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Hydrogels based on carboxymethylcellulose and poly (vinyl alcohol) for controlled loading and release of chloramphenicol

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Hydrogels based on carboxymethylcellulose (CMC) and poly (vinyl alcohol) (PVA), crosslinked with epichlorohydrine in basic medium, are polymeric materials interpenetrated network type, designed for development of polymer-drug systems with sustained release. CMC and PVA are polymers (natural, respectively synthetic) characterized by biocompatibility and biodegradability, important conditions for the polymers used for medical applications. The preparation of this new type of hydrogels is aimed to obtain a new type of macromolecular supports able to include and release in a sustained way hydrosoluble drugs (chloramphenicol). By the variation of the crosslinking reaction parameters there were developed networks with different crosslinking degrees, respectively with different swelling degrees, which influence the drug loading degree. For obtaining diffusional systems, the hydrogels with maximum swelling degree, characterized, *a priori*, by zero order release kinetics, were selected. The same systems were characterized from the point of view of antimicrobial activity by microbiological tests.

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Keywords: CMC/PVA hydrogel, Interpenetrated network, Drug delivery, Microbiological test

1. Introduction

In the last years there were developed, except the classical forms (solutions, emulsions, ointments, suppositories), new drug formulations by technologies which confer them an fundamental attribute: the release of the drug in a specific amount and with a specific rate for penetrate into the body, or the transport of the drug at the pharmacologic action site where it is released in an exact amount and rate accordingly with biological processes. The association of the drugs with macromolecular compounds can modify their release rate into the body and make possible a possible prolonged action. The preparation studies of the polymer-drug systems have attracted in the last years a large interest due to their advantages compared with free drugs: prolonged action, a constant and therapeutic level of the drug into the body, low drug consumption.

The immobilization of biological active principle on substrates can use support materials like: hydrosoluble polymers; polymers with a high capacity of swelling in water (hydrogels); solid type supports: fibers, woven, membranes, porous structures.

Hydrogels are defined as polymer tridimensional networks with a hydrophilic nature, interconnected by physical or chemical bonds. These networks are able to include large amounts of water or biological fluids. Chemical hydrogels are tridimensional networks formed by the introduction of crosslinks formed by covalent bonds; except the case when these covalent bonds can be broken, this type of hydrogels are able only to swell, they are not soluble in water or in other organic solvents not even under temperature.

This study presents the preparation of a new materials interpenetrated networks type, by simultaneous crosslinking reaction of a mixture based on carboxymethylcellulose (sodium salt)-CMC and poly (vinyl alcohol)-PVA in the presence of epichlorohydrine-EpCl [1,2]. The influence of crosslinking reaction parameters on the properties of obtained hydrogels was studied:

- Concentration of CMC in initial polymer mixture (%CMC);
- The ratio between polymers and crosslinker amounts (epichlorhydrine) (r_{pE});
- The concentration of polymers solution (C_p);
- Crosslinking time (t_r).

For hydrogels characterization next aspects were taken into account:

- The composition of obtained interpenetrated network;
- The swelling properties in water;
- The loading and release kinetics of chloramphenicol hemisuccinate;
- Microbiological tests on chloramphenicol hemisuccinate loaded hydrogels.

2. Experimental

2.1 Materials

For hydrogels preparation next substances were used:

Carboxymethylcellulose (sodium salt)- CMC (FLUKA) - polysaccharide, respectively cellulose ether which contains acidic groups; it is usually used as sodium salt The CMC structure is based on the 1,4-β-D-glucopyranose polymer of cellulose (figure 1). Different preparations may have different degrees of substitution, but it is generally in the range 0.6 - 0.97 derivatives per monomer unit.

CMC molecules are most extended (rod-like) at low concentrations but at higher concentrations the molecules overlap and coil up and then, at high concentrations, entangle to become a thermoreversible gel. Increasing ionic strength and reducing pH both decrease the viscosity as they cause the polymer to become more coiled.

As sodium salt (CMC-Na) it is water soluble.

CMC-Na tends to form complexes with some pharmaceutical substances by flocculate or precipitate them. Also, CMC-Na decreases the activity of antimicrobial agents.

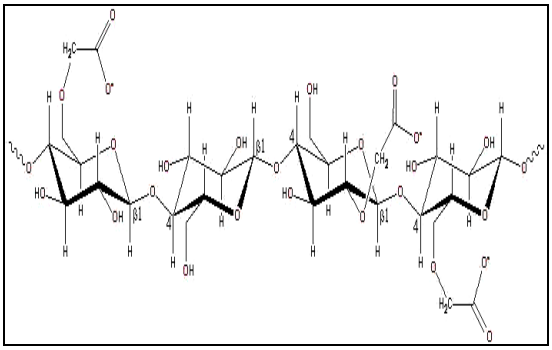

Fig. 1. Chemical structure of carboxymethylcellulose.

Poly (vinyl alcohol)- POLINOL P-05S-PVA (Oriental Chemical Industry, KOREA, M_w=38000, substitution degree 5.1×10^{-3}mol/g);

PVA is a thermoplastic polymer obtained by alkaline hydrolysis of poly(vinyl acetate) (Fig. 2).

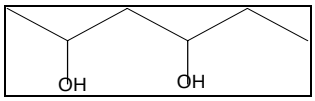

Fig. 2. Chemical structure of poly (vinyl alcohol).

In the case of an incomplete hydrolysis the obtained polymer contains hydroxyl groups and also acetyl groups, nonuniformly distributed all along the macromolecular chains.

The presence of the numerous hydroxyl groups in the polymer structure (group with a high polarity) explains the PVA solubility in water, polyols, formamide, ethanolamine and DMSO.

PVA water solubility varies with the temperature and also depends on the polymerization and hydrolysis degrees, having maximum values at 86-89 % hydrolysis degree.

The viscosity of aqueous solutions of PVA depends on the concentration, temperature and also on preserving time. At concentrations higher than 10% PVA usually gelifies, phenomenon which in time become more intense [3].

PVA is frequently used in pharmaceutical technology like excipient for gels, emulsion stabilizer, but especially like vehicle for collyriums because it is able to increase their viscosity being also compatible with many drugs used in ophthalmology like: zinc sulphate, nitrogen based pilocarpine, ephedrine chlorohydrate, neomycin, polymixine, sulphacetamide, chlorbuthanol, etc.

Epichlorohydrine – EpCl (ALDRICH);

M=92,53; ρ=1,181;

EpCl is a colorless liquid. The water solubility in 100g water is less then 5,0g.

It is used like solvent and in synthesis.

Chloramphenicol (Chloramphenicol sodium hemisuccinate) -ClPh (S.C. ANTIBIOTICE IASI – ROMANIA).

ClPh is the sodium salt of 2,2-dichlor-N- [(1R,2R)-2-hydroxy-1-hydroxymethyl-4-nitro-phenethyl] acetamide.

The molecular formula: $C_{15}H_{15}Cl_2N_2NaO_8$;

M_w=445,2

The most important properties of ClPh are: microcrystalline white or white - yellowish powder, odourless, bitter taste and strong hygroscopic; is easily soluble in ethylacetate, acetone, propylenglycol, alcohols and less soluble in ethers; presents antibacterial activity; it is used as antibiotics [4].

2.2. Methods

Hydrogels based on CMC and PAV-preparation method

The principle of the method consists in obtaining the hydrogels interpenetrating network type, based on a polysaccharide-CMC and a synthetic polymer-PVA, by covalent crosslinking method, using epichlorohydrine as crosslinker.

Protocol

In a beaker the exact amounts of CMC and PVA (the total amount of polymer is 1g) and distilled water (7.5 ml) are added. The mixture is strongly stirred with a glass stick until a uniform gel is formed. Then, are added drop by drop 0.8 ml NaOH 10N and the specific amount of crosslinker (EpCl) under a continuous homogenization for avoiding the gelification and separation of PVA from the polymer mixture. The resulting composition is laid on glass stencils and kept in the oven at 45 ^oC.

After the crosslinking, the hydrogels films are removed in distilled water for removing the non reacted components and sodium chloride resulted from crosslinking reaction.

The hydrogels were washed three times by their immersion for 24 hours in 400 ml distilled water at 45 ^{o}C. Finally, the hydrogels were purified by acetone extraction (for the complete extraction of nonreacted EpCl and of the water) and then dried in the oven at 45^{o}C.

Determination of hydrogels composition based on CMC and PVA

Gravimetric method

The principle of the method is based on the transformation of sodium from CMC sodium salt in sodium sulphate, which is gravimetrically measured.

Protocol

In a porcelaine capsule, brought before at a constant weight, are weighted 3g of hydrogel sample; above this is added 5 ml of concentrated sulphhydric acid and is bring to dry. The capsule is cooled, then are added few more drops of acid and then carefully evaporated. The residue, composed by sodium sulphate, is calcinated for 1 hour at 600^{o}C.

FT-IR spectroscopy

The FT-IR spectra were set out using FT-IR BONEM 104 B Canada spectrometer using the KBr disk method.

Scanning Electron microscopy

The SEM images were obtained using TESLA BS 301 microscope. The sample, partially swollen in ethylenglycol, fixed with a electroconductor paste, is passed to a catodic evaporation process and finally covered with a thin layer of gold.

Determination of maximum swelling degree and swelling kinetics

Swelling degree was determined with Dogadkin device using purified and dried hydrogel samples.

$$Q_t = \frac{m_s - m_d}{m_d} \cdot 100 \text{, (\%)}$$

where:
Q_t-swelling degree at the timepoint t;
m_s-the weight of swollen hydrogel at the timepoint t;
m_d- the weight of dried hydrogel.

All measurements were done at 25^{o}C using distilled water as swelling agent.

Methods for loading and release of Chloramphenicol

The chloramphenicol loading study was performed by diffusion using two metods.

First Method – after purification the hydrogel was dried at 45 ^{o}C for 24 hours and then immersed in 25 ml chloramphenicol solution 0.2%.

Second Method – the hydrogel was first swollen in distilled water to the maximum swelling degree and then immersed in 25 ml chloramphenicol solution 0.2%.

The measurement of Chloramphenicol solution concentration was realized by UV-VIS (UV-VIS spectrometer CECIL) by using an absorption calibration curve (A) - concentration (C_{ClPh}) (figure 3).

For drawing the calibration curve there was used different solutions with known concentrations of Chloramphenicol in distilled water. The absorption wavelength of Chloramphenicol is 279 nm.

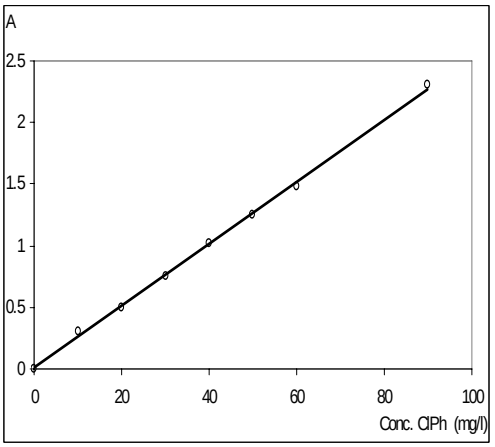

Fig. 3. Chloramphenicol calibration curve.

The kinetics of drug diffusion in hydrogels was determined by measuring the concentration of the drug left in the supernatant, at the different time points. By making the difference with the initial drug concentration the amount of the drug included in hydrogel was determined.

The chloramphenicol release study

For both methods of Chloramphenicol loading the kinetics of release in static regime was studied.

The dried loaded sample was precisely weighted and suspended in 25 ml distilled water. The variation of the drug concentration in the supernatant was established by measuring the concentration (according to calibration curve) of well determined volumes of supernatant taken out at certain time intervals.

The influence of some crosslinking reaction parameters on swelling characteristics and also on those of drug loading and release processes was studied.

The studied parameters are (table 1): CMC concentration in initial polymer mixture (%CMC); the ratio between polymer mixtures and the amount of crosslinker (w/w); the concentration of polymer solution (C_P); crosslinking time interval (t_r).

The determination of antimicrobial activity of the chloramphenicol loaded hydrogels

The antimicrobial activity of drug loaded hydrogels based on CMC and PVA was evaluated by studying the

inhibition capacity of bacterial cultures of *Pseudomonas aeruginosa ATCC 27853*.

The cultures were sowed on glass plates using Muller-Hinton method with incubation 24 hours at 37^0C. The diameters of the bacterial cultures inhibition zones were measured.

Table 1. Crosslinking reaction variable parameters.

Matrice 1P: ***The % of CMC in initial polymer mixture***: (Cp=12,78%; r_{PE} =10; T=45°C; t_r=4h)
Matrice 2P: ***The radio between the total amount of polymers and the amount of crosslinker (w/w):*** (Cp=12,78%; CMC=60%; PVA=40%; T=45°C; t_r=4h)
Matrice 3P: ***The crosslinking time interval***: (Cp=12,78%;CMC=60%;PVA=40%; T=45°C; r_{PE} =10)
Matrice 4P: ***The initial concentration of polymers solution:*** (CMC=60%; PVA=40%; T=45°C; r_{PE} =10; t_r=4h)
The common sample for all matrix: P-hydrogels (1P, 2P, 3P, 4P) – V5 hydrogel (CMC=60%; PAV=40%; r_{PE} =10; t=4h; Cp=12,78%) **The VI hydrogel - matrice 3P:** (CMC=60%; PAV=40%; r_{PE} =10; t=2h; Cp=12,78%)

3. Results and discussion

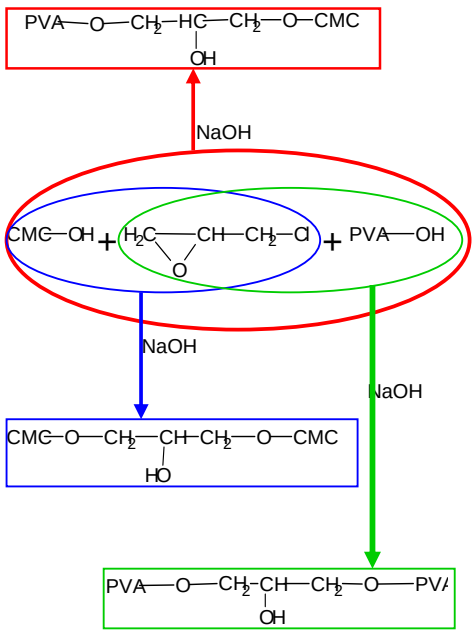

Fig. 4. Crosslinking reaction of CMC and PVA using EpCl in alkaline medium.

The crosslinking reaction takes place between functional groups hydroxyl type of polymers and epoxydic cycle of the crosslinker (epichlorohydrine in alkaline medium) (Fig. 4).

FT-IR spectra of CMC, PVA and those belonging to IPN type hydrogels based on the two polymers (Fig. 5) emphasis the presence of characteristic absorption bands for CMC and PAV, showing that the crosslinking reaction take place (Table 2) [5-7].

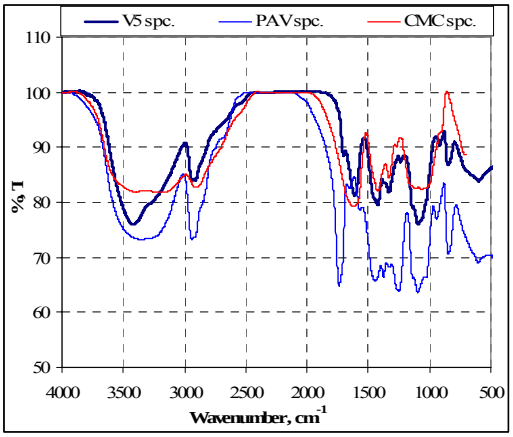

Fig. 5. Tramsmittance FT-IR spectra for sampleV_5.

Tabel 2. Hydrogel based on CMC and PVA crosslinked with EpCl in alkaline medium.

Wavenumber (cm^{-1})	Corresponding functional groups
3423,64	hydrogen bonded band
2937,58	C-H alkyl stretching band
1699,28	C=O band
1610,56	-COOH; C=O asimetric band
1450,47	-$COOCH_3$; CH_3 asimetric band
1423,46	- CH_2-COO-; CH_2 band
1373,31	-$COOCH_3$; CH_3 simetric band
1330,88	-COO^-; C=O and CH band
1234,44	-CO-; C=O asimetric band
1141,86	This band has been used as an assessment tool of poly(vinyl alcohol) structure because it is a semicrystalline synthetic polymer
1093,64	C-O-C aliphatic or cyclic band

From morphological point of view, the hydrogels are macroporous type as it is showed in the SEM image below (Fig. 6).

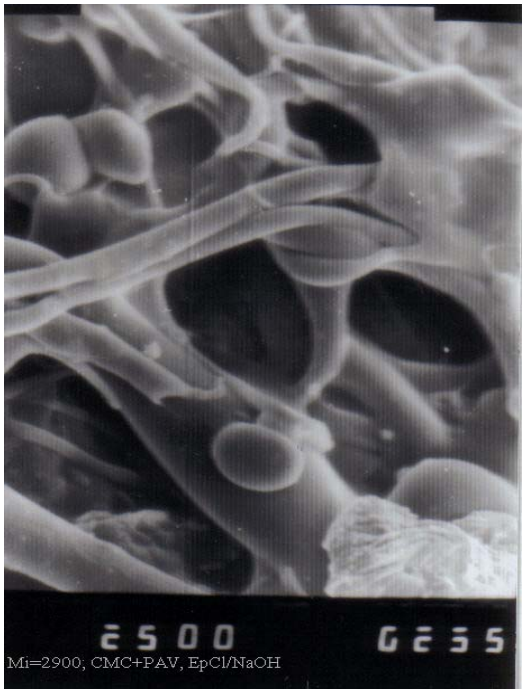

Fig. 6. SEM image for the sample V_5.

3.1 Hydrogels composition

Chemical composition of hydrogels (determined by evaluation of CMC proportion in crosslinked product) depends on the crosslinking reaction parameters (Figs. 8-11).

CMC is a polysaccharide with a double helix structure which is degraded under the alkaline condition and temperature influence. As a consequence, part of hydroxyl groups which are originally stabilizing the helicoidally structure through hydrogen bonds take part at the crosslinking reaction. PVA is a synthetic polymer which in alkaline condition takes a globular conformation [8]. As result, the crosslinking is taking place between PVA hydroxyl groups which are situated at the exterior part of the macromolecular coils and stretched CMC chains. This was proved by SEM image presented in Fig. 6.

After the destruction of the helicoidally double layer the evolution of the crosslinking reaction causes the network compactness [9].

The explanation for the influence of crosslinking reaction parameters on hydrogels composition is forward detailed. For 1P experimental program (Fig. 7) can be observed that for a CMC/PVA ratio higher than 1, the initial amount of CMC can be approximately refund in the final product. This fact suggests that the polysaccharide is more reactive than the synthetic polymer during the reaction with epichlorohydrin.

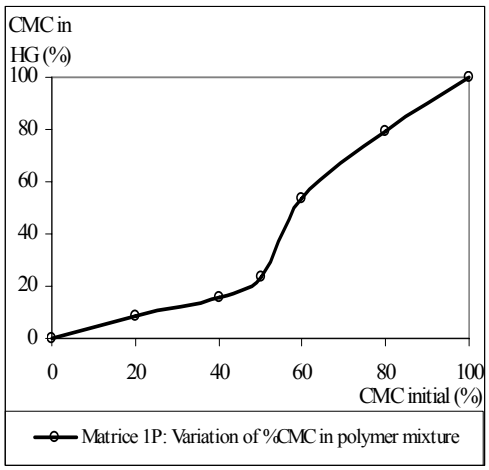

Fig. 7. The variation of CMC percentage in hydrogel in function of CMC percentage in initial polymers mixture.

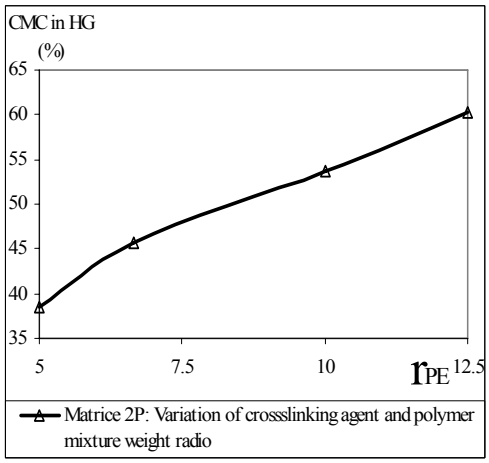

Fig. 8. Variation of CMC percentage in hydrogel function of the polymer/crosslinker (w/w) ratio.

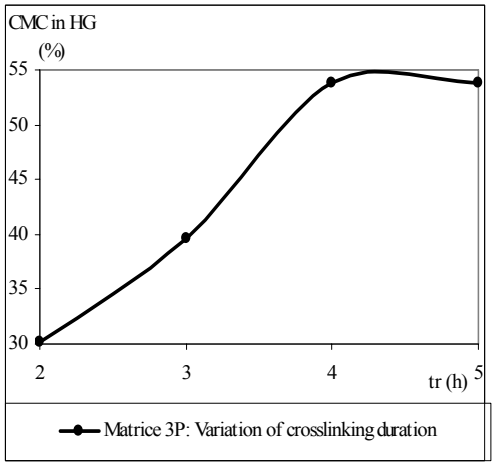

Fig. 9. Variation of CMC percentage in function of crosslinking time interval.

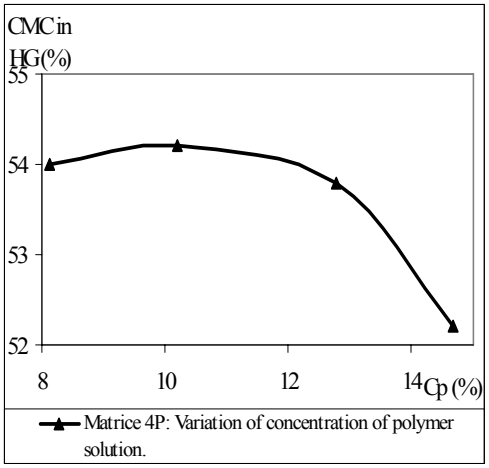

Fig. 10. Variation of CMC percentage in function of the concentration of initial polymer solution.

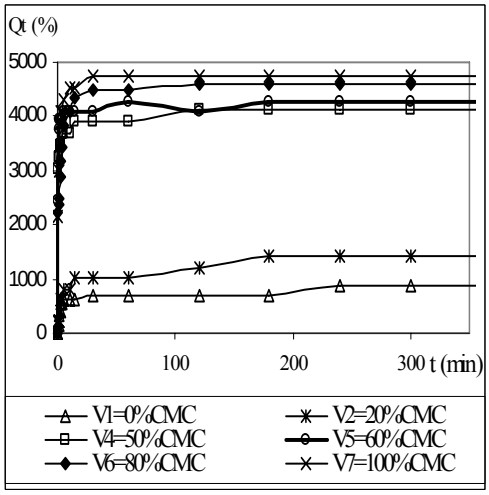

Fig. 11. The variation in time of swelling degree for the hydrogels belonging to 1P experimental program.

In the case of experimental program 2P (Fig. 8), the decreasing of CMC quantity in hydrogel with the increasing of the crosslinker amount, respectively, the decreasing of r_{pe} is due to the intensification of the crosslinking which determines the increase of CMC amount in final product.

In Fig. 9 it was proved that increasing the crosslinking time interval the quantity of CMC in final hydrogel increases. This phenomenon is explained by the fact that destruction of helicoidally double layer take place in time, under the alkaline medium and temperature influences, which determine, after a shorter period of time, a smaller number of hydroxyl groups to participate at the crosslinking reaction; for the bigger time intervals, when the polysaccharide macromolecules take a quasilinear conformation, the hydroxyl groups released from hydrogen bond are involved in the crosslinking reaction.

It was also shown that the initial concentration of polymers solutions does not really influence the quantity of CMC found in hydrogel composition (Fig. 10).

3.2. Determination of swelling degree

Davidson and Sittig (1962) have studied the CMC higroscopicity of CMC in respect with PVA and they have proved that CMC (in general, the polysaccharides) can absorb more water than CMC (hydrophilic synthetic polymer).

This effect is also underlined in Fig. 11 where the evolution of swelling degree in respect with time is presented for the hydrogels having as variable parameters the ratio between the two polymers in the initial mixture.

Can be also noticed that increasing the amount of CMC in hydrogels the maximum swelling degree will increase (Fig. 12).

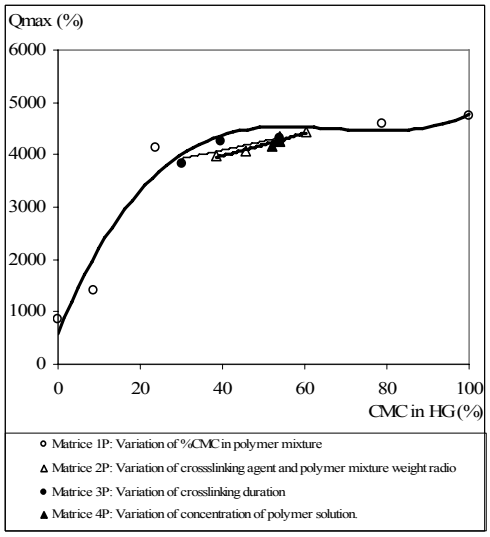

Fig. 12. The variation of the maximum swelling degree in function of CMC % in hydrogels.

3.3. Loading of Chloramphenicol in hydrogels

The Chloramphenicol loading in hydrogels was realized by diffusion from aqueous solution, the kinetic profile of loading curves being similar with the one of an absorption process of a dissolved substance by a solid support.

It was followed the evolution in time of drug concentration until the equilibrium for the two methods of hydrogels loading (Fig. 13).

First method (Fig. 13-(V5-4h) - curve)

From the graphs can be noticed that the loading process takes place in two stages:

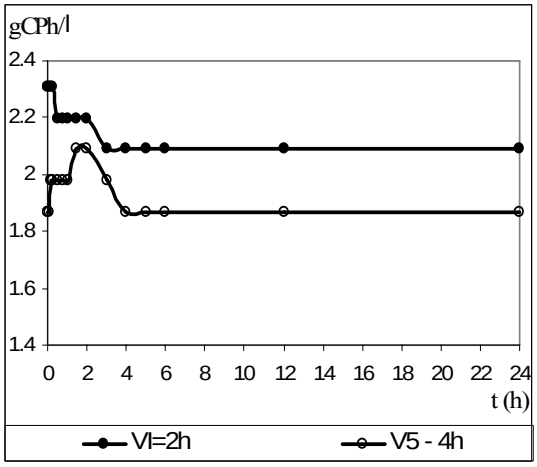

Fig. 13. The variation of Chloramphenicol concentration in the supernatant.

Stage 1 – Due to the fact that water enters first inside of the film, the concentration of drug solution (supernatant) will increase. The duration of this stage corresponds to the time that hydrogel needs to reach the maximum swelling degree.

In the same time, the limited diffusion of the drug takes place, but with a lower rate because of the big dimensions of drug molecule in respect with water, so the global effect is the concentration of Chloramphenicol solution.

Stage 2 – The drug concentration decreases due to diffusion in already swollen hydrogel. This phenomenon takes place after 2 hours, interval which corresponds to the necessary time needed for reaching the maximum swelling degree for V5 hydrogel (Fig. 11).

Second method (Fig. 13-(VI-2h) - curve)

In this case, the concentration of drug solution doesn't take place because the hydrogel is already swollen; the concentration of Chloramphenicol solution will continuously decrease from the first moments of the process due to the diffusion of the drug.

After the equilibrium was attained, from the initial and final values of drug concentration, the quantity of the drug immobilized in respect with 1 g dried hydrogel was calculated (Fig. 14).

For the studied parameters of crosslinking reaction, the amount of drug included in hydrogel usually varies in a similar manner with the swelling degree (Fig. 12 and 15).

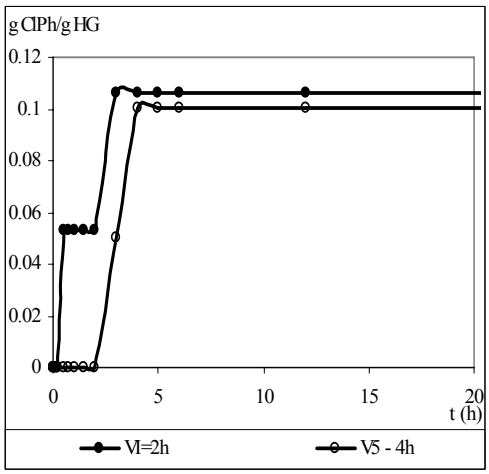

Fig. 14. – The variation in time of the drug quantity included in 1 g hydrogel obtained.

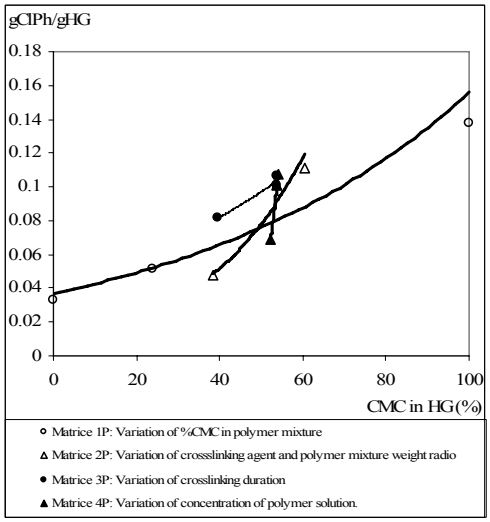

Fig. 15. The variation of the maximum amount of drug included in hydrogels having different CMC amount in composition.

3.4. Release of the drug from cloramphenicol loaded hydrogels

The release kinetics of the drug in distilled water in stationary regime was studied for the both ways of loading.

The variation of drug quantity released by diffusion from hydrogels is shown in Fig. 16.

The release of the drug is preceded first by the dissolution step of the drug in the water which swells the hydrogel up to a maximal quantity. In the first minutes of the releasing process it can be observed a pronounced increasing of the drug concentration in release medium due to the diffusion of the surface adsorbed drug and also to the drug situated in the superficial layers of the hydrogel.

The increase of the swelling degree determines the release of the drug included in the deeper layers of the hydrogel but at a lower rate. The equilibrium state is reached rapidly as the hydrogel reaches the maximum swelling degree.

The release rate of the drug by diffusion from the loaded hydrogels is described in Fig. 17.

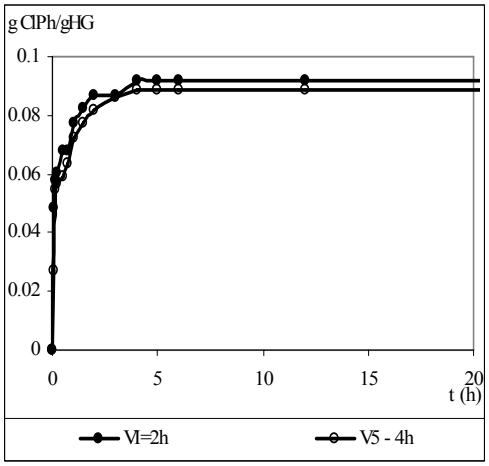

Fig. 16. The variation in time of drug amount included in hydrogels.

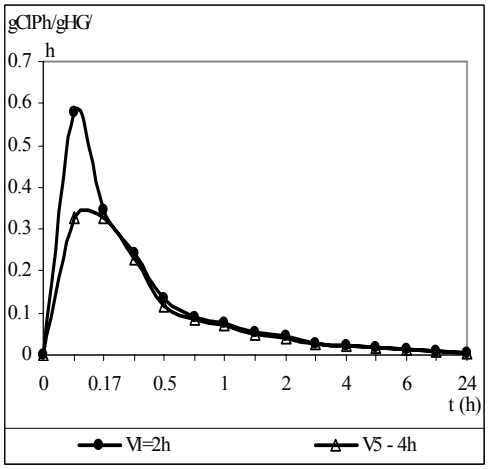

Fig. 17. The release rate of chloramphenicol from loaded hydrogels.

The obtained values for the release rate of Chloramphenicol are high for the first hours of the process due to the release of the drug present in the superficial layers of the hydrogel. This is followed by a decrease in release rate until the zero order kinetics is reached demonstrating the retard character of the system [10].

The variations of the maximal quantities of drug released from the synthesized composite hydrogels with the variation of the crosslinking parameters are shown in the Fig. 18.

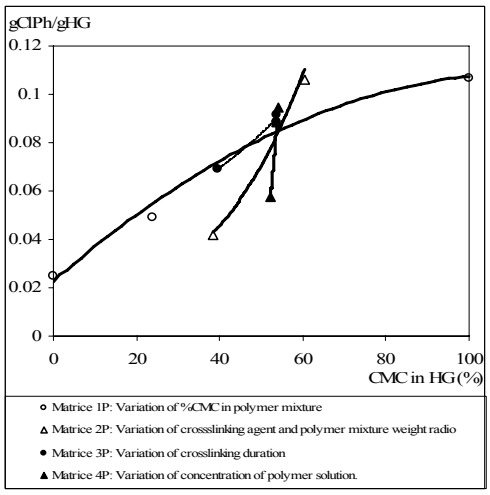

Fig. 18. The variation of maximal values of the released drug from hydrogels in respect with CMC %.

3.5. Antimicrobial activity of the obtained polymer-drug systems

As it is presented in Fig. 19 the drug included in hydrogels based on CMC and PVA, crosslinked with EpCl (alkaline pH) maintains its antimicrobial properties [11-12].

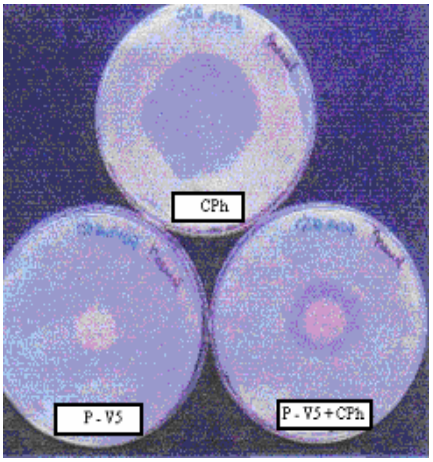

Fig. 19. Microbiological tests made on V5 hydrogel with and without Chloramphenicol on Pseudomonas aeruginosa ATCC 27853 cultures.

4. Conclusions

1. There were synthesized hydrogels in a film form, by the crosslinking with EpCl of mixtures of CMC and PVA in alkaline media.
2. The CMC amount from the synthesized hydrogels is influenced by the crosslinking reaction parameters (time, quantity of the crosslinking agent, quantity of

CMC from the initial mixture of polymers, the total quantity of polymers from the reaction mixture).

3. The swelling degree of the synthesized hydrogels is influenced by the percentage of CMC from the hydrogel and in the same manner by the reaction parameters.
4. CMC/PVA crosslinked hydrogels can be used as supports for inclusion of Chloramphenicol by diffusion.
5. Chloramphenicol release behavior proves the capacity of controlled release of biologic active principles by zero order kinetics.
6. The retaining capacity of drugs depends on swelling degree of hydrogels in water and, moreover, the inclusion and release capacity of the drug is influenced in the same manner by the crosslinking reaction parameters.
7. The microbiological tests effectuated on *Pseudomonas aeruginosa* ATCC 27853 attest the antimicrobial activity of the obtained polymer-drug systems.

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