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Polymer-drug conjugates based on a new 6-amino penicillanic acid derivative

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The paper presents the synthesis of new bioconjugate based on gellan and a 6-amino penicillanic acid derivative as a material with controlled release properties. Drug have been obtained by the decycling of 2-(m-nitrophenyl)-4-(β-carboxyethyl)-Δ2-oxazolinone-5 with 6-amino-penicillanic acid (6-APA) and the product (Ox-Pen) immobilization on polysaccharides (gellan, xanthane, carboxymethyl cellulose sodium salt, sodium alginate) by using esteric bonds forming, in presence of dicyclohexyl carbodiimide (DCCI) as activator. N-(m-nitrobenzoyl)-α-L-glutamyl-penicilline (Ox-Pen), as well as immobilization products, have been characterized by elemental analysis, IR and NMR spectroscopy and toxicity (lethal doses, LD50). For the gellan-active principle conjugate (the system with the highest percentage of immobilised drug, obtained by using a centered, rotator, composed, second order experimental program), there was calculated the regression equation describing the dependence of the amount of drug chemically bonded to the support on the reaction parameters. The efficiency of the immobilisation reaction is maximal at 1.5 mol/mol molar ratio DCCI/Ox-Pen, long reactions' duration, and lower molar ratio support/drug (1:5 mol/mol) respectively. The release kinetics of the active from the polymer-drug system, under alkaline hydrolyses conditions, has been studied too.

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Keywords: Drug-polysaccharide conjugate, Penicillin, Biological activity, Controlled release

1. Introduction

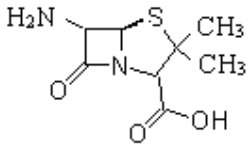
Polysaccharides have been used as supports for the drugs' immobilisation due to their properties such as biocompatibility, biodegradability and lack of toxicity [1-3]. They have been studied as supports for erodible tablets and capsules, mucous adhesive tablets, transdermal devices, films, tablets with controlled releasing, etc. Moreover, due to their particular structure, they can get implied in the forming of esteric bonds with hydroxyl groups [4-8], nearby the active principle that is providing the carboxyl group activated by DCCI.

A great number of drugs have been immobilized on polymeric supports by ester bonds between polysaccharide's hydroxyl groups and drug's carboxyl groups or between drug's hydroxyl groups and some carboxyl groups of activated support [4, 5, 9, 10].

Glutamic acid and its derivatives are compounds with antimethabolic, antimicrobial and antifungal activity. A special role is detained by the acyled derivatives from aminoacids having the m-nitrobenzoyl radical, their oxazolones and other derivatives, due to their remarkable biological activity [4, 9, 11-19].

The high reactivity of Δ²- oxazolinones-5 makes them proper for decyclization reaction with various nucleophiles [5-7, 10, 20] such as benzoxazole and its derivatives, N-yperites, 6-APA, etc., hence forming new biologically active products.

The 6-amino-penicillanic acid (6-APA, Scheme 1)) is a heterocycle having a free NH_2 group, and its reaction with oxazolones, may generate semi-synthetic penicillins.



Scheme 1. Structure of 6-amino-penicillanic acid.

Penicillins are β-lactamic antibiotics heavily used against bacterial infections, the same name being used for antibiotics derived from penicillins. Natural pennicilins have limited antimicrobial action and therefore they have been replaced with semi-synthetic penicillins, much more active.

The paper studies the synthesis of glutamic acid's penicillanic derivative, obtained by the opening of the 2-(m-nitrophenyl)-4-(β-carboxyethyl)-Δ²-oxazolinone-5 cycle with 6-APA and the penicillin's immobilization on polysaccharides (gellan, xanthan, CMC and alginic acid's sodium salt), by esteric covalent bonds. The products can be easily hydrolizable within human digestive tractus, also ensuring the controlled release of active principle.

2. Experimental

2.1. Materials

The 2-(m-nitrophenyl)-4-(β-carboxyethyl)- Δ²-oxazolinone-5 (Ox) have been obtained by treating N-(m-nitrobenzoyl)-L-glutamic acid (NBGA - a compound synthesized according to method described elsewhere [4]) with acetic anhydride [21], by heating up to 85-90 °C.

The 6-aminopenicillanic acid (6-APA) was purchased from Biochimie Company (Spania).

Gellan-supplied by KELCOGEL Company-polysaccharide produced from a microbial culture [22], and commercially prepared by aerobically fermentation of *Sphingomonas elodea* [23].

Xanthan - a polysaccharide produced from microbial synthesis by KELCO Biopolymers Company, commercially prepared by aerobic submerged fermentation from *Xanthomonas campestris* [24].

Carboxymethyl celulose sodium salt (CMC) – supplied from Fluka BioChemika Company.

Used sodium alginate was manufactured by KELP Company, and prepared from *Macrocystis pyrifera*.

Dicyclohexyl carbodiimide, (DCCI), the activator of the drug-polysaccharide reaction, was purchased from Merck Ltd.

2.2. Methods

2.2.1. Decyclization of 2-(m-nitropheyil)-4-(β-carboxyethl)-Δ2-oxazolinone-5 (Ox) with 6-amino-penicillanic acid (6-APA)

To a stirred suspension of 1.08g 6-aminopenicillanic acid (0.05 mol) in 22 ml methylene chloride, at 20-25°C, was added 0.85g triethylamine (0.01 moles;1.38 ml). Mixture was stirred for 45 min, and then cooled to 0-5°C. A 1.4 g oxazolone (0.05 mol) was then slowly dropped, under continuously stirring and the same temperature. After other 60 minutes a 5ml of distilled water, previously cooled at 10°C, was introduced in reaction. The corresponding penicillin was obtained in an acid form by precipitation with HCl 1N to pH=4. Aqueous phase was separated from the organic phase and a viscous product appeared in the first stage, which, after several washings with distilled water (previously cooled to 10°C), became a creamy colored powder, with a specific odour of penicillin. The final product was dried for several hours at room temperature, next at 40-45 0C for 5 hours, under vacuum. The synthesised N-(m-nitrobenzoyl)-α-L-glutamil-penicillin (Ox-Pen), a compound with a melting point of 143-145°C (η=78.94%), was analysed for structure elemental analysis (nitrogen and carbon) and spectral (FT-IR and 1H-NMR spectra).

2.2.2. Ox-Pen's immobilization on polysaccharides

In a first stage there have been performed drug immobilizing tests on various supports in the same reaction conditions (0.5 g drug, DCCI/drug ratio = 1.4 mol/mol, support/ drug ratio = 0.2 mol/mol and 24 h of reaction).

An experimental program has been developed for the system having the highest percentage of drug immobilized on support.

To a stirred solution of Ox-Pen (0.5 g in 10 ml DMSO) was added an amount of gellan (in compliance to experimental program), and stirring for 15 minutes, in order to allow the polysaccharide's swelling and the diffusion of active principle in the support.

Separately, the activator (DCCI) solution was prepared by solving the amount foreseen in the experimental program, in 2 ml DMSO. The DCCI solution was added over the initial mixture and that was considered the zero moment of the reaction. The reaction mass was stirred at 18-20 ^{o}C, for a time, which is foreseen in the la experimental program.

The obtained product was separated by precipitation with acetone (10 ml acetone, under intense stirring, for 10 minutes), the formed suspension centrifugation (5 minutes, 5000 rpm) washing twice with acetone (15 ml) and left for 24 hours in 60 ml of acetone, under continuously stirring. Then a Soxhlet acetone extraction was performed, for 24 hours. The final product was dried under high vacuum at 40 ^{o}C, till the next day.

Table 1. Amount of penicilline immobilized on various polysaccharidic supports.

Support	Reaction time	DCCI/ Ox-Pen (mol/mol)	Support/ Ox-Pen (mol/mol)	% Ox-Pen immob.
gellan	24h	1.4	0.2	40.63
xanthan	24h	1.4	0.2	2.64
CMC	24h	1.4	0.2	3.26
sodium alginate	24h	1.4	0.2	4.46

2.2.3. The experimental program

Preliminary studies regarding the drugs immobilization on various polysaccharide supports [4, 9, 22, 25-27] have shown that coupling reaction's efficiency is influenced by some factors.

For this system there have been selected the following parameters: the activator/active principle ratio, the support/active principle ratio and the reaction's duration. In order to gather information related to the coupling's efficiency (expressed as percentage of chemically bonded active principle) as function of these factors, a centered, rotating composed, 2nd order experimental program have been used. The program makes mandatory, in order to more easily process results, the encoding of variables. Analyzed variables into study and their encoding are shown in Table 2.

Table 2. Encoded variables and variation domain for the Ox-Pen-gellan system.

Real	Encoded variable

variable	-1.682	-1	0	1	1.682
DCCI/Ox-Pen mol/mol : x_1	1.1	1.18	1.3	1.42	1.5
Gellan/Ox-Pen mol/mol : x_2	0.2	0.26	0.35	0.44	0.5
time (h) : x_3	8	14.5	24	33.5	40

The regression equation which describe the amount of coupled Ox-Pen (y,%) in function of considered parameters is the following:

$$Y= a_0 + \sum ai\ xi + \sum aij\ xi\ xj \quad \text{with} \quad i \leq j$$

where: a_0-free term

ai, aij- regression coefficients

xi, xj – variables meaning the process' parameters.

Experimental results listed in Table 3 have been processed by means of the multiple regression method.

2.2.4. The release of active principle from support

The "in vitro" drug release's kinetics was indirectly performed by monitoring the pH's evolution during the basic hydrolysis of immobilisation products, because of difficulty to dose Ox-Pen by UV spectroscopy or other available methods. The binding of Ox-Pen on gellan, via ester bonds, makes possible to have a gradual release of the drug, in acid or basic environment, able to hydrolyse these bonds. Subsequently, the variation in time of pH has been defined (determined by the acid/base consumption when ester bonds hydrolysis takes place).

In order to study the active principle's release from the drug-gellan system, there has been selected a product containing 33.07% Ox-Pen. A 0.150 g gellan-drug compound has been suspended under stirring 37±0.5 °C in a NaOH solution (100ml, pH=10.99). The goal was to study the system's pH variation in time, starting from the idea that the hydroxide within reaction environment gets partially consumed by the ester bonds hydrolysis, reaction that determines the release of active biological product. Certain factors were taken into account, as the variation in time of hydroxyl ion's concentration at 37±0.5°C and the modification of solution's pH when polysaccharide is added [13].

Table 3. Experimental values forOx–Pen immobilized on gellan.

Nr. crt.	X_1	X_2	X_3	% N	% Ox-Pen pract.	%Ox-Pen calc.
1	-1	-1	-1	3. 93	34.66	34.67
2	+1	-1	-1	4. 91	43.31	35.99
3	-1	1	-1	3.22	28.40	23.16
4	+1	1	-1	3.86	34.04	31.78
5	-1	-1	1	4.68	41.28	40.66
6	+1	-1	1	5.15	45.42	40.18
7	-1	1	1	3.77	33.25	27.54
8	+1	1	1	3.89	34.31	34.36
9	-1.682	0	0	3.81	33.60	32.59
10	1.682	0	0	4.37	38.54	39.44
11	0	-1.682	0	5.40	47.63	40.26
12	0	1.682	0	3.75	33.07	25.69
13	0	0	-1.682	3.34	29.46	28.31
14	0	0	1.682	4.38	38.63	35.52
15	0	0	0	3.97	35.01	35.13
16	0	0	0	3.93	34.66	35.13
17	0	0	0	3.98	35.10	35.13
18	0	0	0	4.05	35.72	35.13
19	0	0	0	4.00	35.28	35.13
20	0	0	0	3.97	35.01	35.13

The following data were analysed:

a. pH variation in time for the NaOH solution (100 ml , pH initial=11), at 37±0.5 °C

b. pH variation in time for the NaOH solution (100 ml , pH initial=10.99), when gellan is added (0.1g) at 37±0.5°C

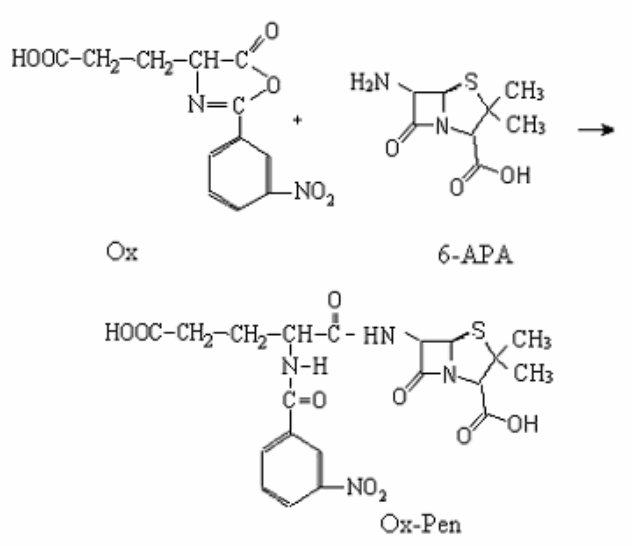
3. Results and discussion

3.1. Synthesis and characterization of Ox

Progresses recorded in the antibiotics industry are due to the activities in the field of penicillin synthesis, as well as to the enhancing of testing methods for products.

Δ2-oxazolinones-5 are behaving, in most of cases, when reacting with nucleophile agents, as anhydrides of N-acyl-aminoacids.

Due to >C=N- group's capacity to attract electrons, the carbonyl group in position 5 is behaving more like a cetonic group than like a lactonic carbonyl, showing a strong affinity for nucleophile reagents. Nucleophile agents are able to attack the C5 electrophilic center, and break and form a stable amide bond.



Scheme 2. Synthesis of N-(m-nitrobenzoyl)-α-DL-glutamil-penicilline (Ox-Pen) by the decycling of 2-(m-nitrophenyl)-4-(β-carboxyethyl)-Δ2-oxazolinone- 5 (Ox) with 6-aminopenicillanic acid (6-APA).

Ox-Pen has been synthesized by decycling Ox with 6-APA in presence, of triethylamine, at cold, being precipitated by acidulation with HCl 1N, in aqueous solution.

Ox–Pen is a crystallized product, cream coloured, with a melting point of 143-145°C.

% $N_{practical}$= 11.45 (%$N_{theoretic}$= 11.33).

% $C_{practical}$= 49.30 (%$C_{theoretic}$= 48.58).

The Ox-Pen structure was analysed by FT-IR spectroscopy, (Fig. 1), which confirms the presence of β-lactamic bond, typical for penicillins, at 1774 cm^{-1}, of group >C=O from carboxylate at 1624 cm^{-1}, presence of group NO_2 at 1340 cm^{-1} and 1529 cm^{-1} (symmetrical and asymmetrical), of two carboxyl groups at 2978 cm^{-1} and 2941 cm^{-1}, for the secondary amine at 3414 cm^{-1}, for the amidic group at 1529cm^{-1} (strong), for the1,3-disubstituted aromatic ring at 719 cm^{-1} and the disappearance of peak at 1076cm^{-1}, typical fact for the C-O-C bond within oxazolone.

Ox-Pen 1H-RMN spectrum (DMSO,d6, 300MHz) proved that product is pure (Fig. 2).

The signals of 1H-RMN spectra indicate the protons presence in β-lactamic cycle, at δ values between of 2.5-7.7 ppm. The methyl groups bonded by the synthesized antibiotic penem ring presents the signals in 1H-RMN specters at δ = 1.30-1.35 ppm . The aromatic protons from N-acylated aminoacid, grafted on antibiotic penem ring are indicated at the following values: δ = 7.7-8.8 ppm. The amidic -NH-groups give the signals in 1H-RMN spectrum at δ = 7.8 ppm, and carboxyl groups at δ = 11-12.8 ppm.

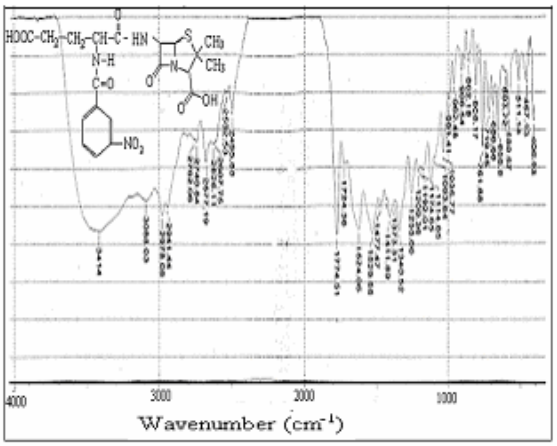


Fig. 1. FT-IR spectra for Ox-Pen.

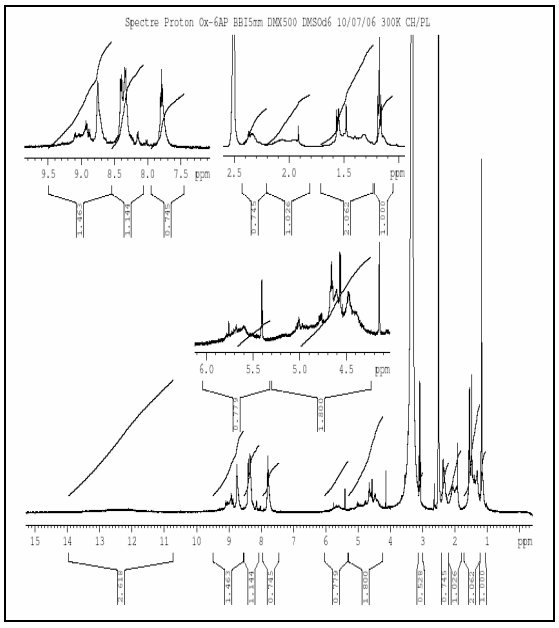


Fig. 2. 1H-NMR spectra for Ox-Pen.

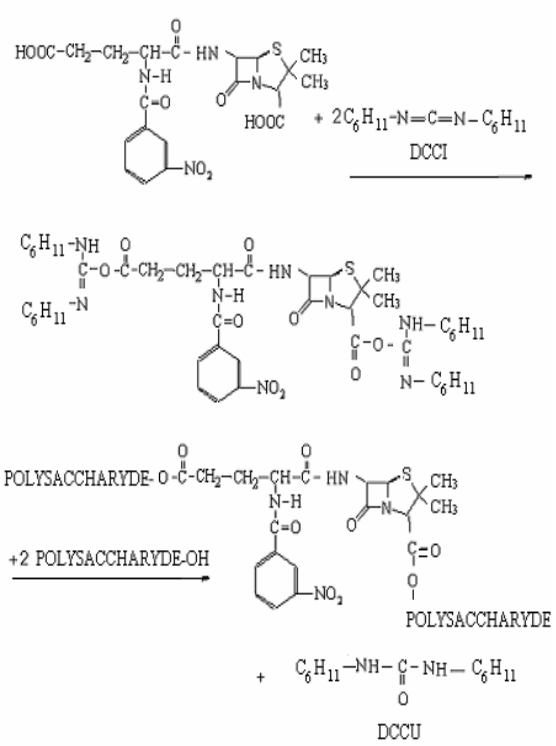
3.2. Ox-Pen immobilization on polysaccharides

The coupling of Ox-Pen on polysaccharides is based on the esterification of active principle's carboxyl groups with support's hydroxyl groups, activated with DCCI, according to scheme 3.

The penicillin's particular structure (compared to oxazolone) is comprising two competitive carboxylic groups for DCCI activation, respectively to the forming of esteric bonds with polysaccharidic support's OH groups.

Xanthan's structure is more complex than gelan's and therefore a lower percentage of pennicilin was immobilized on xantan.

In addition, CMC's relatively high substitution degree, with acidic chloride type groups, was significantly decreased the number of primary hydroxilic groups able to react with penicillin.



Scheme 3. Ox-Pen's immobilization reaction on polysaccharides with active OH (gellan, xanthan, CMC and sodium alginate).

Fig. 3 presents FT-IR spectra of Ox-Pen's coupling products, the characteristic bands being the next: it appears at 1234-1250 cm^{-1}, and 1740 cm^{-1} the corresponding band for esteric groups; the active principle presents typical bands of I and II amidic group at 1619- 1625 cm^{-1} si 1531-1572 cm^{-1}, 1734 cm^{-1} typical for the β-lactamic bond within penicillin, and for >NH group at 3325- 3424 cm^{-1}. The support's presence is evidenced in FT-IR specters by the characteristic bands appearance for the free OH group at 3325-3424 cm^{-1}, for methyl or methylen groups at 2928-2929 cm^{-1} and for C-O-C group within the macromolecular chain at 1079-1091 cm^{-1}.

If experimental results are processes, fro Ox-Pen-gellan system there has been obtained the following regression equation:

$$\%Ox\text{-}Pen = 35.13+2.03X_1 - 4.33X_2 + 2.14X_3 + 0.31X_12 - 0.76X_22\text{-}1.13X_32 + 1.82X_1X_2 - 0.45X_1X_3 - 0.40X_2X_3$$

If two variables, in the center of experimental domain, are to be particularized, then we can obtain data concerning the influence of the third, this being the graphs shown in Fig. 4-6.

Fig. 4 shows the influence of the activator amount, expressed as the DCCI/Ox-Pen ratio (mol/mol) upon the efficiency of the coupling reaction. It can be noticed that if amount of DCCI increases, the amount of penicillin immobilised on support also increases. Therefore, in order to have high coupling efficiencies, it is mandatory to work with an excess of activator.

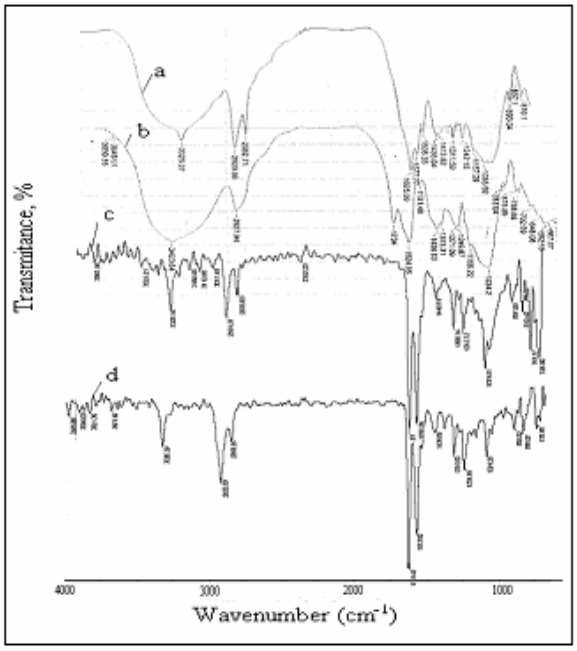


Fig. 3. FT-IR specter for a. Ox-Pen-gellan; b. Ox-Pen-xanthan; c. Ox-Pen-CMC; d. Ox-Pen- sodium alginate.

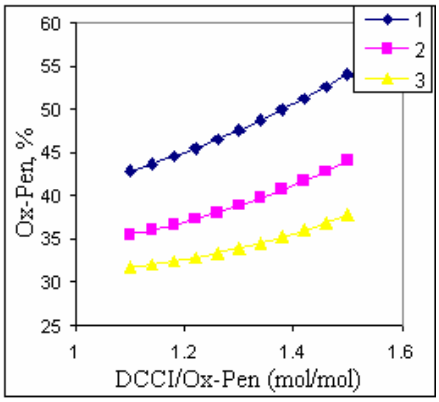


*Fig. 4. Influence of DCCI/Ox-Pen ratio upon the percentage of immobilized Ox-Pen at 24h for gellan/Ox ratios: **1** - 0.2 mol/mol; **2** - 0.29 mol/mol; **3**- 0.38 mol/mol.*

The amount of immobilized Ox-Pen is decreasing when the amount of support increase (against Ox-Pen), see Fig. 5.

It is then obvious that, in order to have maximal coupling efficiencies have to use a minimal amount of gellan. The great number of hydroxyl groups comprised by 1 mol of polysaccharide (10 groups–OH) provides a sufficient number of reactive sites for the Ox's esterification and its coupling, in huge amounts, to the support.

Compared to oxazolone or its benzimidazole derivative, which comprise only one free carboxylic group

at the active principle's molecule, in Ox-Pen we can notice the existence of two carboxylic groups. This could explain the lower percentages of active principle in the cases of penicillin-CMC, penicillin-sodium alginate and penicillin–xanthan systems, when polysaccharide's structure allows both carboxylic groups esterification.

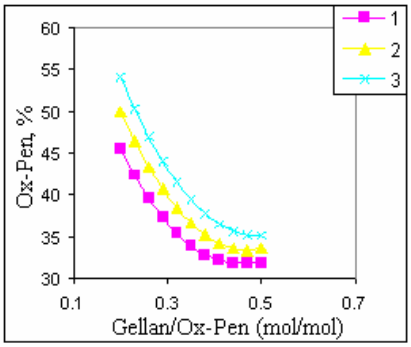


*Fig. 5. Influence of molar gellan ratio /Ox-Pen upon the percentage of immobilized Ox-Pen at t = 24 ore for DCCI/Ox ratios: **1**- 1.22 mol/mol; **2** - 1.34 mol/mol; **3**- 1.5 mol/mol.*

The esterification reaction's duration is a crucial parameter of the synthesis. Its influence on the contents of Ox-Pen bonded to support is shown in Fig. 6.

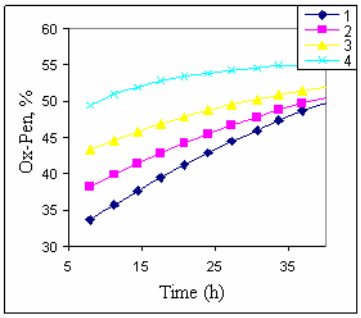


*Fig. 6. Influence of reaction time upon Ox-Pen percentage within immobilization product, for gellan/Ox-Pen ratio = 0.2 mol/mol, for various DCCI /Ox-Pen molar ratios : **1**- 1.1 ; **2**- 1.22 ; **3**- 1.34 ; **4**- 1.5.*

No matter the other process' parameters, maximal efficiencies are obtained at long reaction duration. It can be noticed that if amounts of activator increase, the increase of immobilised active principle is lower and lower significant. One explanation could be the fact that when amounts of DCCI is higher the Ox-Pen's esterification at gellan's hydroxyl groups is competed by the intermolecular esterification of the polysaccharide, which contains also carboxylic groups. Another fact is the esterification at both drug's carboxylic groups.

DCCI activates Ox-Pen's carboxylic groups, but also support's ones (partially getting consumed in this last reaction, fact that leads to the gellan's crosslinking).

Results shown in Figs. 4-6 are confirmed in Fig. 7-8, which illustrate the correlated influence of two grouped parameters upon the immobilization's efficiency (assessed by means of Ox-Pen percentage within immobilization products) in three-dimensional space.

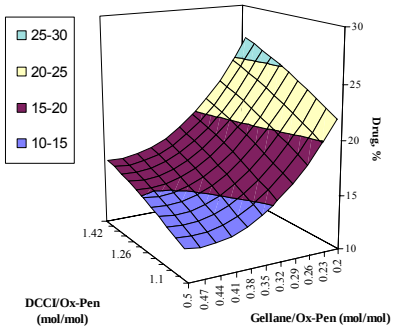


Fig. 7. Influence of DCCI/Ox-Pen ratio (mol/mol) and of gellan / Ox-Pen ratio (mol / mol) at a 24 h reaction duration (three-dimensional graph).

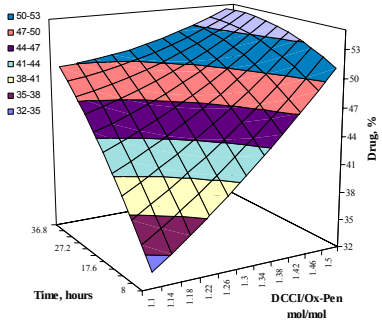


Fig. 8. Influence of DCCI/Ox-Pen ratio and of reaction time upon the amount of drug which gets immobilized at a gellan/Ox-Pen ratio of 0.2 mol/mol.

It results that, in order to obtain a maximal content of active biological product within coupling compounds, synthesis is to be achieved in the conditions shown below:

DCCI/Ox-Pen = 1.5 mol/mol

Gellan/Ox-Pen = 0.2 mol/mol

t =40 hours.

A synthesis that has been performed in these conditions lead to a product with a content of 54.45% Ox-Pen.

3.3. The release of Ox-Pen from the gellan-active principle system in alkaline environment

A study of the active biological principle release capacity has been performed by means of hydrolysis of ester groups in alkaline environment, results obtained in previous works [18] suggested that the release of biologic compounds immobilized on polysaccharide supports is slower in these conditions. The acid hydrolysis is proven to occur very fast, generating a *burst effect*, which is

leading to a very fast increasing of the amount of drug released in the first moments of the hydrolysis.

If, for the NaOH solution, the pH variation in time is monitored, it will be noticed that in the lapse of time 0...600 minutes, pH practically remains constant [5].

In the second case we can notice a variation of 0.06 units for pH in the first 15 minutes, afterwards pH remaining practically constant at 10.93, up to 600 minutes. This pH variation means NaOH consumption, perhaps due to its reaction with some carboxylic groups in gellan which have not been converted in the sodium salt form.

A consequence was that the curve of pH variation in the Ox-Pen – gellan system has been rectified, in the time lapse of 0-15 minutes with values established from the same curve for the black sample (b). According to experimental data, it can be seen that in the first 8 hours the pH variation follows a straight line (that is, a constant decrease in time).

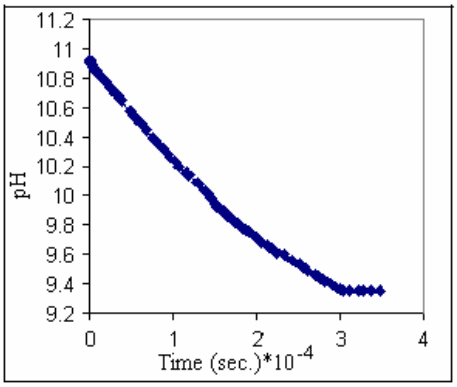


Fig. 9. pH variation, in time, for the alkaline hydrolysis of Ox-Pen's immobilization product on gellan (33.07% Ox-Pen).

On basis of pH variation curve for the alkaline hydrolysis of the immobilisation product Ox-Pen on gellan, there has been calculated and graphically represented the variation in time of the amount of Ox-Pen during the drug's releasing process (Fig. 10) and the drug release velocity (Fig. 11).

It can be seen from the graph related to the amount of drug released in time, the reaching of a constant plateau after approximately 250 minutes. This is the proof of a "zero order" release kinetics, which is typical for the polymer-active principle systems (with controlled release of active principle). The process is perhaps dominated by the phenomenon of immobilized Ox-Pen's diffusion.

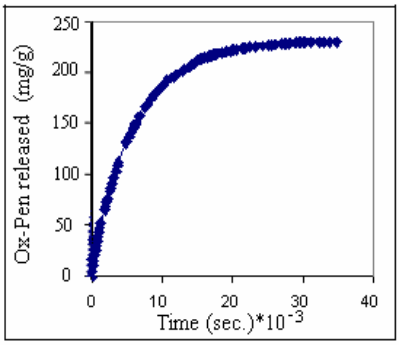


Fig. 10. Amount of Ox-Pen released in alkaline environment from immobilization product, at temperature of 37±0.5°C (33.07% Ox-Pen).

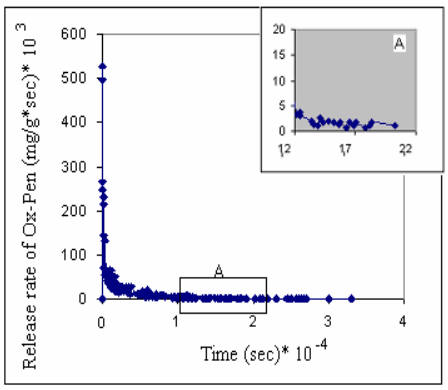


Fig. 11. Variation, in time, of releasing velocity for Ox-Pen released from immobilization product, in alkaline environment, at T= 370C (33.07% Ox-Pen).

3.4. The toxicity tests and antibacterial activity for Ox-Pen and immobilization product on polysaccharides

Hence, their toxicity degree has been tested and the lethal dose (LD50) was calculated. The acute toxicity has been evaluated by intra-peritoneally injecting the compounds, as Twen 80 suspensions, to groups of three mice (20-25g), according to the classic method (Korber method).

Animals were then followed up and mortality has been recorded after 7 days (Table 4). Analysis of data in table 3 leads to the conclusion that the products (Ox-Pen, Ox-Pen-gellan and Ox-Pen-xanthan) featured toxicity parameters adequate for medical use. The much lower toxicity of the immobilization product, compared to Ox-Pen, recommends it for tests, in order to further use it as antibiotic against experimental infections.

The natural polymeric support decreases the active principle's toxicity, having also the role to provide the controlled/sustained release of drug, respectively to ensure a relatively constant concentration of it within human body, for a longer period.

Table 4. Toxicity of tested compounds.

Compound	Lab animal	Administration path	LD50 (mg/kg body)
Ox-Pen	mice	i.p.	1850
Ox-Pen-gellan	mice	i.p.	3210
Ox-Pen-xanthan	mice	i.p.	3540

The gellan-Ox-Pen conjugate have an important antibacterial activity against of *Escherichia coli,* producing a inhibition zone with 17 mm diameter.

4. Conclusions

1. Ox's high reactivity towards nucleophile agents allow its decyclizing with 6-aminopennicilanic acid, this leading to a derivative with potential biological properties.

2. The chemo-therapeutically characteristics of active substance can be enhanced by its immobilization on macromolecular supports, especially of polysaccharide nature. The possibility to achieve an immobilization reaction has been proven by spectral and elemental analysis of obtained products.

3. The immobilization reaction's efficiency is influenced by the ratio activator/Ox-Pen, the ratio support/Ox-Pen and by reaction duration. Percentage of immobilized Ox-Pen increases if the amount of DCCI increases, amount of support is lower and reaction duration increases.

4. In the same reaction conditions, if gellan is used as support, we have noticed a higher percentage of immobilized drug on support, compared to other polysaccharides used in tests.

5. The release of active principle from coupled product is achieved by the breaking of ester bonds by acid hydrolysis, as well as by alkaline one, in compliance to the pH within various segments of the human digestive tractus.

6. All results are recommending the conditioning of the conjugate gellan-Ox-Pen as antibiotic suppositories.

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