Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 20s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Green and chemical synthesis of silver nanoparticles: Study of biological properties and toxicity concerns related to chemically synthesized nanoparticles

Pradnya Salunkhe[1], Tushar Borse[2], Girish Pathade[1], Dhanashri Patil[1]

[1]Krishna Institute of Allied Sciences Krishna Vishwa Vidyapeeth

[2](Formerly Krishna Institute of Medical Sciences Deemed To be University) Karad. Vidya pratishthan school of biotechnology, Baramati 413133, Maharashtra, India.

*** Corresponding Author**

Email ID: salunkhepradnya068@gmail.com

Cite this paper as: Pradnya Salunkhe, Tushar Borse, Girish Pathade, Dhanashri Patil, (2025) Green and chemical synthesis of silver nanoparticles: Study of biological properties and toxicity concerns related to chemically synthesized nanoparticles. *Journal of Neonatal Surgery*, 14 (20s), 667-674.

ABSTRACT

Metallic nanoparticles, particularly silver nanoparticles, are widely used in science, engineering, and medicine due to their small size. However, conventional synthesis methods pose biological hazards, prompting the development of environmentally friendly methods like green synthesis using plant-derived components. Present study used eco-friendly, cost efficient and easy method for synthesis of silver nanoparticles (AgNPs) using Withania somnifera extracts as reducing and capping agents. In another approach, chemical synthesis of AgNPs has been carried out using tri-sodium citrate and polyethylene glycol as reducing agents. Measurement on UV-Vis absorption spectroscopy at 415 nm confirmed the synthesis of AgNPs in the solution. Both the AgNPs were tested for their antibacterial properties against Bacillus subtilis, antioxidant and antifungal properties against A. niger. The chemically synthesized AgNPs were tested on the seed germination of Zea mays for detecting their toxic effect. Moreover, the treated plantlets were also evaluated for their protein and chlorophyll content and results confirmed that as the concentration of chemically synthesized AgNPs increases the growth of the Zea mays plantlet decreases.

Keywords: *Antibacterial potential, Antifungal properties, Green chemistry, Silver nanoparticles*

1. INTRODUCTION

Small size and high surface area for reaction is an important advantage of metallic nanoparticles. Several noble metals have unique features in chemical, magnetic, optical and electronic properties (*Jeong et al., 2005*). Toxic chemical was used in the traditional chemical methods, whilst green syntheses of nanoparticles eliminate the hazardous substances. Silver nanoparticles' anti-inflammatory and antioxidant qualities have made it one of the most alluring metals. Silver nanoparticle biogenesis has recently been investigated using a variety of plants such as olive, mulberry, *Thymbra spicatas*, *Stachys lavandulifolia*, green tea, *Sesamum indicum*, banana, oak fruit bark, and *Eucalyptus chapmaniana*.

Silver has been used in cooking for ages to make flatware products that can securely contain water and prevent food spoilage. As a result, it is the metal that is the subject of the most extensive investigation to discover its unique properties. Silver nanoparticles have also been the subject of substantial nanotechnology study, mostly as a result of their antimicrobial properties (*Suman et al., 2013*). It exhibits widespread antibacterial action. The continuous release of Ag+ ions from silver nanoparticles exhibits improved antibacterial activity, demonstrating the effectiveness and benefits of silver nanoparticles. AgNPs may mitigate the emergence of plaque biofilm in dental applications while boosting the antibacterial impact of dental materials.

Photoplate-1 *Withania somnifera (*Ashwagandha) plant species.

(Image Source: Adobe stock)

Withania somnifera (Ashwagandha) possesses numerous medicinal uses such as memory boost and improves brain and nervous system efficiency. Furthermore, it aids in times of stress by enhancing reproductive function (*Albrecht et al., 2006*). In also serves in biomedical applications including antimicrobial, anticancer, wound healing, bone healing, inflammatory inhibition, dental materials and vaccines (*Chaloupka et al., 2010).* The present study focused on the green as well as chemical synthesis of silver nanoparticles. leaves of *Withania somnifera* were assessed for green synthesis of AgNPs. Additionally, the antifungal, antibacterial and antioxidant effects of the produced nanoparticles were studied.

2. METHOD FOR GREEN SYNTHESIS OF SILVER NANOPARTICLES

2.1 Collection of plant specimen

The collection of leaves of *W. somnifera* was done from the natural resources by following the relevant national and international guidelines. The plant doesn't fall under any IUCN category and grows naturally throughout the country (India).

2.2 Preparation of aqueous extract of plant specimen:

The leaves of plant specimen were dried in sunlight for 30^0C for about a week. 10 g of leaf powder of *W. somnifera* were taken separately in a beaker containing demineralised water 100 ml. The extract was then kept in hot water bath for about 15 min at 65˚C. Thereafter, extract was filtered and stored at 4˚C to be used as a reducing agent (*Ahmed et al., 2016*).

2.3 Green synthesis of silver nanoparticles

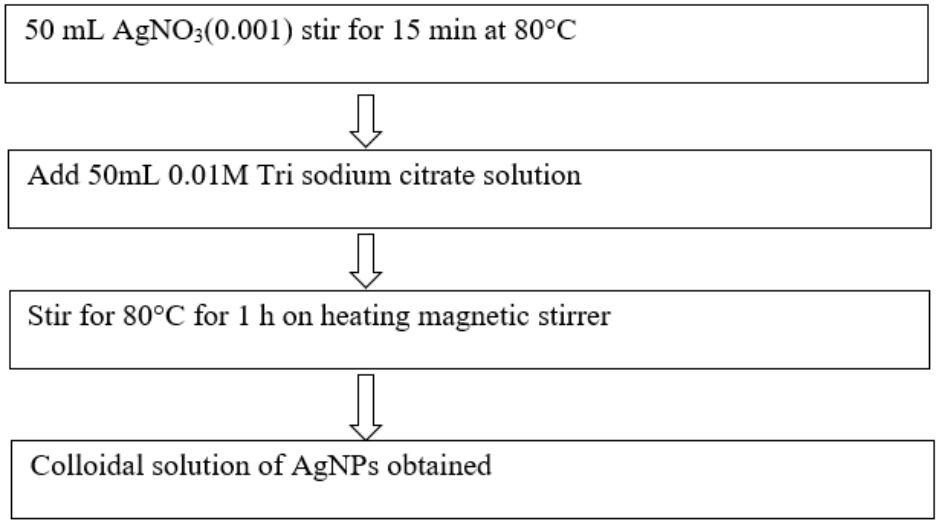
Prepared plant extract were used as a reducing agent and were added drop wise to boiling colourless solution of 50 mL of 1mM $AgNO_3$ till color changes from colourless to pale yellow. The solution was centrifuged twice at 6000 rpm to obtain the pellet. The pellet was then crushed at room temperature to obtain fine powder in glass container *(Ashraf et al., 2016).*

2.4 Chemical synthesis of silver nanoparticles

2.4.1 Preparation of chemicals

1M $AgNO_3$ (stock) {1.6987gm in 10 mL mili-Q-water} 1M Tri-sodium citrate (stock) {2.941gm in 10mL mili-Q-water} Take 0.001M AgNO3 50mL (50µL from 1M stock then make up the volume up to 50mL by using mili-Q Water. Take 0.01M Tri-sodium citrate 50mL (500 µL from 1M stock then made the volume up to 50 mL by using mili-Q-water.

2.4.2 Chemical synthesis of silver nanoparticle's reaction (Flow Chart)



2.5 Characterization of green and chemically synthesized silver nanoparticles

Characterization of the green and chemically synthesized silver nanoparticles was carried out using a UV-visible Spectrophotometer by measuring absorption spectra in the range 300-600 nm. DMSO (Dimethyl sulfoxide) was used as control. The maximum absorption for silver nanoparticles lies between 410-440 nm which is its unique characteristic property.

2.6 Antibacterial activity of the synthesized AgNPs

Agar well diffusion method was used to determine the antibacterial activity of the green and chemically synthesized AgNPs against *Bacillus subtilis*. Filter sterilized 30µL of the AgNPs were added to the well, 30µL distilled water was used as the negative control and the AgNPs were allowed to diffuse (*Nejatzadeh-Barandozi et al., 2013*). The plates were incubated at 30°C for 48 h and the diameter of the zone of clearance was measured in millimetres.

2.7 Antifungal activity of AgNPs using Potato Dextrose Agar diffusion method

Potato dextrose agar cup method was used to determine the antifungal activity of the synthesized AgNPs using leaves extracts against *Aspergillus niger*. The filter sterilized extract (30 µL) of the AgNPs were added to the well. 30 µL of distilled water was used as negative control. The AgNPs were allowed to diffuse as the plates were incubated at 30°C for 24 h. The diameter of the zone of inhibition was measured in millimetres.

2.8 Antioxidant activity analysis and percentage of antioxidant activity

DPPH radical scavenging activity was tested Briefly, the DPPH solution (0.1 m Mol/L) was freshly prepared by dissolving DPPH in ethanol and homogenizing in an ultrasonic bath for 30 s. Then, 2 mL of the sample solution was mixed vigorously with 2 mL of the DPPH solution, and the mixture was incubated in the dark at room temperature for 30 min. Then, the absorbance of the solution was measured at 517 nm. The DPPH radical scavenging activity

$(A_0 - A_1)/A_0 \times 100$,

Where A_0, A_1, and A_0 are the absorbance values of the sample, control (in which the DPPH solution was replaced with ethanol), and blank (in which the sample was replaced with ethanol), respectively.

3. TOXICITY ASSESSMENT OF CHEMICALLY SYNTHESIZED AGNPS *ZEA MAYS* PLANTLETS

The seeds of *Zea mays* were soaked in water containing chemically synthesized nanoparticles. Different test tubes were labelled as per the AgNPs treatment such as control (without AgNPs), 100 µL, 200 µL, 300 µL and 400 µL respectively. The seeds were allowed in 20 mL of solution for overnight. The seeds were then raised in cocopeat and recorded the morphological changes after 1 week. Moreover, the protein and chlorophyll content were also measured from the leaves of *Zea mays* plantlet.

3.1 Determination of protein content in leaves of *zea mays* by Lowry method

Preparation of reagents:

Reagent A: 2% Na_2CO_3 in 0.1 N NaOH (200mL) 4gm Na_2CO_3 in 200 mL 0.1N NaOH.

Reagent B: 1% NaK tartrate in 50 mL D/W

Reagent C: 0.5 % $CuSO_4$ reagent 0.25 gm in 50 mL D/W

Reagent D: 10 mL Folin reagent in 10 mL D/W

BSA: 200 mg BSA in 100 mL D/W

Procedure

Took 12 test tubes and labelled them. 1st tube was used as a blank. Pipetted out BSA as 0.2, 0.4, 06, 0.8, 1.0, and 0.5mL respectively from 2nd to 7th tube. made up the volume up to 2 mL by addition of distilled water. Added 1 mL of reagent (Alkaline copper sulphate) in each test tube. Allowed for incubation at RT for 10 min. Add 0.5 mL of FC Reagent in each tube; allowed the tubes for incubation for 30 min at 30^0C. Measured the absorbance at 660 nm and plotted standard graph conc. Vs absorbance. Determined the conc. of unknown protein in sample.

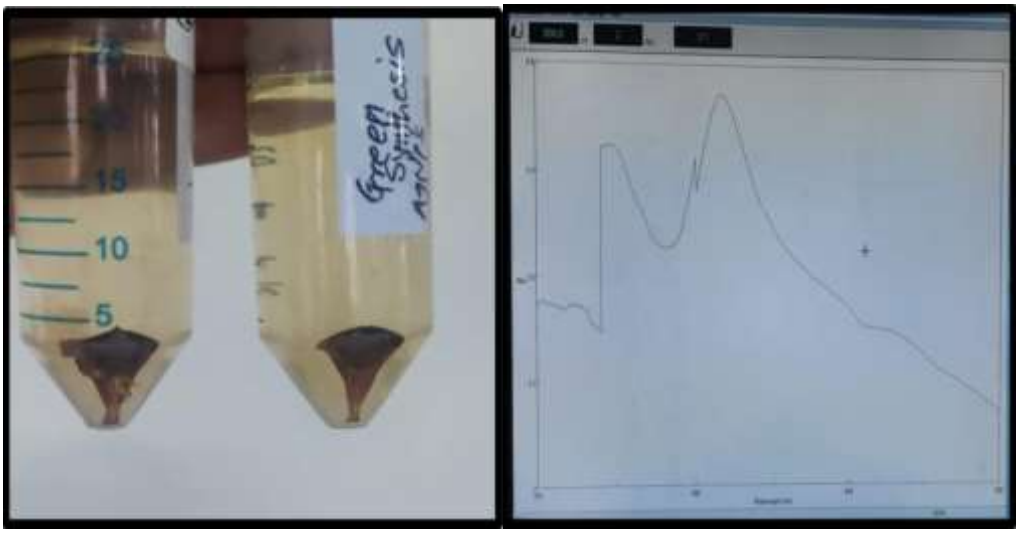
3.2 Chlorophyll content test

Taken leaves from tray as control, 100 µL, 200 µL, 300 µL and 400 µL respectively and crush them in 5 mL 80% chilled acetone and then centrifuge at 5000 rpm for 15 min. Then use the supernatant to check the OD at 645 nm and 655 nm to check the chlorophyll content. Then calculated the value using formulae.

4. RESULTS AND DISCUSSION

4.1 Synthesis and characterization of silver nanoparticles

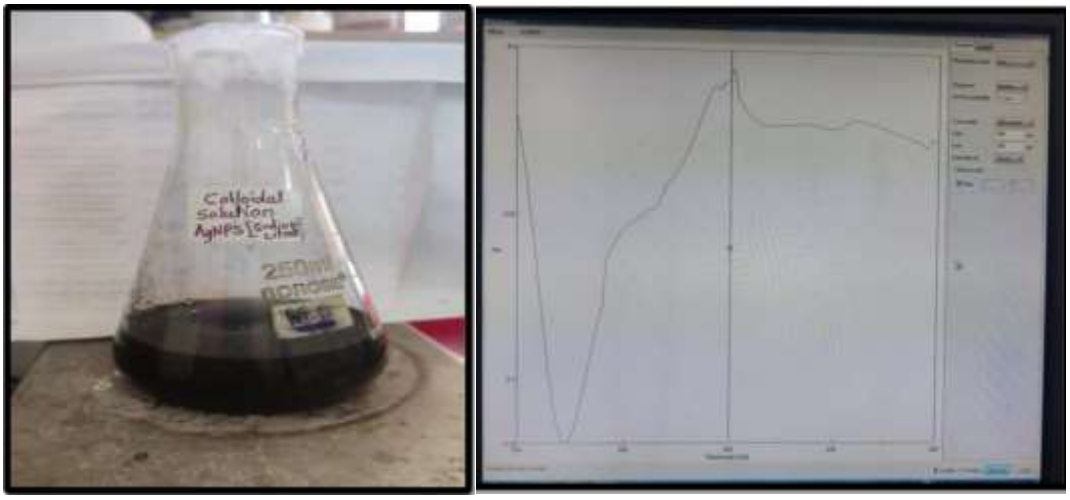
The root and leaf of *W. somnifera* synthesized nanoparticles via aqueous extract shown the pellet containing nanoparticles (Photoplate 2). Silver nanoparticles synthesized from *Withania somnifera* plant as sources of reducing agents showed a peak wavelength around 415 nm (Figure 1) in both root and leaf mediated nanoparticles. If a deviation from these maxima occurs, either decrease or increase in the absorption indicates the presence of impurities. Impurity in this case could be the components of the biological extract or the capping and stabilizing agents present inherently (*Bryan et. al., 2017).*



Photoplate:2 Green synthesis of AgNPs. Pellet containing AgNPs,

Figure:1 UV-Vis Spectra.

Tri-sodium citrate and polyethylene glycol (PEG), two distinct reducing agents, coupled in the chemical method produced a dark brown to blackish solution (Photoplate 3). By analyzing the absorption spectrum between 300 and 600 nm, the synthesis was verified. Silver nm. (Figure2).



(A) **(B)**

Photoplate: 3 Obtained Colloidal solution of AgNPs,

Figure 2: UV-Vis Spectra.

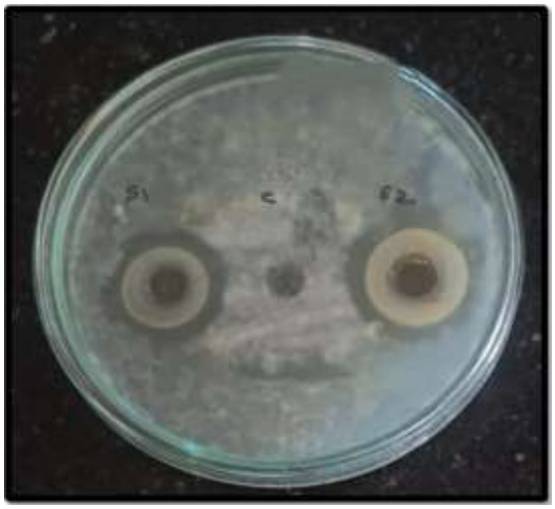
4.2 Antimicrobial and Antifungal properties of AgNPs

Both plant-based and chemically developed AgNPs were tested for their antibacterial efficacy against *Bacillus subtilis* and *Aspergillus niger*. The zone of clearance observed around wells inoculated with plant-based AgNP's against *B. subtilis* and *A. niger* is represented in figure 4. Diameter of zone of clearance of nanoparticles synthesized via leaves of *Withania somnifera* is 8 mm. antimicrobial targets can range from preventing the growth of certain types of microorganisms such as bacteria to inactivating microorganisms, to either reduce the levels and types present or to completely eliminate all (*Ahmed et al., 2016*).

(A) **(B)**

Photoplate 4: Antimicrobial properties of plant-based AgNPs. Zone of clearance against *B. subtilis*

Photoplate 5: Zone of clearance against *A. niger* of green synthesized nanoparticles.



Photoplate 6: Antifungal properties of chemically synthesized AgNPs against *A. niger*.

Chemically synthesized AgNPs were tested against *A. niger*. The zone of inhibition observed around wells inoculated with AgNP's are presented in photoplate 6.

4.4 Antioxidant properties of Synthesized silver nanoparticles:

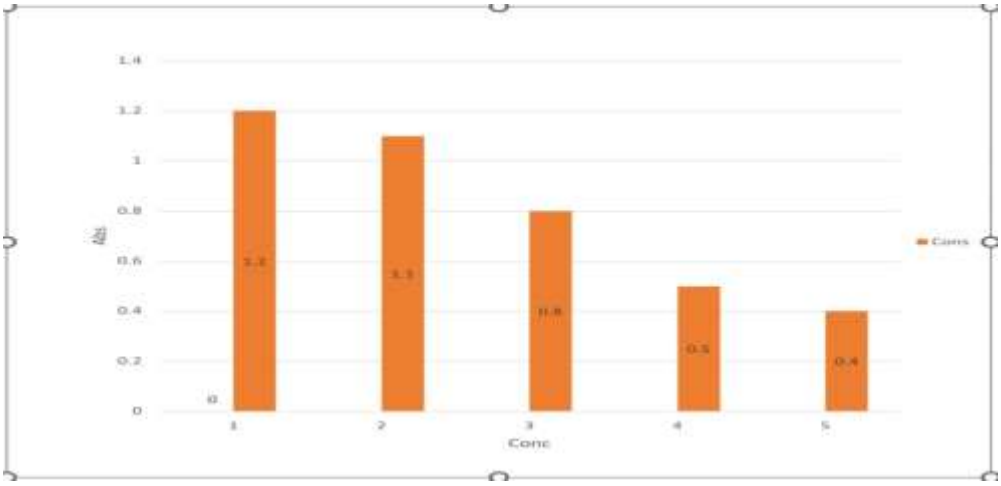
AgNps antioxidant qualities were evaluated by testing their ability to scavenge DPPH radicals. The DPPH solution (0.1 m Mol/L) was made by dissolving DPPH in ethanol and homogenizing it for 30 seconds in an ultrasonic bath. After that, 2 ml of the sample solution and 2 ml of the DPPH solution were well combined, and the mixture was allowed to sit at room temperature for 30 minutes in the dark. Next, at 517 nm, the solution's absorbance was measured. The percentage of green produced nanoparticles with antioxidant activity is **4.2678%.**

4.3 Effect of chemically synthesized AgNPs on seedling growth of *Zea mays*

The findings indicate that the growth rate of plantlets decreases as the concentration of chemically produced silver nanoparticles increases, demonstrating the harmful effects of these nanoparticles. (Photoplate 7).

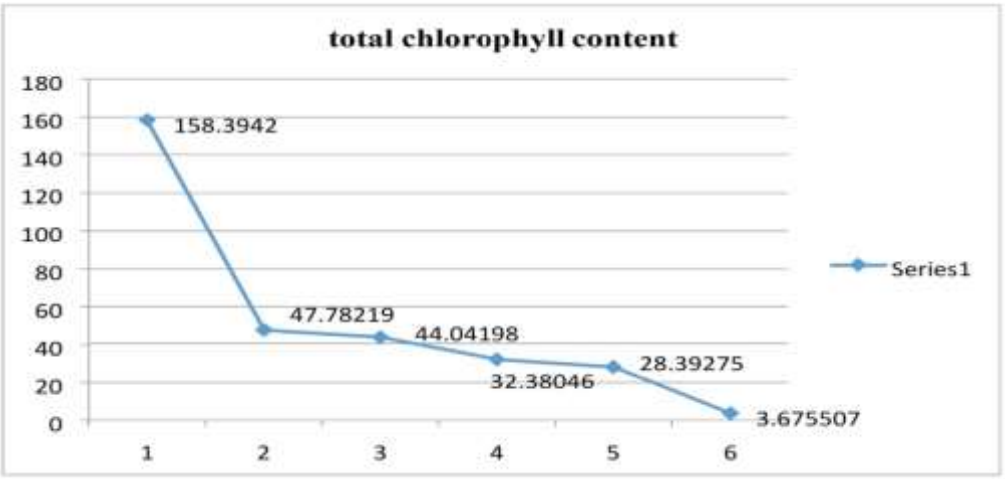
Photoplate 7: Growth performances of *Zea mays* seedlings against different treatments of chemically synthesized AgNPs.

The adverse impact of chemically produced AgNPs wasalso measured by calculating the values of protein and chlorophyll contents in AgNPs treated plantlets. Lowry's method confirmed (Figure 3) the decreasing level of proteins while increasing the AgNPs dose. Similarly, dose enhancement also decreased the chlorophyll content in AgNPs treated seedling of *Z. mays* (Figure 4).



(A)

Figure 3 Protein concentrations of leaf samples in plantlets of *Zea mays.*



(B)

Figure 4 Total chlorophyll content of leaves sample in plantlets of *Zea mays.*

5. CONCLUSION

The environmentally benign and relatively inexpensive process of producing silver nanoparticles is called "green synthesis." The produced AgNPs shown strong antifungal and antibacterial properties against Aspergillus niger and Bacillus subtilis, respectively. They can consequently be utilized for medicinal and environmental purposes since they are thought to be less harmful in eukaryotes than in prokaryotes. Because the chemically produced nanoparticles reduced the amounts of protein and chlorophyll, they had a harmful effect on Zea mays. AgNPs mediated by *Withania somnifera* have comparatively superior biological qualities to those of chemically produced nanoparticles.

REFERENCES

[1] Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications. J Adv Res 7:17- 28

[2] Albrecht AM, Evans CW, Raston CL (2006) The health implications of nanoparticles. Green Chem 8:417-432

[3] Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I (2016) Green synthesis of silver nanoparticles characterization of their inhibitory effects on AGEs formation using biophysical techniques. Sci Rep 6:20414

[4] Bryan CJ, Monique EJ, Antonio RMB, Karen EM, Michael RW, José RVB (2017) Silver Nanoparticles: Technological Advances, Societal Impacts Metrological Challenges. Front Chem

[5] Chaloupka K, Malam Y, Seifalian AM (2010) Nano-silver as a new generation of nano-product in biomedical applications. Trends Biotechnol 28:580-8

[6] Jeong S, Yeo S, Yi S. (2005) J Mater Sci 40:5407.

[7] Nejatzadeh-Barandozi F, (2013) Antibacterial activities and antioxidant capacity of *Aloe vera*. Org Med Chem Lett 3:5

[8] Suman TY, Radhika Rajasree SR, Kanchana A Elizabeth SB (2013) Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morinda citrifolia* root extract. Colloids Surf B Biointerfaces 106: 74-8.