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Xanthan and Poly(vinyl alcohol) - Based Composite Films, as Supports for Chloramphenicol Immobilization

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Abstract

The paper discusses a method for the realization of some polymer - drug systems in which the macromolecular support is represented by a three-dimensional network based on xanthan and poly(vinyl alcohol). Knowing that the drug (chloramphenicol) was to be inserted through a diffusion process, the support – selected according to an experimental program – had the highest degree of swelling.

Several variants of chloramphenicol inclusion into the synthesized support are analyzed by studying the process kinetics. The study of chloramphenicol release from the inclusion products, in the form of films, indicated the installation of a "zero order" kinetics.

The tests devoted to the system's antimicrobial activity evidenced their biological action.

Introduction

The studies devoted to the systems of drug controlled release continue to be of special interest, as assuring the interdisciplinarity between the medical field and that of macromolecular chemistry. Such systems have been created for stimulating the processes occurring in the organism through the drug release towards an organ or a tissue, with a certain transfer rate, and over a determinated time interval, so that an optimum therapeutical effect, with minimum secondary effects, should be obtained.

In the preparation of such systems, several aspects should be considered, among which the chemical nature of the polymeric support and the interactions between it and the biologically - active compound are of special importance. Natural polymers and their derivatives, especially polysaccharides, characterized by biocompatibility, biodegradability, absence of toxicity towards the living organisms, are mainly preferred [1-3]. The preparation of the polymer-drug conjugate may be made through various methods, which involve chemical (ionic or covalent) bonding of the drug, insertion into three-dimensional networks, encapsulation in micro - or nano-particles [4-10].

The present paper studies the preparation of some polymer - drug systems by the insertion of the drug into the three-dimensional network of interpenetrating type, as a film, based on xanthan (Xan) and poly(vinyl alcohol) (PVA). The literature in this field makes mention of the possible formation of complexes between PVA and macromolecular compounds having substituents with anionic character [11].

The drug retention on the support is caused by the hydrogen bonds determined by the presence of hydroxylic groups in the chemical structure of all components involved in the system.

The study is structured into two parts; thus, the former one is dedicated to the preparation of the Xan - and PVA - based macromolecular support, while the latter approaches the kinetic study of inclusion through diffusion of chloramphenicol (CLP) in the hydrogel structure and, respectively of its *in vitro* release. The biological activity of the synthesized system is evidenced by standard tests of antimicrobial activity.

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Methods

For the synthesis of the macromolecular support, the following substances have been used: xanthan ("Keltrol F", Kelko/AIL Int.Ltd.), poly(vinyl alcohol) ("POLINOL" P-17S, Oriental Chemical Industry, Korea) and epichlorhydrine (S.C. ANTIBIOTICE S.A. Iasi, Romania). Chloramphenicol (sodium succinate) is produced by S.C. ANTIBIOTICE S.A. Iasi, Romania.

The hydrogels were characterized from the point of view of the maximum swelling degree (Q_{max}), determined by using the relation (1):

$$Q_m = \frac{m - m_0}{m_0} 100 = \frac{m_1}{m_0} 100 \, , (\%) \qquad (1)$$

where: Q_m = swelling degree; m_0 = sample's weight before swelling; m = sample's weight after swelling; m_1 = weight of the solvent absorbed by the sample.

This property was studied by means of a centered, rotatory, second order experimental program, depending on three factors: the PVA/Xan weight ratio, the reaction temperature and the time of reaction. Codification and values of these factors are listed in Table 1.

Table 1
Real and coded values of the factors studied in the synthesis of hydrogels.

Code variable	-1.682	-1	0	1	1.682
PVA/Xan ratio	0	0.203	0.5	0.797	1
Temperature, °C	40	48.11	60	71.89	80
Time, min	120	217	360	503	600

The hydrogels have been synthesized as 0.5 mm thick films, from 13.4 % concentrated Xan and PVA solutions, in distilled water, which have been combined so that to respect the weight ratio between polymers given in Table 1. The reaction with epichlorhydrine was performed in alkaline medium, at a polymers/epichlorhydrine weight ratio = 2. After the reaction, the film has been purified through repeated washings with distilled water (for NaOH removal) and methanol (for epichlorhydrine removal), then dried at room temperature. The study of hydrogels' swelling in distilled water was developed on a Dogatkin apparatus. For the subsequent experiments of immobilization and controlled release of CLP, a hydrogel with a maximum swelling degree in distilled water (1116 %) synthesized in the following conditions: PVA/Xan = 0.2 (g/g), 72°C temperature reaction and 210 min. time of reaction was selected. Chloramphenicol's inclusion into the structure of hydrogel was realized through the diffusion, in three variants, as follows:

1. After purification, the hydrogel was dried at 50°C for 12 h, then introduced into 25 mL solution of CLP (0.2 %);
2. The hydrogel was swollen in distilled water up to a maximal swelling degree (1116 %), then introduced into 25 mL solution of CLP (0.2 %);
3. A precisely - weighed amount of CLP was deposited on the hydrogel film swollen at the maximal swelling degree in distilled water, seen as being dissolved and absorbed very quickly in the structure of support.

The drug amount included in the support (variants 1 and 2) was determined by UV-VIS spectroscopy (SPEKORD UV-VIS spectrophotometer, Germany), the value of extinction corresponding to a 360 nm wavelength being established. To this end, the constant volumes of solution (which, after their dilution to a certain amount have been photocolorimetered) have been taken over from the supernatant (in which a precisely – weighed amount of suspended at well – determined time intervals). Based on the previously - plotted reference curve (Fig. 1), the concentration of the drug in the samples taken over has been determined, while the amount of CLP included into the support was deduced as a difference.

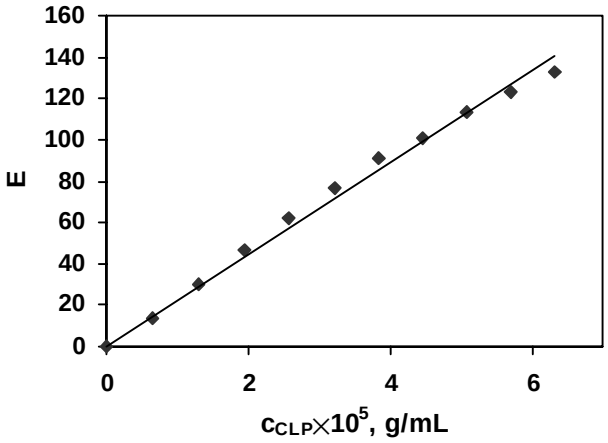

Fig. 1. Reference curve, plotting the variation of extinction as a function of CLP concentration in solution

For all the three variants, the kinetics of drug's release in distilled water was studied, in either static or continuous elution regime. In a static regime, the dry, precisely - weighed drug sample was introduced

into 25 mL of distilled water; periodically, precisely - weighed volumes of solutions - the drug concentration of which had been determined by the above mentioned technique - have been taken over. In the second variant, an exactly - weighed amount of film swollen with the inserted drug was introduced into 10 ml eluent (distilled water), continuous elution being assured, at a constant flow (1 mL/h), using the installation presented in Fig. 2.

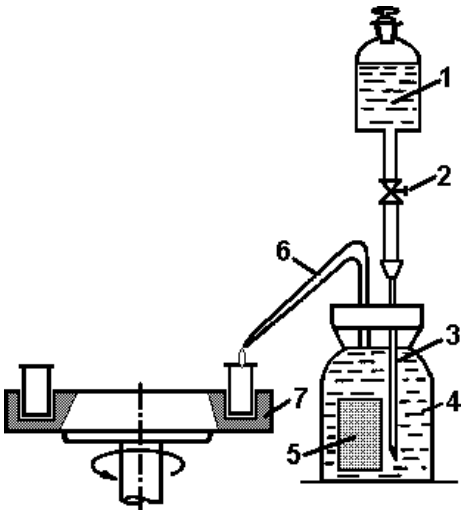

Fig. 2. Dynamic in vitro drug elution from Xan - and PVA-based hydrogel with CLP: (1) eluent reservoir, (2) setting valve, (3) capillary tube, (4) reservoir, (5) hydrogel with CLP, (6) capillary tube, (7) automatic sample collector

The time variation of the concentration of CLP accumulated in the eluent was followed for 24 h by means of the previously described technique.

The microbiological tests on *Staphilococsus aureus* and *Bacillus subtilis* (agar plates) attest the antimicrobial activity of the product.

Results and discussion

The two polymers employed in the synthesis of the macromolecular support have been selected according to their compatibility and also due to the fact that each of them may be used, individually, in the preparation of systems with controlled release of pharmaceutical substance. The chemical structure of polymers is given in Fig. 3.

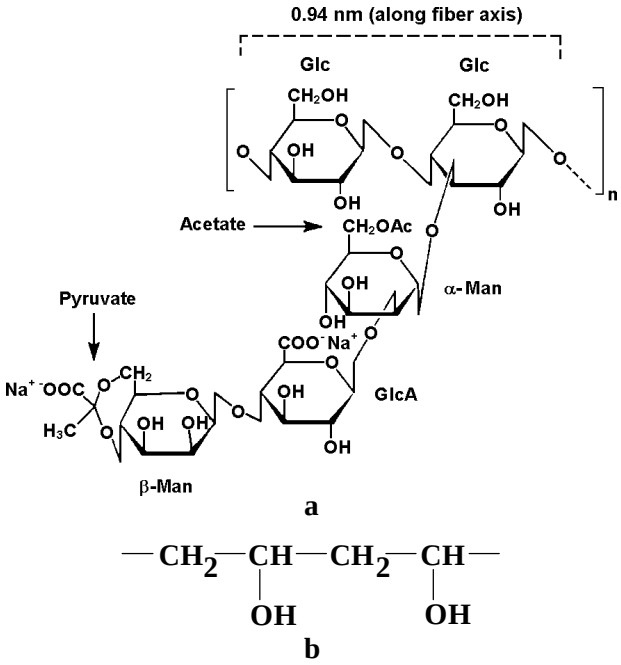

Fig. 3. Chemical structure of xanthan (a) and PVA (b).

The presence of hydroxylic groups in both polymers assures their compatibility in solution. In order to prepare the hydrogels, the mixture of the polymers was reacted with epichlorhidrine. The scheme of the reactions is presented in Fig. 4:

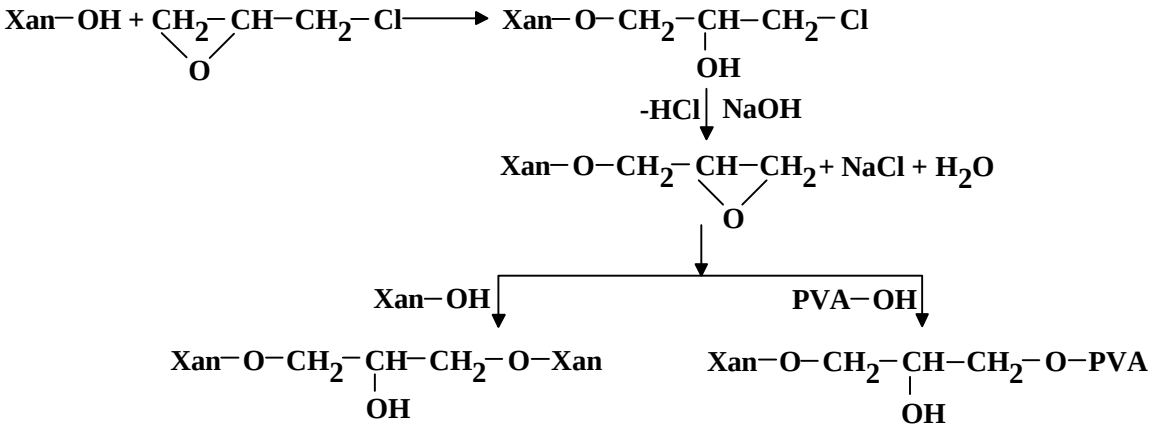

Fig. 4. Chemical reactions involved in the preparation of Xan - and PVA - based composite hydrogels

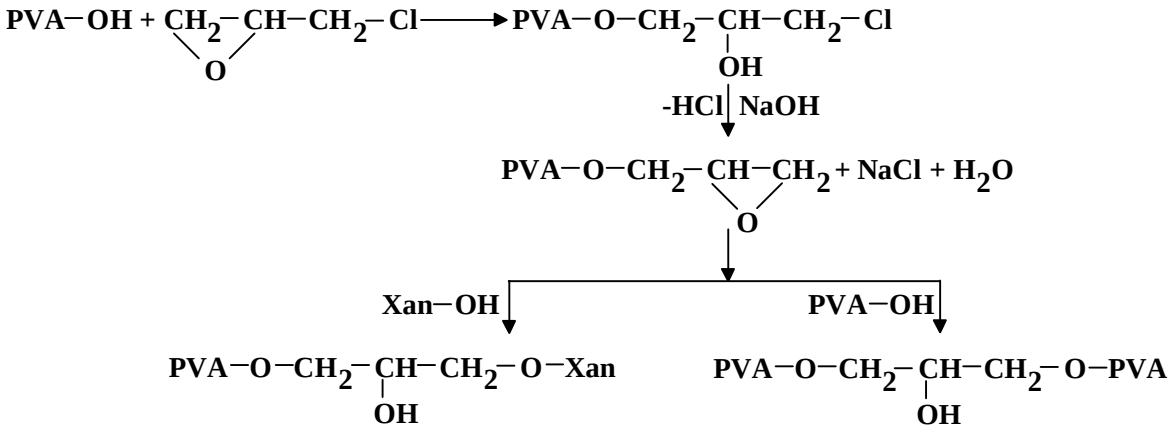

Fig. 4. *(Continued)*

In the first phase of the reaction, the hydrophobization of the two polymers occurs, as manifested by a reduction of their solubility in water, a phenomenon which is intensified with the system's shifting into the second phase of the reaction, when crosslinking of the two macromolecular compounds takes place. Finally, interpenetrating networks, whose crosslinking degree depends on the synthesis conditions were obtained [12]. The main characteristic of hydrogels, known as depending on crosslinking density, is their swelling degree in water, which is influenced by time and temperature of reaction.

Starting from the experimental of included in the research program, the hydrogel with the highest swelling degree (synthesized as shown above) has been selected for subsequent tests. In similar conditions of reaction, the hydrogels of some individual polymers have been synthesized, their recorded swelling degrees being found as lower than those of the composite hydrogel. Also, the impossibility of obtaining of films based exclusively on PVA by application of this technique was demonstrated.

1. In the first variant of CLP immobilization, the evolution of the concentration of drug was followed in time, up to the installation of equilibrium (Fig. 5).

Diffusion of the drug occurs in two stages. Thus, in the first 2 h, a concentration of the solution in the drug is noticed, as a result of swelling of the hydrogel into water. The duration of the first stage corresponds to the time necessary for the swelling of the hydrogel up to its maximal value. In parallels, the limited diffusion of drug into the macromolecular support occurs, too, so that the global effect is that of the solution concentration of the CLP.

In the second stage of the process, a slight decrease of drug' s concentration may be observed, as a result of its diffusion in the already swollen hydrogel.

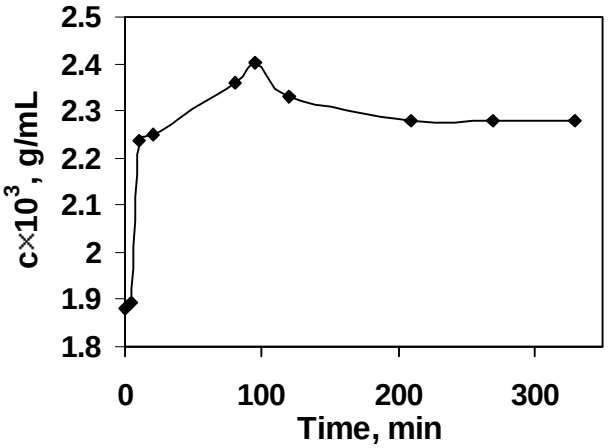

Fig. 5. Diffusion of CLP in the Xan - and PVA - based dry hydrogel: 0.5 g film immersed in 25 mL CLP solution (0.2 %)

After the installation of equilibrium the amount of CLP immobilized in 1 g of dry hydrogel (5.2 mg/g) and also the ratio of immobilized CLP (5.53 %) *versus* the initial amount have been calculated from the values of the initial and final amounts of drug in solution.

The kinetics of CLP release in distilled water was developed in static regime (Fig. 6), being described in the following section.

In this situation, the release of the drug is preceded by its dissolution into water, which assures the swelling of the hydrogel up to its maximum value. In the first minutes of the release process, a pronounced increase of the CLP concentration in the releasing medium is recorded, as a result of the diffusion of the drug absorbed on the surface of the hydrogel and in its superficial layers, too.

Increase of the swelling degree induces, too, release of the CLP immobilized in the deeper layer of support, although at lower rate. The equilibrium is installed quite quickly, in about 3 h.

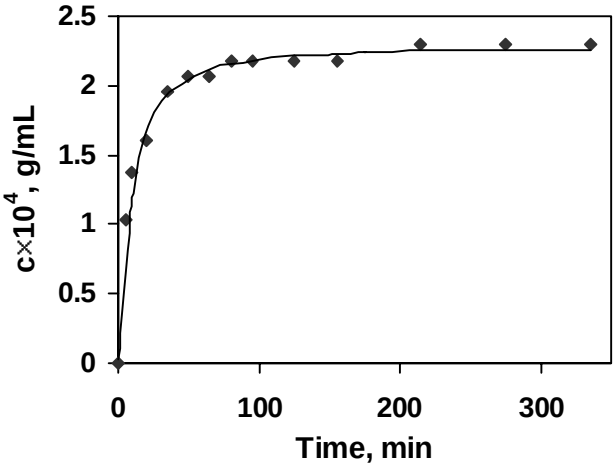

Fig. 6. Release of CLP from the Xan –and PVA-based hydrogel, in static regime: 0.4732 g dried hydrogel with 2.4 mg immobilized CLP, immersed in 25 mL distilled water

2. In the second variant of introduction of the CLP in the structure of the hydrogel, the previous process of swelling of the hydrogel to the maximal value is eliminated. In this way, the CLP concentration in solution decreases continuously, from the first moments of the process, as a result of its diffusion into the support (Fig.7)

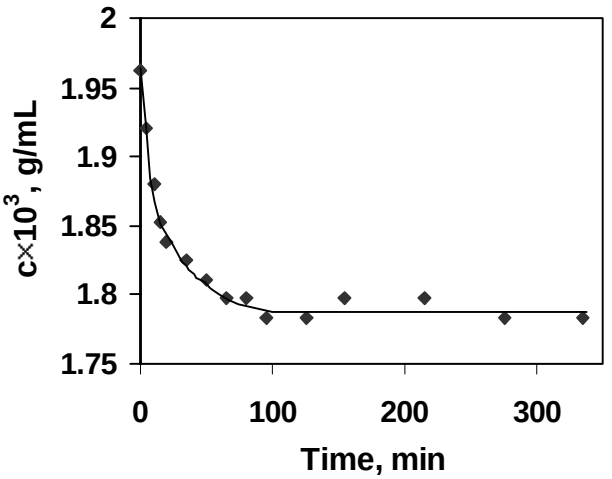

Fig. 7. Diffusion of CLP in the Xan – and PVA - based swollen hydrogel: 0.57 g hydrogel swollen in distilled water (1116 %), immersed in a 25 mL CLP solution

In this variant, 9.18 % of the initial drug amount is inserted, a system with 7.8 mg CLP/g hydrogel being thus obtained. After establishment of equilibrium in the system, the macromolecular support with inserted drug was separated and, without being dried, was immersed in a well - determined volume of distilled water, for the determination of the release kinetics. Thus, Fig. 8 plots the amount of drug released in 24 h, at a solution flow of 1mL/h. The release rate is higher in the first 2 h (Fig. 9), on behalf of the CLP immobilized in the film's superficial layers, which is followed by a decrease, up to the installation of a "zero order" kinetics for about 10 h, which is indicative of the system's retard character.

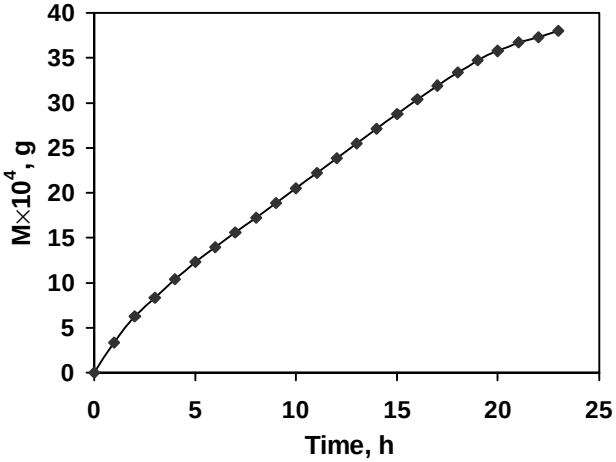

Fig.8. Amount of CLP released in 24 h, in a regime of continuous elution: 0.57 g swollen hydrogel, with a content of 4.45 mg CLP, immersed in 10 mL distilled water; eluent flow = 1 mL/h

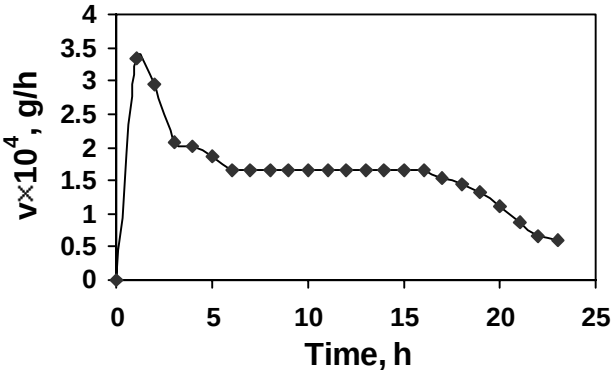

Fig. 9. Variation - in time - of the release rate of CLP, from the Xan - and PVA - based hydrogel, in a regime of continuous elution: 0.57 g swollen hydrogel, with a CLP content of 4.45 mg, immersed in 10 mL distilled; water eluent flow = 1mL/h

3. For the third immobilization variant, which permits an exact control of the content of drug included into the support, the release kinetics was determined in a regime of continuous elution, too. Graphical representation of the drug amount released in a 24 h time interval (Fig. 10) evidences two time intervals, characterized by relatively constant although slightly different rates.

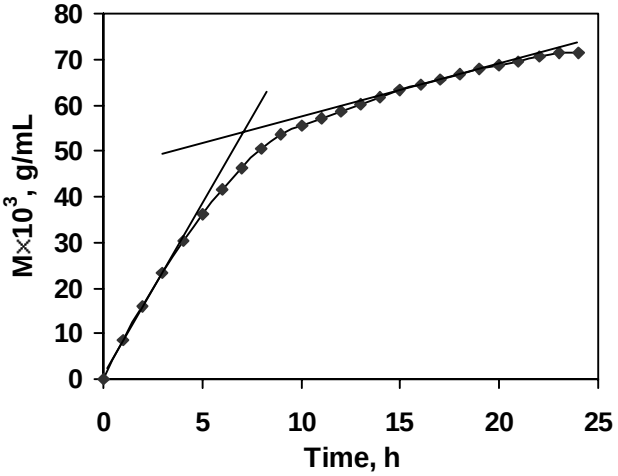

Fig. 10. CLP amount released in 24 h of continuous elution regime: 1.9414 g swollen hydrogel, with a 74 mg chloramphenicol content, immersed in 10 mL distilled water; eluent flow = 1mL/h

Suprasaturation of the macromolecular support in the drug may be possibly induced by two factors, namely: the good solubility of CLP – occurring as a sodium salt in water - and the support' s high swelling degree. In this way, in the support there will exist a certain amount of drug linked through physical interaction at the polymeric chains, and another amount, dissolved in water, from the network's meshes, which is not involved in physical links with the polymers, at all. This might explain the existence of two time intervals, characterized by relatively constant release rates. In the first interval (showing higher rates), the CLP dissolved in water from the networks' meshes is released, while in the second (characterized by lower rates), it is released the CLP involved in direct physical links with the polymers' network.

The tests of antimicrobial activity [13, 14] performed on this system have evidenced the capacity of the polymer - drug systems obtained of inhibiting the development of some microorganism structures (*Staphilococcus aureus* and *Bacillus subtilis*, agar plates), having an efficiency comparable to that of the free drug.

Conclusions

Composite hydrogels of the IPN network - type, based on Xan and PVA, with various swelling degrees, superior to the individual polymers crosslinked by the some method, have been realized.

CLP' s insertion into Xan – and PVA - based supports as a film, has been realized by various techniques.

The retard character of the hydrogel - CLP systems has been demonstrated by the installation of a "zero" order kinetics, over certain time intervals, during drug's *in vitro* release.

The microbiological tests on *Staphilococcus aureus* and *Bacillus subtilis* (agar plates) attest the antimicrobial activity of the product.

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