Characterization of Copper Oxide Nanofluids in medical applications: Cases of Hospital Infections

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Abstract

Health acquired infections (IACs) is a major public health problem that cause an alarming number of victims in worldwide. Copper oxide (CuO) nanoparticles have several properties, such as their strong antimicrobial potential. CuO nanofluids were prepared and applied in an Escherichia coli culture to evaluate their antibacterial potential. The effect of increasing the temperature and concentration of the nanofluids on their electrical conductivity and their pH was also evaluated, showing that the increase in temperature and the increase of the nanoparticles in volume, promote the increase of the electrical conductivity of the nanofluids. It has been found that the temperature makes the nanofluids quite reactive, which is reflected in the inconstancy of their pH value. As for the microbiological test performed, it was not possible to prove the antibacterial potential of CuO nanofluids. The same happened with the hospital disinfectant used, which may suggest that the microbiological test used was not the most adequate.

1. Introduction

IACs are one of the major causes of morbidity and mortality worldwide. Besides of the various complications they bring to patients, IACs are also synonymous of a direct increase in the cost of medical care, as well as in the spread of multiresistant microorganisms (source of dissemination of infections) [1-4]. In a European context, a 2005 study concluded that IACs affects about 1 in 10 inpatients and causes around 5.000 deaths per year [5]. According to the Directorate General of Health in Portugal, the number of deaths associated with IACs (2013) reached 4.606, that is, more than 12 cases per day and seven times more than the number of deaths due to road accidents. Projections from the same entity indicate that by 2050 some 390.000 people in Europe and 10 million on the planet will die victims of this public health problem [6].

There are other pathogenic organisms responsible for IACs, however, bacteria, such as *Escherichia coli*, are the organisms with the greatest capacity for survival in dry hospital surfaces for long periods. These bacteria have the ability to create biofilms, that is, extremely organized colonies, even under conditions of nutrient deficiency [7, 8]. In the reducing of environmental burden of these microorganisms, traditional methods, such as daily cleaning of hospital surfaces, using disinfectants, and improved cleaning and self-cleaning systems are used [8-11]. However, even after successive cleanings, the elimination of pathogenic organisms doesn't occur effectively [8]. The use of disinfectants is a regular practice in all health units that requires strict guidelines due to the chemical composition of disinfectants, which includes toxic substances capable of causing sensitization and irritation of the skin and mucous membranes, but also damage of materials surfaces [12, 13].

In this context, new solutions, promoters of IACs reduction, have been developed, namely the application of CuO nanofluids on hospital surfaces. Due to the antimicrobial potential of copper oxide and the fact that it doesn't present danger to humans when used in low amounts (skin sensitization and irritation), it is thought that the creation of antimicrobial solutions from CuO nanofluids for disinfection of surfaces will be a strategy that will offer a theoretical advantage to conventional cleaning with disinfectants [8, 14].

2. Materials and methods

The nanofluid samples were prepared by the application of CuO nanoparticles in ethylene glycol base fluid.

2.1. CuO nanoparticles

The nanoparticles used (99.9% of purity and 6.4g/cm^3 of density), provided by mkNANO®, were in the form of black powder of nanometer size, 70nm of diameter.

2.2. Ethylene glycol

The base fluid used was ethylene glycol, with a density of 1.108-1.118g/cm^3 at 20°C.

2.3. Preparation of samples

The samples of CuO nanofluids were prepared using an ultrasonic homogenizer. To the flask was added the base fluid and then, gradually, the nanoparticles. The samples were maintained at 25-30°C. The homogenization time chosen, a preponderant factor for the stability of nanofluids, was determined after the experimental tests, since few authors refer to the existence of an optimal time of homogenization. A study refers that the homogenization time can reach up to 30 hours, but for CuO nanoparticles in ethylene glycol the optimum time should be 9 hours [15].

2.4. Measurement of electrical conductivity

Electrical conductivity is the least investigated thermophysical characteristic of nanofluids [16-18].

To evaluate the effect of increasing the volume percentage of nanoparticles and the effect of temperature increase on the electrical conductivity of CuO nanofluids, samples with 0.20%, 0.40%, 0.60%, 0.80% and 1.00% of nanoparticles in base fluid volume were prepared. Each sample was subjected to a thermostatic bath, with temperature increments of 5°C. Measures are performed at 30ºC, 35ºC, 40ºC, 45ºC and 50ºC.

2.5. pH measurement

There are authors who defend the existence of an optimum pH for which the nanofluid stability is maximum, as well as its thermal conductivity [19]. In this context, similar to the electrical conductivity measurements realized, pH measurements were performed for the same conditions.

2.6. Evaluation of antibacterial potential

The microbiological test used to compare the antibacterial efficacy of a commercially available hospital disinfectant and CuO nanofluids was performed using a bacterial strain of *Escherichia coli*, whose growth was conducted in culture media composed of Plate Count Agar contained in Petri dishes and incubated at a constant temperature of 36°C for 24 hours. In the preparation of the microbiological test, 60µL of bacteria were added to all Petri dishes. The control used was acetic acid, an inhibitor of *Escherichia coli*. The following table allows to systematize the test performed.

Control (2 replicas)	Disinfectant (2 replicas)	Nanofluid (2 replicas)
CuO nanofluid (10µL)[1] + Acetic Acid (10µL)	Zeta 3 Wipes Total (10µL)	CuO nanofluid (10µL)[1]

1 - nanofluids extracted from a sample with 1.00% in volume of nanoparticles in demineralized water.

Table 1. *Microbiological test.*

2.6.1. *Escherichia coli*

Escherichia coli is a gram-negative bacterium, present innocuously in the intestinal tract of humans, however, it may have pathogenic activity, being a major cause of IACs [20, 21].

2.6.2. Hospital disinfectant

The hospital disinfectant used was Zeta 3 Wipes Total (Bactericide), compound based on alcohols and with an alkaline pH (10.49).

3. Results and discussion

3.1. Evaluation of stability

For an agitation time of 60 minutes, it was possible to obtain stable nanofluids during 8 hours (figure 1). These results are associated with the fact that ethylene glycol is a nonionic surfactant [22-24].

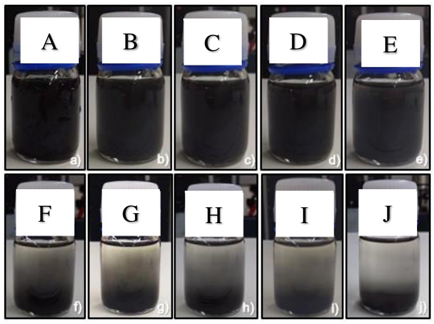

Figure 1. *Stability evaluation of CuO nanofluids samples in ethylene glycol (0.25%) subjected to 60 minutes of ultrasonic agitation. A) immediately after agitation; B) after 2 hours; C) after 4 hours; D) after 6 hours; E) after 8 hours; F) after 22 hours; G) after 24 hours; H) after 26 hours; I) after 28 hours; J) after 48 hours.*

3.2. Evaluation of electrical conductivity

As proved by Azimi H. R. and Taheri R., it is observed that with the increase of temperature and concentration of nanofluids (figure 2) there is an increase of electrical conductivity [25].

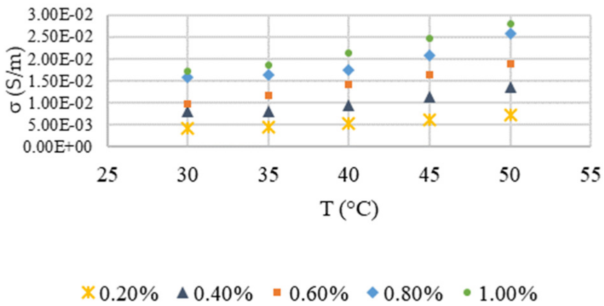

Figure 2. *Effect of temperature (T) increase in the electrical conductivity (σ) of nanofluids samples with different percentages of nanoparticles in volume.*

These results are in agreement with the predicted one, since with the increase of the temperature an increase of the mobility of the ions of a given solution occurs, which allows increased conduction of electric current [26]. Furthermore, a study carried out in 2013 showed that the electrical conductivity of CuO nanofluids in ethylene glycol increases with the increase of the quantity of nanoparticles present in the dispersion [27].

3.3. Evaluation of pH

The pH value of nanofluid samples was very unstable, indicating that the increase of temperature makes the nanofluid samples quite reactive. The pH variation detected may be associated to the fact that the samples show some instability when removed from the thermal bath (sedimentation). A study with CuO nanofluids allowed to conclude that the higher the pH value variation, the higher the thermal conductivity of nanofluids (thermal conductivity) [28].

3.4. Evaluation of antibacterial potential

The acetic acid (control) inhibited bacterial growth, as expected. However, the hospital disinfectant and the nanofluids did not reveal antibacterial activity (figure 3).

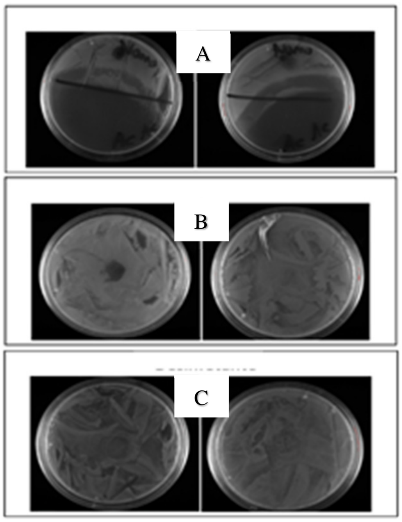

Figure 3. *Evaluation of antibacterial potential of CuO nanofluids. A) Control; B) Nanofluids; C) Disinfectant.*

Although the hospital disinfectant used is an antibacterial, the bacteria proliferated normally. Considering the results obtained, it is thought that the microbiological test performed maybe wasn't the most indicated. A study carried out in Brazil proved that the success of the application of alcohols in cultures of *Escherichia coli* is directly associated with the type of technique used [29]. It was hypothesized that the inefficiency of nanofluid is associated with its alkaline pH, considering that only the acid has proved to be an inhibitor. This hypothesis was rejected, since the hospital disinfectant, such as others commercialized, has a very alkaline pH (10.49).

4. Conclusions and future goals

In the present work CuO nanofluids and hospital disinfectant did not reveal antibacterial activity, it is believed that the microbiological test carried out may be not the most adequate and a new methodology must be developed through a different protocol. The results allowed to conclude that the choice of an adequate ultrasonic agitation time is a fundamental aspect to obtain stable dispersions. It was also verified that with the increase of temperature and concentration, there is an increase in electrical conductivity. On the other hand, it was realized that subjecting the samples of nanofluids to different temperatures increases their reactivity, which is reflected in the inconstancy of its pH and it may be associated to the increase in thermal conductivity.

In the future, it is intended to continue the studies carried out, improving the points where difficulties have occurred, namely, increase the number of replicates, in order to have a significant statistics results, and to improve the microbiological methods used.

Acknowledgements

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