Rev. Inst. Med. Trop. Sao Paulo
2016;58:73
http://dx.doi.org/10.1590/S1678-9946201658073

BRIEF COMMUNICATION

SILVER NANOPARTICLES-DISK DIFFUSION TEST AGAINST *Escherichia coli* ISOLATES

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SUMMARY

Nanotechnology can be a valuable ally in the treatment of infections. Silver nanoparticles (AgNPs) are structures that have antimicrobial activity. The aim of this study was to produce AgNPs by green methods, characterize these structures, and assess their antimicrobial activity against *Escherichia coli* associated with the antibiotic ciprofloxacin. AgNPs were characterized by spectroscopic and microscopic techniques. Antimicrobial activity was evaluated by the disk diffusion method against 10 strains of *E. coli*. The synthesized AgNPs showed a spherical shape and a size of 85.07 ± 12.86 nm (mean ± SD). AgNPs increased the activity of ciprofloxacin by 40% and may represent a new therapeutic option for the treatment of bacterial infections.

KEYWORDS: Silver nanoparticles; Antimicrobial activity; *Escherichia coli.*

INTRODUCTION

Nanotechnology involves the study of bodies that have dimensions of up to 100 nm in any direction and possess properties that differ from bulk material[1]. This peculiarity represents a new area of knowledge that can open a technological frontier, with the possibility of developing new compounds that could help to improve people's lives[1,2].

There is an immense perspective of the use of nanoparticles in the diagnosis and treatment of human and animal diseases[3]. The main products available are those for personal hygiene, such as toothpaste, shaving creams, and deodorants[2]. These products mainly contain silver nanoparticles (AgNPs), due to their known antiseptic action[2]. AgNPs have various applications, including antiseptic, antibacterial, and antifungal properties. Silver is a cheaper metal than gold, which makes it attractive for research. Moreover, silver in the nanoparticle state has peculiar optical properties, allowing its use in technological products[4,5]. The use of medical catheters covered with a nanometer-thin layer of AgNPs can prevent microorganisms colonization and thus decrease the length of hospital stay as it prevents infections[2,6].

The antibacterial susceptibility test using AgNPs disk diffusion is well known[7]. These particles act by causing damage to membranes and to DNA, therefore preventing reproduction of microorganisms, leading to death[8]. In addition to its intrinsic bactericidal property, the microorganism-killing power of AgNPs can be increased when associated with antibiotics[8]. This mechanism may be useful in the treatment of multidrug-resistant infections, and may represent an important therapeutic alternative[9]. Ciprofloxacin is a very useful antibiotic that acts by inhibiting bacterial DNA synthesis, resulting in the death of microorganisms[10].

The aim of this study was to synthesize, characterize and evaluate the activity of AgNPs produced by green synthesis and associated with the antibiotic ciprofloxacin against strains of *Escherichia coli* using the diffusion disk method.

AgNP synthesis was performed using glucose as a reducing agent and sodium dodecyl sulfate (SDS) as a stabilizing agent. Briefly, 1.0 g of glucose and 0.5 g of SDS were added to 500 mL of a $AgNO_3$ solution (5 mM). The solution was continuously stirred and the temperature was maintained at 50 °C to favor the reaction. Then 1.0 mL of 0.2 M NaOH was added to the mixture. The reaction was maintained under these conditions for 30 min, then stirred, heated and suspended[11,12]. The AgNPs were purified by ultracentrifugation at 8,500 xg for 20 min, and characterization was carried out using spectrophotometric reading at 300 to 700 nm (UV-Vis). The size and shape were determined by atomic force microscopy (AFM), and scanning electron microscopy (SEM)[13], (National Institutes of Health, USA, version 1.48v).

This study used 10 strains of *Escherichia coli*; provided by the collection maintained at the Microbiology Laboratory of the Department

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of Clinical Analysis, School of Pharmacy, Federal University of *Ceará,* Brazil. The strains of *E. coli* were isolated from urine samples of patients and they are all sensitive to ciprofloxacin. The tests were performed by the disk diffusion (DD) method on Müller-Hinton agar. In this experiment we used three types of discs: (1) paper disc (10 mm) saturated with 10 µL of AgNPs; (2) ciprofloxacin discs with (Ciprofloxacin) 5 µg (Cecon®); and (3) ciprofloxacin disks saturated with 10 µL of AgNPs. *E. coli* strains were suspended in saline solution and plated in the culture medium, and then the discs were placed. The plates were incubated at 35 °C for 24 hours, after this time the inhibition zones were measured[12]. The synergism was evaluated by the formula $\{(C^2 - B^2) / B^2\} \times 100$, where, B = the inhibition zones of the ciprofloxacin alone and C = ciprofloxacin + AgNPs[14]. This formula allowed us to evaluate the increment of the inhibition zone around the bacteria caused by the antibiotic in association with AgNPs[9,14].

Processes that use sugars to obtain AgNPs are called green synthesis, because of the absence of toxic compounds and no formation of toxic waste[7]. Furthermore, sugars are cheap and affordable[7]. In our study, we used glucose for the synthesis and SDS for the stabilization. Nanoparticles were formed with an average size of 85.07 ± 12.86 nm (average ± SD) (Fig. 1a); UV-VIS spectra confirmed the efficient synthesis of AgNPs; these particles absorbed energy at 420 nm and exhibited a spherical shape, as can be seen in Fig. 1b and 1c. AgNP synthesis using glucose is a recurring theme in the literature[7,15]. The particles are spherical and stable, and SDS enhances the antimicrobial activity[16].

AgNPs stand out for their antimicrobial activity[17]. The mechanism of action involves the inactivation of enzymes and the DNA damage of microorganisms[6]. In our study, we evaluated the effect of AgNPs alone and associated with the antibiotic ciprofloxacin (cipro). Ciprofloxacin alone produced an inhibition zone of 45.9 ± 7.4 mm; AgNPs alone showed an inhibition zone of 17.1 ± 5.9 mm; and the combination of cipro + AgNPs showed an inhibition zone of 54.3 ± 8.6 mm (Fig.1d). The association of cipro + AgNPs produced a 40% increase in the inhibition zone when compared with the antibiotic alone (Table 1). The association AgNPs and antibiotics against bacteria and fungi have shown good results[9,14,18]. In Figure 1(d) we can observe the action of this association; the inhibition zone on the disc containing cipro + AgNPs is much larger than the disc with cipro alone and AgNPs alone. The AgNPs bind proteins

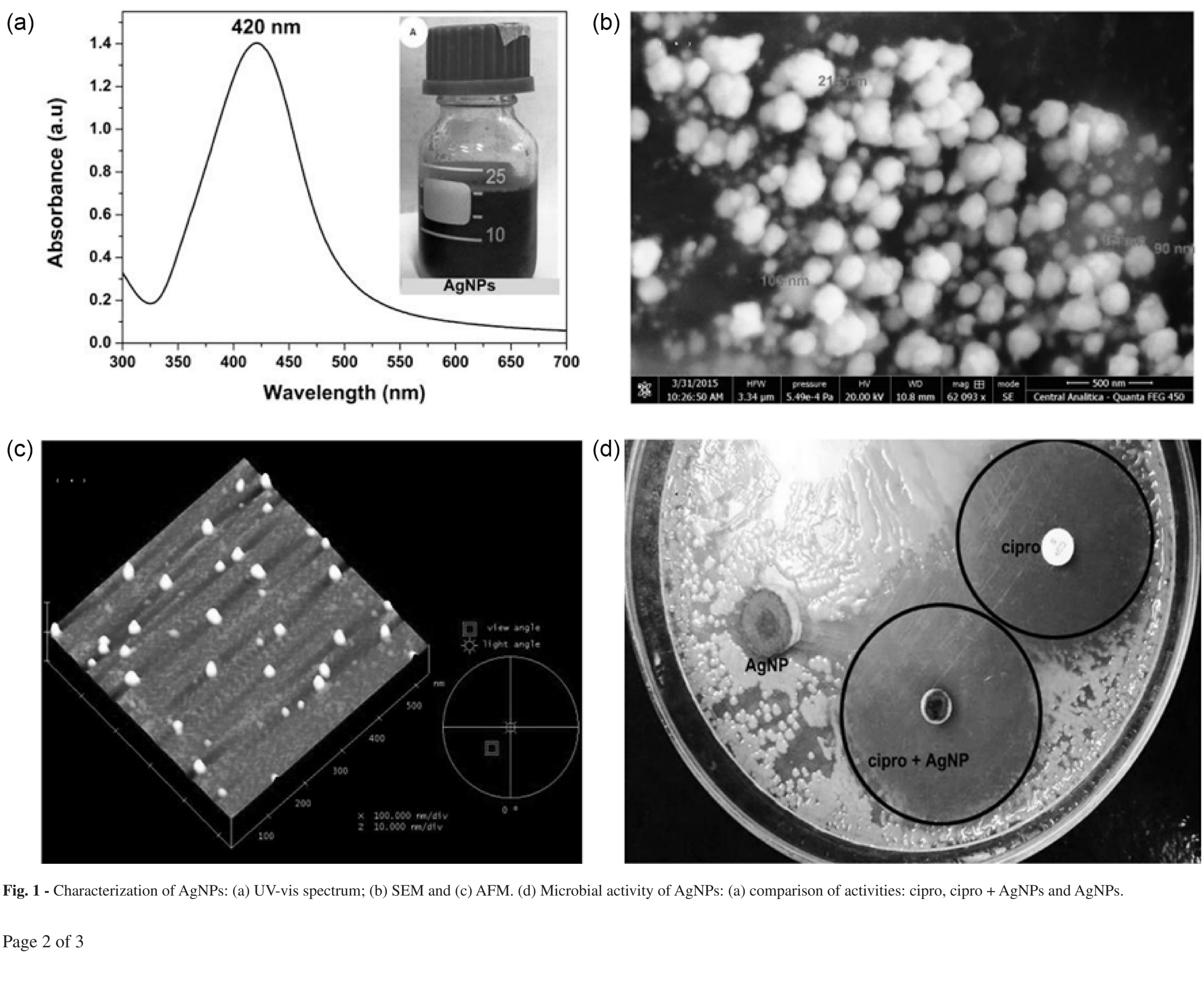

Fig. 1 - Characterization of AgNPs: (a) UV-vis spectrum; (b) SEM and (c) AFM. (d) Microbial activity of AgNPs: (a) comparison of activities: cipro, cipro + AgNPs and AgNPs.

Table 1
Antibacterial activity of AgNPs, cipro discs and cipro + AgNPs against *E.coli*

Bacteria *(n)*	Inhibition Zone (mean ± SD) mm			Increased fold area (%)
	A	B	C	$(C^2 - B^2)/B^2)$x100
	AgNPs	cipro	cipro + AgNPs	
E. coli (10)	17.1 (±5.9)	45.9 (±7.4)	54.3(±8.6)	40%

Increased fold area was calculated using ($C^2 - B^2)/B^2$ x 100), where B and C are the inhibition zones for B and, C, respectively.

and DNA of the bacteria and ciprofloxacin damages the DNA, and this combination enhances the effects on *E. coli* strains[10].

AgNPs were synthesized using glucose and SDS and were characterized through physical-chemical techniques: UV-VIS, SEM, and AFM; they showed a size of 85.07 ± 12.86 nm and a spherical shape. The AgNPs showed activity against *E. coli* isolates and an increased activity of the antibiotic ciprofloxacin. AgNPs associated with antimicrobial agents can be a therapeutic option for the treatment of bacterial infections. The disk diffusion method has limitations so that new tests using different methodologies should be used to confirm the results found in our study.

ACKNOWLEDGMENTS

The authors are grateful to *Central Analítica* - UFC/CT-INFRA/MCTI-SISNANO/*Pró-Equipamentos*, UFC Department of Physics, CAPES, CNPq and Funcap, for the grant provided to support this research on nanoparticles.

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Received: 15 October 2015
Accepted: 29 March 2016