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ELECTROCHEMICAL EVALUATION OF CONTROLLED RELEASE PROFILE OF CEFOPERAZONE FROM MAGNETIC NANOPARTICLES

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Cefoperazone is a third generation cephalosporin often used to treat infections caused by Gram-negative and some Gram-positive microorganisms. Controlled release dynamics of this antibiotic from the C-Fe (Benzen-Aniline) and Fe_3O_4/oleic acid nanostructured supports was studied by two analytical methods: conductometry and UV-VIS spectrophotometry. After comparing the two sets of experimental results with a control sample consisting of Cefoperazone alone, the analytical data confirmed a higher retention in the case of adsorption of Cefoperazone on the two type of nanostructured support.

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1. Introduction

In recent years, the research has aimed at obtaining nano-biomaterial synthesis with possible use in multiple medical and pharmacological applications. Due to their outstanding physical, chemical and biological properties, nanomaterials could be used in establishing early diagnosis, as contrast agents, targeted therapy and, not least, as curative therapy [1, 2, 3]. Possible applications in medicine include: the release of targeted drugs in the body, prevention, diagnosis and treatment.

Controlled release systems for drug substances are a success for the pharmaceutical industry, due to the development of new technologies capable of delivering the active substance in the body over a prolonged period of time, such as: hours, days, or weeks [4].

Newly developed nanostructured systems for controlled release of various drugs are an ingenious way of solving current pharmacologic issues drug, toxicity on other organs, and difficulty in maintaining therapeutic concentrations, metabolism and excretion [5]. The most studied nanosystems and also the most popular so far are carbon nanotubes, and magnetic nanoparticles like Fe_3O_4 and Fe_2O_3 [6, 7]. Single-wall carbon nanoparticles proved to be useful as bio-carriers to transport biomolecules through cell membranes, like the nuclear membrane, without producing cytotoxic effects [8-12]. Nanoparticles with magnetic properties (Fe_2O_3, Fe_3O_4, etc.) have been widely studied for use as biosensors for chemical and biochemical separation processes as controlled drug delivery systems, enzymatic encapsulation, etc [13-15].

This work is the continuation of previous studies of our team and presents the dynamics of controlled release of the ((6R,7R)-7-[(2R)-2-{[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino}-2-(4-hydroxyphenyl)acetamido]-3-{[(1-methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid - Cefoperazone - adsorbed on nanostructured

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magnetic delivery assemblies, C-Fe (Benzen-Aniline) and Fe_3O_4/oleic acid, using two electrochemical analytical methods.

2. Experimental

All the analytical reagents and solvents were purchased from Merck (Darmstadt, Germany) and Cefoperazone, the cephalosporin adsorbed on magnetic nanoparticles, was purchased as injectable powder from pharmacy. Ultrapure water was obtained with the aid of a Millipore Simplicity UV system.

Synthesis and characterization of C-Fe (Benzen-Aniline), Fe_3O_4/oleic acid – Cefoperazone nanostructured assembly

C-Fe (Benzen-Aniline) magnetic nanoparticles were synthesized by mixing benzene and aniline, followed by plasma processing and purification by solvent extraction (benzene, dichloromethane, o-dichlorobenzene). The last step consists of removal of inorganic impurities, washing with ultrapure water and drying at high temperature [16, 17].

The Fe_3O_4/oleic acid core/shell magnetic nanoparticles were synthesized by adapting the Massart method using Fe^{3+} and Fe^{2+} salts and oleic acid as the surfactant, under microwave conditions [18, 19]. The core-shell/adsorption-shell - Fe_3O_4/oleic acid/Cefoperazone nanoparticles were obtained after the preliminary chloroform dispersion with Cefoperazone. The concentration of Cefoperazone deposited on magnetic nanoparticles was 0.8%. Primary functionalization was aimed at obtaining the non-polar coating (shell) of the nanoparticle (for ferrites), allowing their dispersion in non-polar or weakly polar solvent.

Synthesis of nanostructured nuclei involved obtaining nanostructures with diameters between 5 and 20 nm, achieved through plasma processing for the C-Fe version and by precipitation in aqueous environment for ferrites.

The electronic microscopy analyses were made with a high resolution electrical transmission microscope Tecnai G2F30 S-TWIN, equipped with an energy dispersive detector sensor EDS and an electron energy loss sensor EELS.

The study of the controlled release dynamics of the Cefoperazone from the two magnetic nanomaterials

The dynamics of Cefoperazone controlled release from the nanostructured support was studied using two different analytical methods: the conductometric method, and the UV-VIS spectrophotometric method. For both work methods, the experiments had analytical data recorded in real time by a computer linked to the measuring system of the process parameters.

For the active substance studied - Cefoperazone, the release curve used as reference was recorded in the same conditions, using the same amount of compound used for the nanostructured system.

A. **Conductivity Method** - the experiments were made using a Denver Instruments Conductometer Model 220. We used for calibration a 0.1 mL of KCl standard, with a conductivity of 1408 μS/cm. The analytical instrument was linked to a computer through a RS232 interface, the data acquisition was made at 0.5 second, mediation through 10 experimental, with the help a serial port data acquisition program; the data processing was made in Microsoft Excel, using spline interpolation. The analyzed samples contained 200 mg nanostructure support (0.8% deposited Cefoperazone), 1.6 mg pure Cefoperazone – dissolved in 20 ml ultrapure water and introduced in the flask, were placed in the thermostat at 25^{o}C, under continuously and controlled stirring (500 rpm).

B. **UV-VIS spectrophotometric method -** the experiments were made using Spectro UV-VIS Double Beam PC 8 Labomed, INC spectrophotometer linked to a computer through RS232 interface, the data acquisition was made at 1 second with mediation on seven points experimental with the help of a serial port data acquisition program; the data processing was done

in Microsoft Excel, using spline interpolation. In our study two samples were analyzed: 60 mg pure Cefaperazone and 750 mg nanostructured support (750 mg nanostructured supports with deposited Cefoperazone) dissolved in 20 ml ultrapure water. Samples were thermostated in a flask at 25^{o}C, under continuous and controlled stirring. The work flow has been maintained with the help of the peristaltic pump at a value of 5 mL/minute. To avoid migration of the nanostructured support through the system, the sample was placed in a separate nacelle and separated from the dispersion medium with a low porosity filter paper.

3. Results and Discussion

Characterization of the nanostructured substrates

Characterization of the two nanostructured substrates was achieved by infrared spectrometry (FT-IR) and high resolution transmission electron microscopy (HR-TEM) - methods which prove the existence of the expected nanostructured assemblies, and give the possibility to estimate their size (Figure 1). FT-IR spectra (Figures 2, 3) were obtained using a Thermo - Nicolet 6700 spectrometer operating in the wave number range 650-4000 cm^{-1} with a resolution of 4 cm^{-1} for all nanostructured systems: C-Fe/Cefoperazone, Fe_3O_4/oleic acid/Cefoperazone.

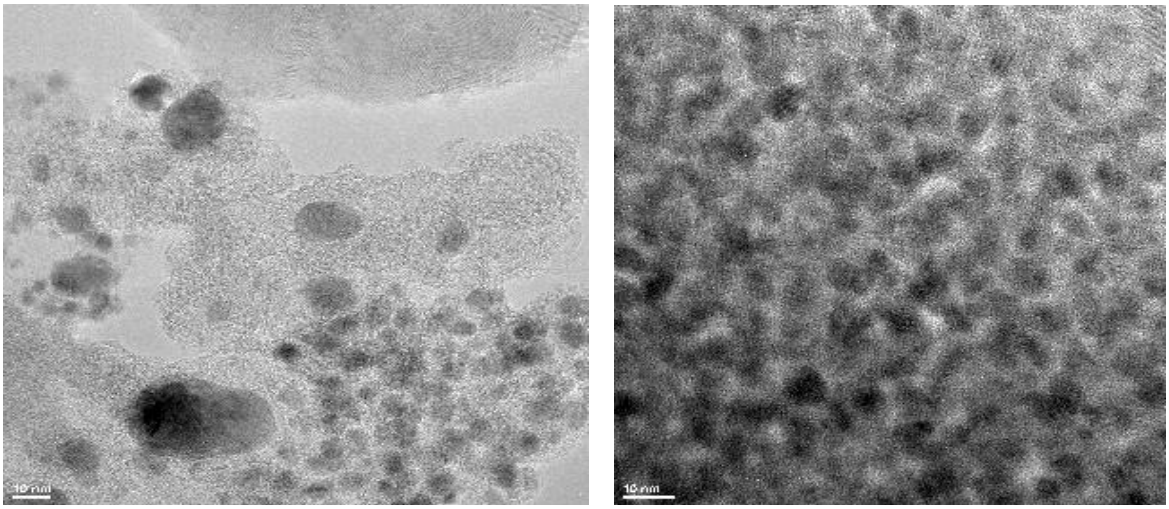

Fig. 1. TEM and HR-TEM images of magnetic nanoparticles: left: C-Fe (benzene/aniline), right: Fe_3O_4/oleic acid

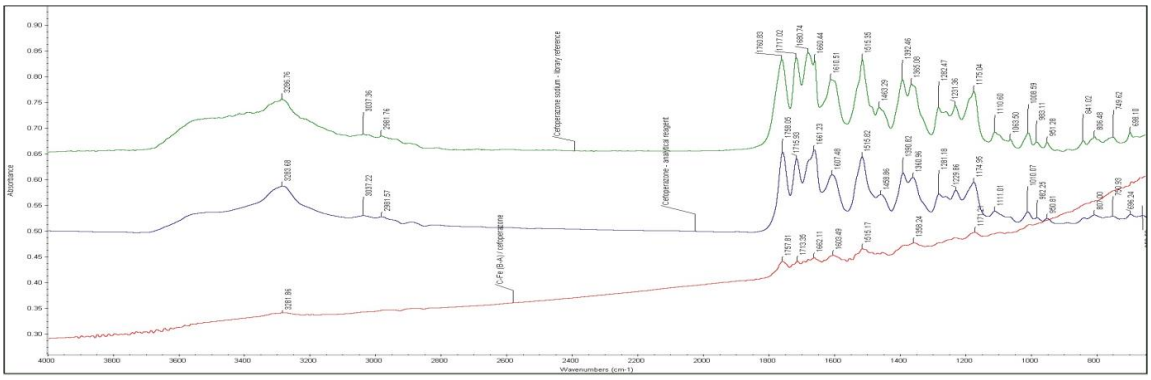

Fig. 2. FT-IR spectra of magnetic nanoparticles C-Fe (B-A)/Cefoperazone

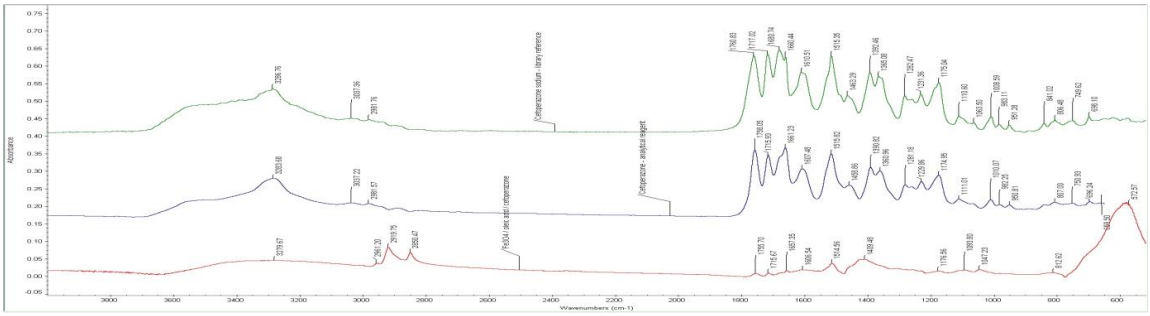

Fig. 3. FT-IR spectra of magnetic nanoparticles Fe_3O_4/oleic ac./Cefoperazone

The FT-IR spectra confirms the nanostructured system expected - IR: secondary amine - 3286 cm^{-1} one characteristic N-H absorption band, overlapped with O-H 3400 cm^{-1} large carboxylic band, 2900 cm^{-1}, 2824 cm^{-1} and 3053 cm^{-1} for the saturated and unsaturated C-H absorption, 1760 cm^{-1}, 1717 cm^{-1}, 1680 cm^{-1} related to amidic, carboxylic and lactamic C=O streching vibration mode. Cefoperazone adsorption on both nanostructured supports is proved by the main active compound absorption bands around 1755, 1715, 1657 and 1514 cm^{-1}.

Controlled release profile of Cefoperazone from magnetic nanoparticles

The experiments showed that, even for times exceeding 4000 seconds, the water solubility level of the pure compound, Cefoperazone, is not reached, demonstrating a gradual release of cephalosporin in the aqueous medium. The analytical data confirmed a higher retention in the case of adsorption of Cefoperazone on a nanostructured support, by comparison with a control sample consisting of Cefoperazone alone. In all cases, this curve presents a maximum after less than 300S, demonstrating the solubilisation of the studied compound. This maximum is followed by a plateau, indicating that the maximum concentration has been reached by complete solubilisation.

From the experimental point of view, the conductometric method is more advantageous having better control on the experiment parameters, and also because the active compound's diffusion in a turbulent regime allows maximum momentary quantities of active compound to be transferred from the nanostructured solid phase to aqueous phase. The experimental results are present in figures 4, 5, in time (s) – conductivity (μS)
coordinates.

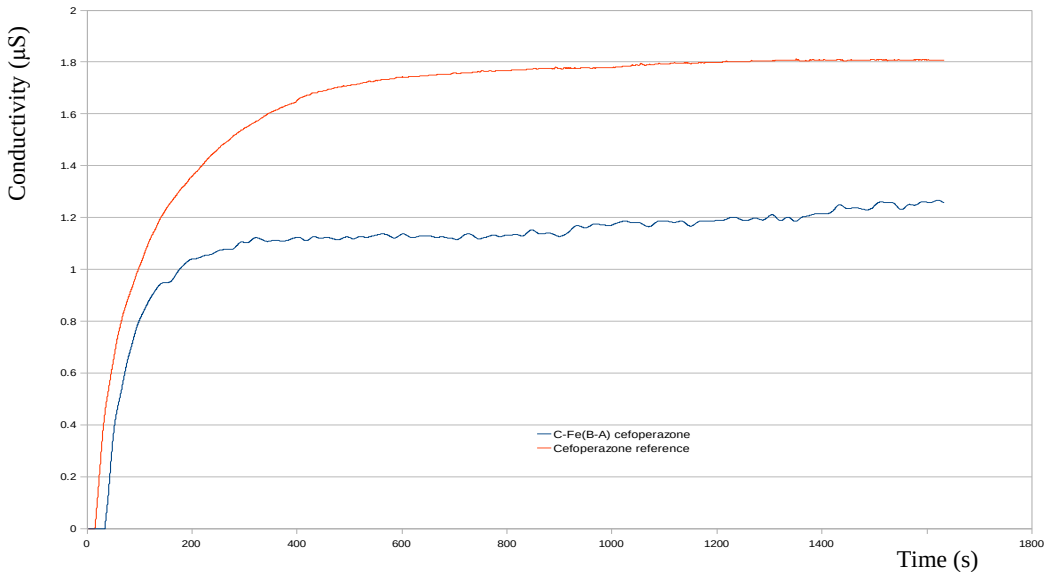

Fig. 4. Controlled release test of Cefoperazone alone (red) and from nanosystem type C-Fe (BA) (blue)

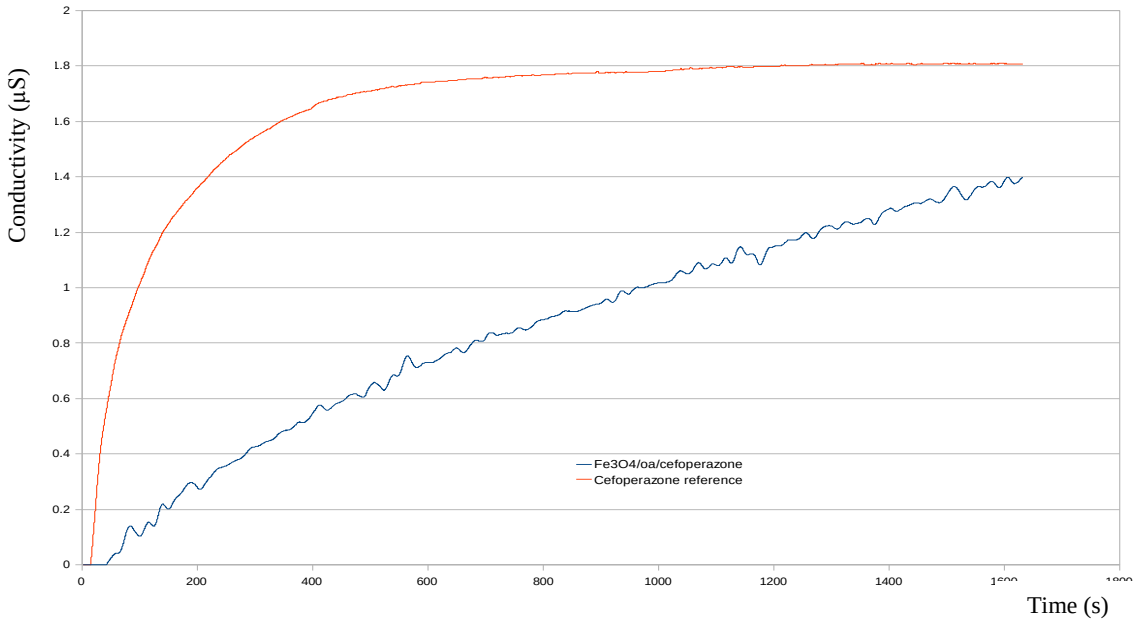

Fig. 5. Controlled release test of Cefoperazone alone (red) and from nanosystem type Fe_3O_4/oleic acid (blue)

The experiments made using the spectrophotometric method involve more complex assemblies, rising issues concerning the immobilization of the nanostructured support. A correct analysis in the conditions of the nanostructured support's transit through the analyser of the UV-VIS spectrometer is impossible.

Another experimental constraint for this second method is represented by the very low concentrations of active compounds, lower than the sensibility limits of a classic liquid cell UV-VIS. The obtained results were grouped according the nanostructured support's nature (in absorbance units), been presented in figures 6 and 7, in time (s) – absorbance (mAu) coordinates.

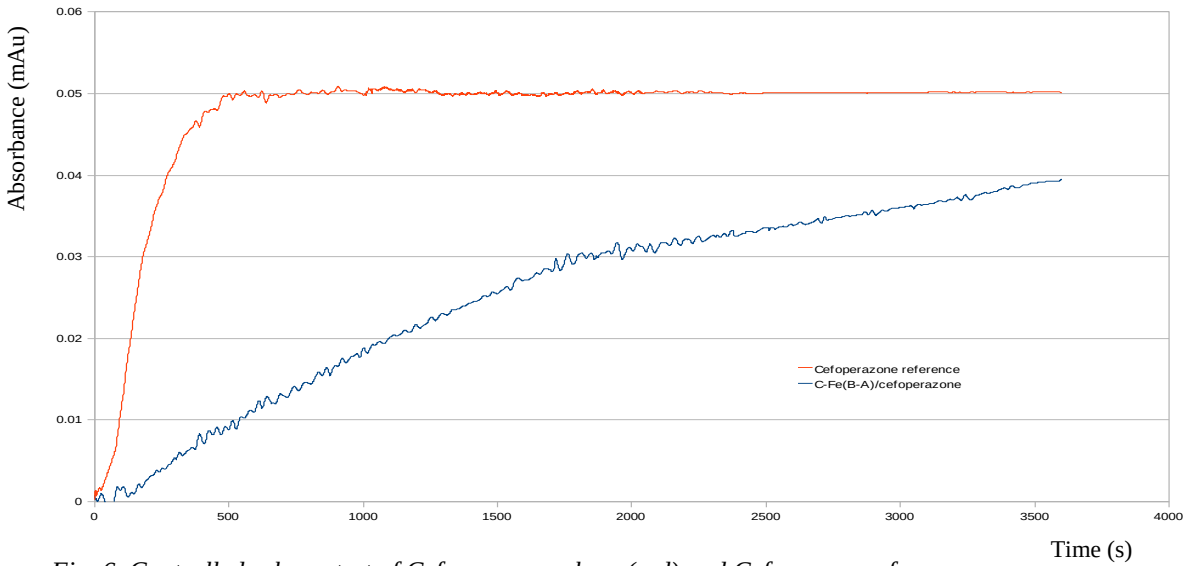

Fig. 6. Controlled release test of Cefoperazone alone (red) and Cefoperazone from nanosystem type core/shell C-Fe(BA) (blue)

Time (s)

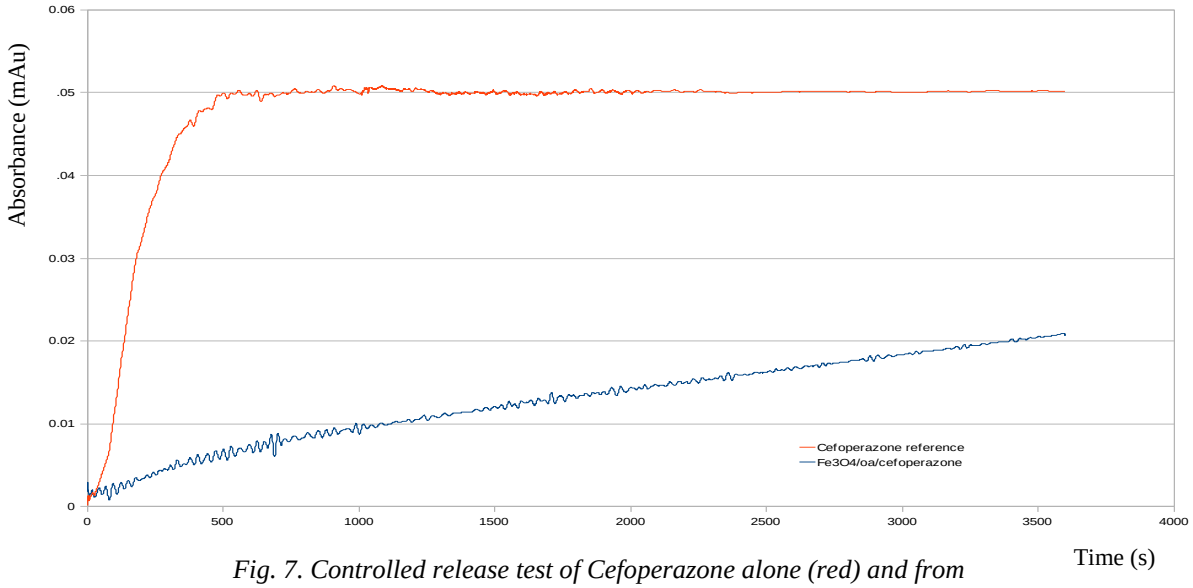

Fig. 7. Controlled release test of Cefoperazone alone (red) and from nanosystem type core/shell Fe_3O_4/oleic acid (blue)

Specific precautions regarding the elimination of possible gas bubbles forming in the liquid cell were necessary. This type of perturbations could possibly lead to the compromise of the experiment. From this point of view, it was necessary to introduce a buffer vessel for collecting any gas bubbles and fitting the liquid cell with vertical input and output ports, for a faster release.

4. Conclusions

The controlled release dynamics of Cefoperazone, third generation cephalosporin, from the two types nanostructured supports was studied by conductometric method and UV-VIS spectrometry. In all cases, at times larger than 4000 seconds, Cefoperazone is far from the solubility threshold, which demonstrates that setting the adsorption in nanostructured complex is a viable option for slow release of active compounds in the dispersion environment.

The full release of the biologically active compounds occurs after a period at least 10 times greater than the total time of 3600s. The great plateaus observed imply changes in the release mechanism. These are attributed to the existence of successive shells of active compound due to the particularities of the synthesis process. Consequently, solvation is sequential, interaction parameters changing as depending on the distance to the core of the nanoparticle. In all studied situations, two or three significant plateaus are noticed. Variations due to different types of nanostructured support have been noted as well.

The UV-VIS method showed significantly larger releases times for Cefoperazone, according to the controlled release curves obtained. This can be explained by diffusion limitation factors such as necessity of introducing the nanostructured material in a nacelle made from a porous material, thus eliminating favourable turbulence mechanisms.

Therefore, it can be concluded that the UV-VIS spectrophotometric method is superior to the conductometric method, being able to eliminate possible electrode reactions of the compounds from the dispersion medium. On the other hand, conductometry is very useful as a quick work method.

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