physiochemical and biodegradational properties of films. *J. Biomed. Mater. Res*.vol(31), pp.439–444.
39. Yang, X., Lin, Z.B., Cui, S.X., 2007. Effect of concentration of wool keratin on the rebuilding of disulfur bond. *Chin. Sci. Bull.*vol(52), pp.2876–2879.
40. Yang, X., Zhang, H., Yuan, X., Cui, S., 2009. Wool keratin: a novel building block for layer-by-layer self-assembly. *J. Colloid Interface Sci*. vol(336),pp. 756–760.
41. Yun-Xian Wang, Xue-jun cao (2012), Extracting keratin from chicken feathers by using a hydrophobic ionic liquid,*Process biochemistry*, vol(47),pp 896-899.
42. Zhang, W., Qiao, X., Chen, J., Wang, H., 2006. Preparation of silver nanoparticles in water-in-oil AOT reverses micelles. J. Colloid Interface Sci. vol(302), pp.370–373.