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Study of Immobilization by Esteric Bond of 2-(m-Nitrophenyl)-4-(β-Carboxymethyl)-Δ²-Oxazolinone-5 on Gellan

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Abstract

The paper studies the coupling reaction, through ester-type covalent bonds, of an oxazolone derived from the N-(m-nitrobenzoyl)-L-asparagic acid, on gellan (a polysaccharide of microbian synthesis), in conditions of activation with dicyclohexyl carbodiimide. Based on a centered, rotatory, composed, second order experimental program, the regression equation describing the dependence of the amount of active principle, chemically bounded to the support, on the reaction's parameters (support/active principle ratio, active principle/activator ratio, duration) is obtained. One may observe that the efficiency of the coupling reaction is maximum when employing the parameters' highest values, over the variation domain established. The coupling product has been characterized through elemental analysis and IR spectroscopy. For the establishment of the capacity of the active principle's controlled release by the polymer-active principle system thus obtained, drug's release kinetics from the polysaccharidic support is studied in conditions of basic hydrolysis. The oxazolone release from the coupling products, by basic hydrolysis proceeds conformely to a zero order kinetics, proving their retard activity.

Introduction

The L-asparagic acid and its acylated derivatives with *m*– or *p*– substituted benzoyl radical evidence a remarkable biological activity [1-4], as participating to animal organisms' metabolism, reducing the toxicity of some drug products and assuring, at the same time, an appreciable bioactivity at cellular level [5-10].

Among the derivatives employed in antibacterian therapy, special place is occupied by oxazolones, the chemo-therapeutical indices of which may be improved through coupling to macromolecular, especially of polysaccharidic nature – supports [11].

The present paper is devoted to the coupling of an oxazolone derived from the N-*m*-nitrobenzoyl-L asparagic acid on gellan, through esteric links, as being easily hydrolyzable in the digestive tract of the human organism, which assures controlled releasing of the active principle. The influence of certain parameters on the efficiency of the coupling reaction is analyzed, along with the most favorable reaction conditions for binding of high drug amounts to the support, as well as the release, in a basic medium, of the coupling product.

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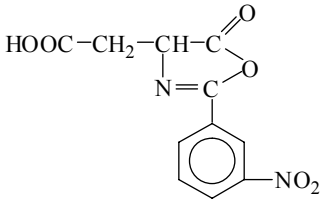
Experimental

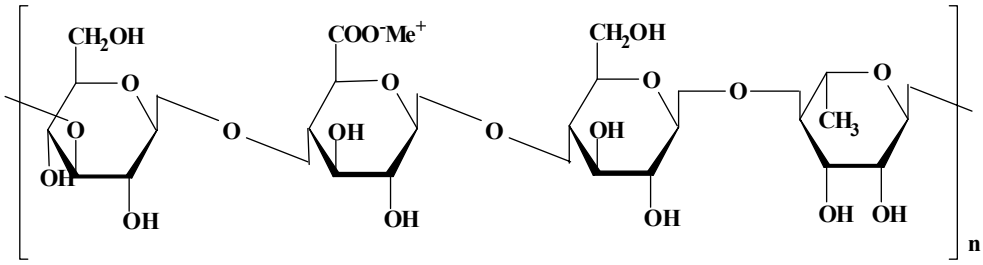
Materials

2-(m-nitrophenyl)-4-(β-carboxymethyl)-Δ²-oxazolinone-5, (Ox) – was obtained through treatment of the N-m-nitrobenzoil-L-asparagic acid with acetic anhydride, according to the method described elsewhere [8].

The product's chemical structure is the following:



Dicyclohexyl carbodiimide, (DCCI), from Merck. Gellan – provided by KELCOGEL Company, is a polysaccharide obtained from a microbial culture [12], with the following formula:



Method

Experimental program

Preliminary studies [11], which made use a different polysaccharidic support (xanthan) [12,13], have indicated that the efficiency of the coupling reaction is influenced by several factors. For the present system, there have been selected, as showing a prevailing influence, the following parameters: the support/active principle ratio, the activator/ active principle ratio and the process' duration. For the obtention of information on the manner in which coupling's efficiency (expressed by the amount of Ox bound chemically) is influenced by these factors, an experimental, centered, rotatory, composed, second order program, which reduces considerably the number of experiments and finally permits optimization of the process, was utilized.

For facilitating results' processing, the program assures codification of the variables listed in Table 1, together with the limits of their variation domain.

The equation proposed for describing the dependence of the amount of coupled Ox (y, %) on the parameters considered has the form:

$$y = a_o + \Sigma a_i x_i + \Sigma a_{ij} x_i x_j \quad \text{with} \quad i \leq j$$

where: – a_o - free term

– a_i, a_{ij} – regression coefficients

– x_i, x_j – variables expressing the process' parameters

The experimental results listed in Table 2 have been processed by the multiple regression method.

Table 1

Codification of variables and their variation domain

Real variable	Codificated variable				
	-1.682	-1	0	1	1.682
DCCI/Ox (mol/mol) - x_1	1	1.1	1.25	1.4	1.5
Gellan/Ox (mol/mol) - x_2	0.192	0.25	0.338	0.425	0.448
Time (h) - x_3	8	14.5	24	33.5	40

Table 2

Experimental values on the content of coupled Ox.

Nr.crt.	Codded variable			% Bounded Ox
	x_1	x_2	x_3	
1	-1	-1	-1	45.80
2	1	-1	-1	51.48
3	-1	1	-1	35.91
4	1	1	-1	40.84
5	-1	-1	1	54.25
6	1	-1	1	60.17
7	-1	1	1	44.71
8	1	1	1	53.58
9	-1.682	0	0	50.75
10	1.682	0	0	59.39
11	0	-1.682	0	67.56
12	0	1.682	0	41.27
13	0	0	-1.682	38.30
14	0	0	1.682	59.26
15	0	0	0	53.37
16	0	0	0	53.04
17	0	0	0	52.85
18	0	0	0	52.76
19	0	0	0	53.16
20	0	0	0	53.21

Release of the active principle from the support

In order to establish the active principle's capacity of controlled release, from the system accomplished there has been selected a product with a content of 67.56% Ox. Testing was performed in conditions of basic hydrolysis of this product, on following the time variation of the system's pH, starting from the idea that the hydroxide from the reaction medium is partially consumed through the hydrolysis of the esteric groups, a reaction that determines the release of the biologically active product.

Results and Discussions

Coupling of Ox to gellan is based on the reaction of esterification of the active principle's carboxylic groups, with the support's hydroxyl ones, activated by DCCI, according to the Figure 1:

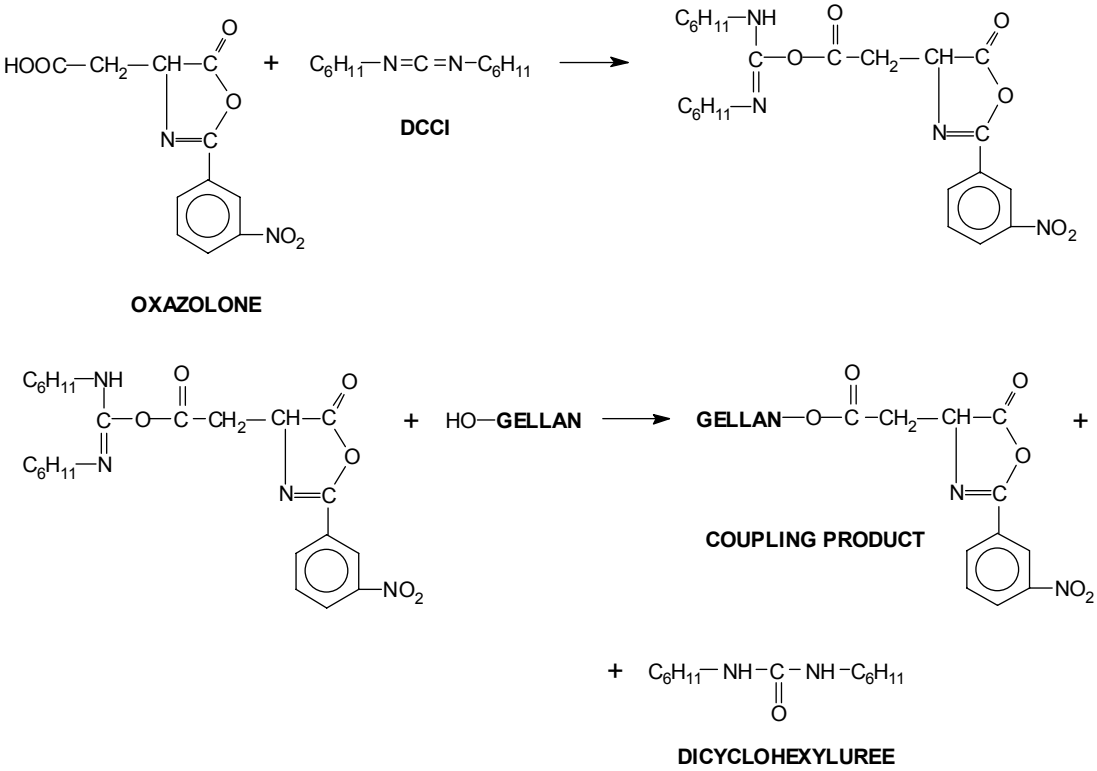


Fig. 1. Coupling reaction of oxazolone on gellan.

Formation of the Ox ester with gellan is evidenced by IR spectra, which attest the presence of the esteric groups at 1735 cm^{-1}, as well as of the functional groups specific to oxazolone:1080 cm^{-1} attributed to C-O-C group, 1360 cm^{-1} and 1540 cm^{-1}, attributed to NO_2 group, both symmetrically and asymmetrically, 1620 cm^{-1}, attributed to C=N group, 880 cm^{-1}, attributed C-H aromatically (disubstituted aromatic ring).

Processing of the experimental results led to the following regression equation:

$$Ox\% = 53.19 + 2.92x_1 - 5.92x_2 + 5.41x_3 - 0.21x_1^2 + 0.28x_2^2 - 0.44x_3^2 + 0.52x_1x_2 + 0.55x_1x_3 - 2.44x_2x_3$$

On particularizing two of the variables at the center of the experimental domain, information on the influence of the third one may be obtained, as the curves plotted in Figures 2-4.

Figure 2 presents the influence of the amount of activator, expressed as the DCCI/Ox molar ratio, on the efficiency of the coupling reaction. The observation to be made is that increase of DCCI amount induces a continuous increase of the Ox ratio in the reaction product, over the variation interval of this parameter.

The effect is normal, being due to the activation of a higher and higher number of functional carboxylic groups and active principle molecules, respectively. Another important observation is that, for at-

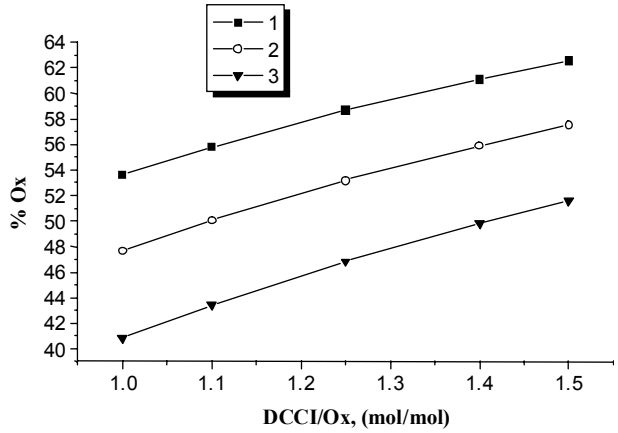


Fig. 2. Influence of the DCCI/Ox molar ratio on the coupled active principle ratio, at t = 24 hours, 1 – gellan/Ox = 0.25 mol/mol; 2 – gellan/Ox = 0.338 mol/mol; 3 – gellan/Ox = 0.425 mol/mol.

taining high coupling yields, one should employ the activator in excess.

The amount of coupled Ox decreases with the gellan/Ox molar ratio (Fig. 3). It is obvious that, for attaining maximum coupling yields, a minimum amount of gellan should be considered. The high number of hydroxyl groups contained in one mole of polysaccharide (10 –OH groups) assured a sufficient number of reactive sites for Ox's esterification.

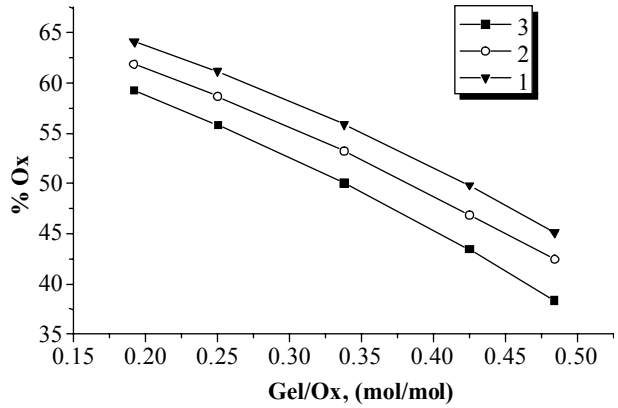


Fig. 3. Influence of the gellan/Ox molar ratio on the coupled drug ratio, at t = 24 hours, 1 – DCCI/Ox = 1.1 mol/mol; 2 – DCCI/Ox = 1.25 mol/mol; 3 – DCCI/Ox = 1.4 mol/mol.

An important parameter in the synthesis is the duration of the esterification reaction (Fig. 4).

One should observe that, regardless of the other parameters of the process, maximum yields are obtained after about 33 hours of reaction. A possible explanation might be that, along the whole duration

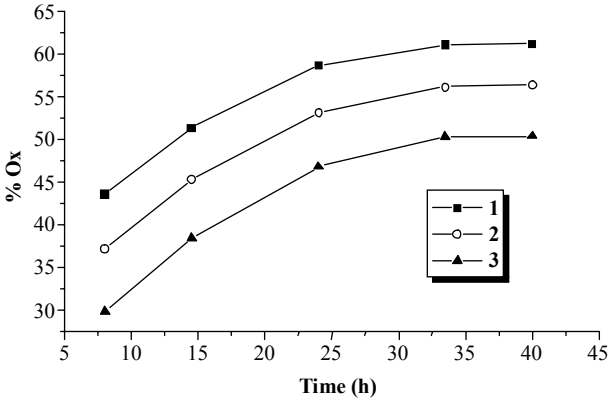


Fig. 4. Influence of the reaction time on the coupled Ox ratio, at a DCCI/Ox molar ratio = 1.25 mol/mol: 1 – gellan/Ox = 0.25 mol/mol; 2 – gellan/Ox = 0.338 mol/mol; 3 – gellan/Ox = 0.425 mol/mol.

of the synthesis, Ox esterification to gelan's carboxylic groups is completed by the intermolecular reaction of polysaccharide's esterification. DCCI activates, too, the support's carboxylic groups, being partially consumed in this reaction, which results in gellan's slight crosslinking. It may happen that, at duration exceeding 30 hours, the amount of activator should be wholly consumed in the two esterification reactions.

Such an explanation is supported, too, by the fact that, on increasing synthesis' duration, the reaction products become more and more rigid, manifesting a lower and lower capacity of swelling in water (which may be also due to hydrophobization through oxazolone's bonding).

The phenomenon is even more obvious through the increase of the activator/oxazolone ratio, versus maintaining constant the amount of gelan, when the reaction products evidence a very reduced capacity of swelling in water and intense rigidity.

Figures 5, 6 confirms the results plotted graphically in figures 2-4, which illustrate, in three-dimensional representation, the influence of each two parameters on coupling's efficiency (estimated by the ratio of Ox in the coupling products).

Analysis of the above discussed data shows that, for attaining a maximum content of biologically active product in the coupling compounds, the synthesis should be developed in the following conditions:

Gellan/Ox = 0.192 mol/mol
DCCI/Ox = 1.5 mol/mol
t = 33 h

In order to study the active principle's release capacity, hydrolysis of the esteric groups in basic

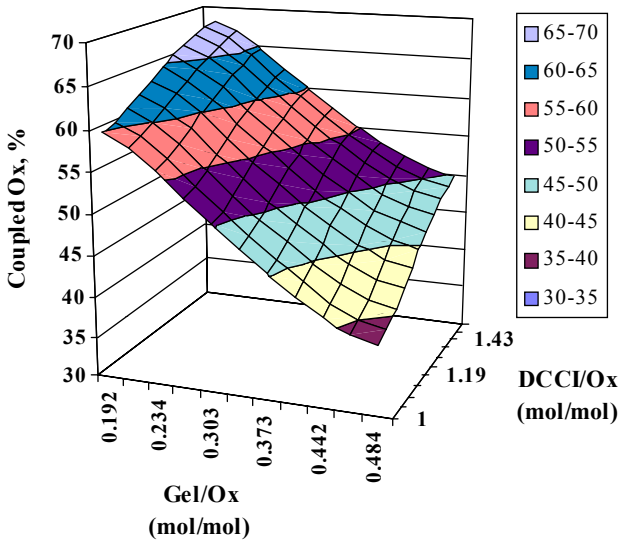


Fig. 5. Influence of the DCCI/Ox ratio and the gellan/Ox ratio on the amount of Ox bound in the coupling product, for t = 24 h.

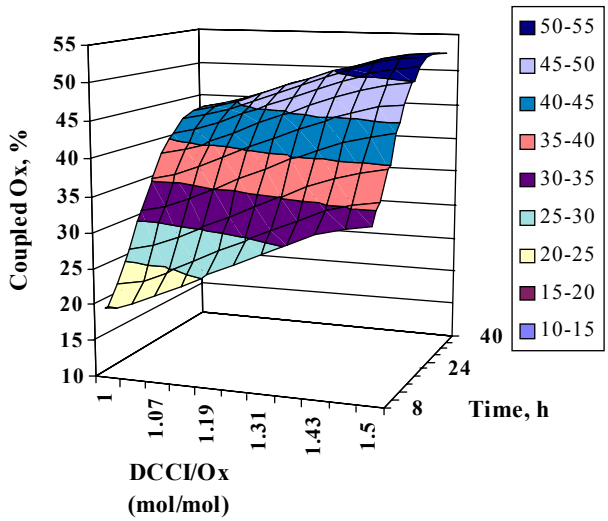


Fig. 6. Influence of the DCCI/Ox ratio and the reaction time on the amount of Ox bound in the coupling product, for a gellan/Ox ratio = 0.484.

medium has been performed, starting from the idea that pH variation represents a suitable method for estimating the kinetics of drug release.

The results obtained are presented graphically in Figures 7-9.

Based on this curve, there could be calculated and represented graphically the time variation of the amount of Ox in the process of drug release (Fig. 8), as well as the time variation of the rate of Ox release from the coupling product.

In the first approximately 300 minutes, the release rate of Ox is higher; over the 300-1500 minutes interval, the release rate gets stabilized, becoming practically constant, up to an almost total elution of the immobilized Ox. This domain, characterized by a zero order kinetics, permits the conclusion that the gellan-Ox system may be considered as belonging to the class of controlled-release drugs.

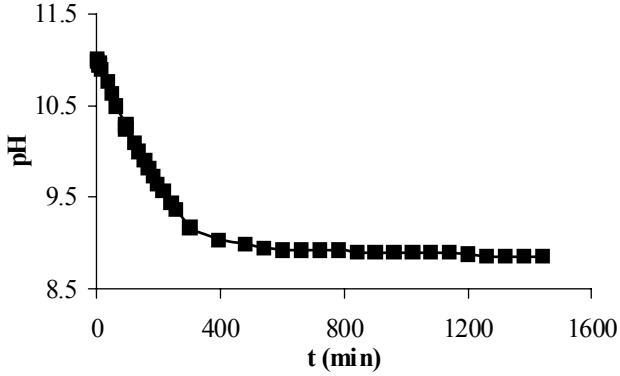


Fig. 7. Time variation of pH for the release, in a basic medium, of Ox from the Ox-gellan system (Ox content = 67.56%).

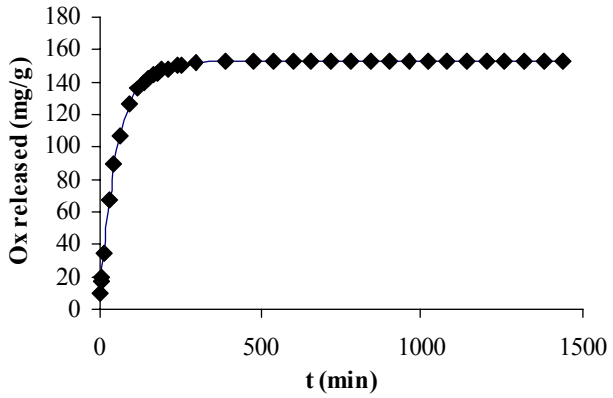


Fig. 8. Time variation of the amount of Ox released from the coupling product, in a basic medium (Ox content = 67.56%).

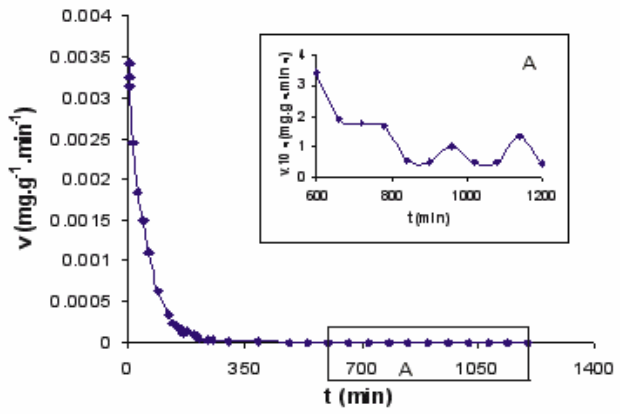


Fig. 9. Time variation of the rate of Ox release from the coupling product, in a basic medium (Ox content = 67.56%).

Conclusions

- Oxazolones based on amino-acids can be coupled be esterification on natural polymers, such as gellan, in the presence of dicyclohexyl carbodiimide as an activator
- The coupling reaction efficiency is influenced by activator/oxazolone. and support/oxazolone ratios, as well as by reaction duration
- The amount of bonded oxazolone in the coupling products increases with the increase of activator/drug ratio and reaction duration and decreases with the increase of gellan/drug ratio.
- The oxazolone release from the coupling products, by basic hydrolysis proceeds conformely to a zero order kinetics, proving their retard activity.

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