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Bioactive polymers
XLII. Coupling of ampicilline on (chlorocarbonylmethyl)cellulose

Cristofor I. Simionescu*, Severian Dumitriu, Marcel Popa, and Maria Dumitriu

Department of Organic and Macromolecular Chemistry, Polytechnic Institute of Jassy, RO-6600 Jassy, Romania

SUMMARY

In the present paper the coupling of ampicilline on (chlorocarbonylmethyl)cellulose is studied. The reaction is influenced by the drug/support mole ratio, duration and solvent volume (DMSO). The dependence of the coupling yield on the above - mentioned parameters is described fairly by a regression equation of the second order whose coefficients were settled by the multiple regression method. The obtained products are polymer drugs with retard antimicrobial action.

INTRODUCTION

In recent years the use of synthetic and natural polymers as polymeric drugs or drug delivery systems has received increasing attention, several Symposiy being organised to discuss the curent state-in-art of research into polymeric drug (DONARUMA et al., 1978), controlled release of bioactive materials BAKER, 1980; LEVIS, 1981), applications of immobilized enzymes and proteins (CHANG, 1977) and the general biomedical applications of polymers (GOLDBERG et al., 1980; GEBELEIN et al., 1981; CHIELLINI et al., 1983). Studies have largely been confirmed so far either to the development of sustained-release systems based on insoluble polymers in the form of powders pellets or devices (HELLER et al., 1980) or to the development of polymeric drugs, i.e. soluble polymers which themselves display pharmacological activity.

The coupling of antibiotics on modified polysaccharides allowed their retardation (SIMIONESCU et al., 1984; 1985a) as well as the obtention of new semisynthetic penicillins (SIMIONESCU et al., 1985b).

In the present paper the ampicilline immobilization on (chlorocarbonylmethyl)cellulose (CMC-Cl) is reported.

EXPERIMENTAL

CMC-Cl was synthesized by literature method (SIMIONESCU et al., 1982) starting from a carboxymethylcellulose of an average viscosimetric molecular weight of 120,000 and substitution degree (DS) = 0.78. A product of a substitution degree of chloroanhydride groups of 0.41 and viscosimetric average molecular weight of 60,000 was thus obtained. The molecular weight of the recurring unit 225 was calculated by additivity taking into account the substitution degrees of chloroanhydride and residual carboxy groups.

The coupling was carried out in DMSO. The ampicilline

* To whom offprint requests should be sent

amount (according to the experimental programme) and 1 ml pyridine were introduced into the required DMSO volume (constituting a reaction parameter). After the complete drug dissolution 0.225 g CMC-Cl (1×10^{-3} mole) were added. The coupling proceeded at $20 \pm 1^{\circ}C$ under good stirring. The reaction product was filtered and washed on the filter with DMSO till the filtrate did not containe any traces of ampicilline (ALICINO, 1961). The product was washed finally with acetone (25 ml) for DMSO removal and dried under vacuum at the room temperature.

The amount of the drug chemically bound was estimated by the nitrogen dosing (Kjeldhal method). The coupling yield was calculated by the relationship:

$$\eta\% = \frac{A}{4.81} \quad 100$$

where: A - nitrogen content in the coupling product as determined experimentally, % ;
4.81 - theoretical nitrogen content corresponding to the total amidation of chloroanhydride groups on the support.

Experiment programming. The efficiency of the coupling reaction is influenced by several factors among which the following were selected for the study: ampicilline/CMC-Cl mole ratio, duration and solvent volume (DMSO). The coupling yield as defined previously was used for its estimation.

In order to correlate the coupling yield with reaction parameters a second order regression equation of the following form was proposed:

$$Y = a_o + \sum a_i x_i + \sum a_{ij} x_i x_j \qquad i \leqslant j$$

where: Y - coupling yield;
a_o - free term;
a_i, a_{ij} - regression coefficients;
x_i, x_{ij} - process parameters.

The rather large number of the experiments required for settling the values of the regression coefficients may be significantly reduced by working according to a composed rotating centrated experimental programme of the second order with the codded variables given in Table 1.

TABLE 1

The codded variables

Actual values	Codded variables				
	-1.682	-1	0	1	1.682
Ampicilline/CMC-Cl (mol/mol) - x_1	0.6	0.76	1	1.24	1.4
Time (h) - x_2	0.5	1.60	3.25	4.90	6.0
Solvent volume (DMSO) (ml) - x_3	10	14	20	26	30

The experimental programme and coupling yield values are given in Table 2.

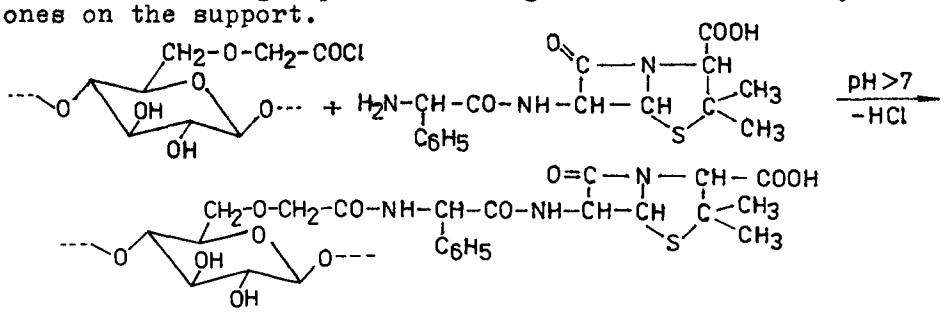
TABLE 2
Experimental design and experimental results

	Experimental design			Coupling yield η (%)
	x_1	x_2	x_3	
1	-1	-1	-1	28.8
2	1	-1	-1	32.8
3	-1	1	-1	31.6
4	1	1	-1	39.6
5	-1	-1	1	18.9
6	1	-1	1	21.1
7	-1	1	1	20.6
8	1	1	1	27.5
9	-1.682	0	0	19.8
10	1.682	0	0	26.1
11	0	-1.682	0	22.2
12	0	1.682	0	24.0
13	0	0	-1.682	25.1
14	0	0	1.682	18.2
15	0	0	0	23.1
16	0	0	0	22.6
17	0	0	0	24.6
18	0	0	0	23.6
19	0	0	0	24.1
20	0	0	0	24.2

The coefficients of the regression equation were determined by the multiple regression method using a Felix C-256 computer.

RESULTS AND DISCUSSION

The ampicilline coupling on CMC-Cl is based on the condensation of amino groups on the drug with the chloroanhydride ones on the support.



The dependency of ampicilline coupling yield on the reaction parameters (in the coded system) is given by the following function:

$$\eta\% = 25.53 + 2.31\, x_1 + 1.52\, x_2 - 4.11\, x_3 + 0.82\, x_1^2 + 0.86\, x_1 x_2 + 0.36\, x_2^2 + 1.07\, x_1 x_3 - 0.36\, x_2 x_3 - 0.19\, x_3^2$$

The values of the multiple correlation coefficient (0.85) and factor F (4.902) indicate the mathematical model to be adequate.

By particularizing two parameters in the center or in any other point of the experimental domain the regression equation simplifies permitting the influence of the third parameter on the yield to be settled.

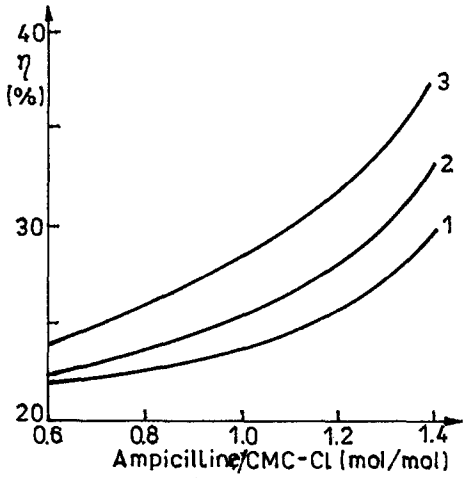


Figure 1. Influence of ampicilline/CMC-Cl molar ratio on coupling yield. 1 - 3.25 h; 2 - 4.9 h; 3 - 6 h; V_{DMSO} = 20 ml.

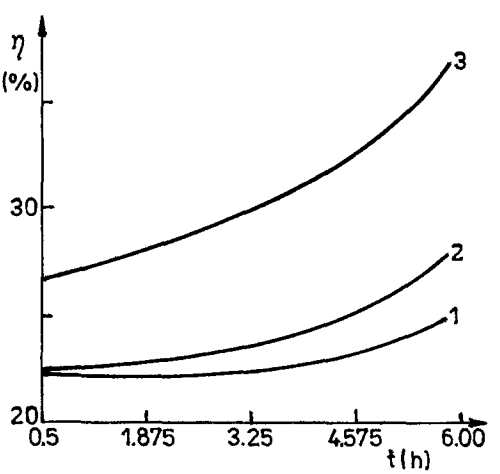


Figure 2. Influence of duration on coupling yield. 1 - ampicilline/CMC-Cl = 0.70 mol/mol COOH; 2 - 1 mol/mol COOH; 3 - 1.4 mol/mol COOH; V_{DMSO} = 20 ml.

The Figure 1 shows tha coupling yield to increase with increasing drug amount in the initial reaction mixture without any stabilization, not even for values of the variables under study larger than the highest one in the experimental domain.

Since the macromolecular support is insoluble in DMSO the reaction proceeds in an heterogeneous system being kinetically controlled by the diffusion. The ampicilline concentration in the reaction mixture increases with increasing amount in the initial mixture which results in the intensification of diffusion into the polymer particles. Consequently, the coupling yields are higher.

The influence of the duration, another important reaction parameter, is illustrated in Figure 2.

Within the 0.5 - 6 h interval the yield is noticed to increase with time which would suggest higher drug amounts to bind at longer durations.

The influence of the solvent volume on the coupling efficiency is depicted in Figure 3.

The coupling yield is noticed to decrease continously with increasing DMSO volume due to the decrease in the drug concentration in the reaction medium which diminishes its diffusion to the functional groups on the support.

By following the simultaneous correlated influence of the parameters an the coupling yield interesting results are noticed. Thus, the reaction efficiency is seen to be maximum at long durations and high drug/support ratios.

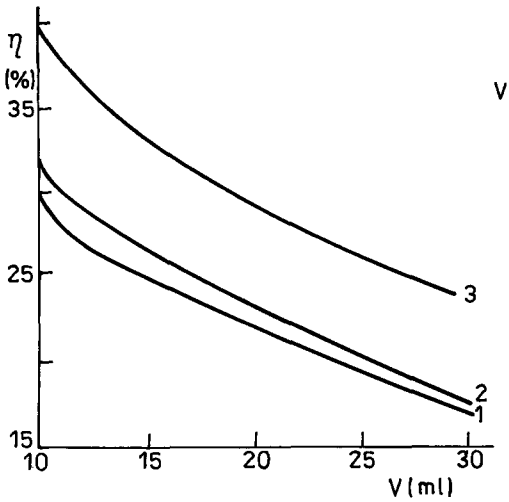


Figure 3. Influence of solvent volume on the coupling yield. 1 - ampicilline/CMC-Cl = 0.6 mol/mol; 2 = 1 mol/mol; 3 = 1.14 mol/mol. Time = 3.25 h.

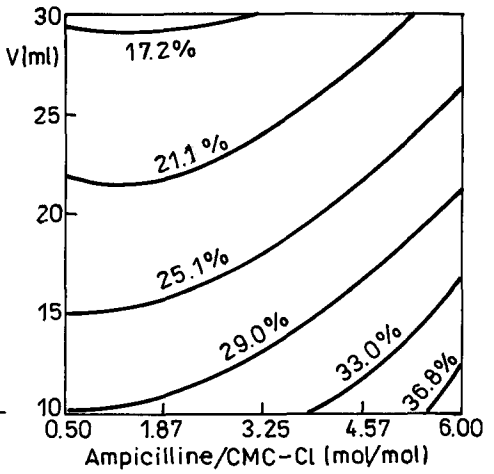


Figure 4. Constant level curves in the drug/support - solvent volume experimental plane. Time = 3.25 h.

Very good resulta are obtained with high drug/support mole ratio and minimum solvent volume (Figure 4). This effect is certainly due to the increase in the ampicilline concentration within the system which is favourable for its diffusion into the particles of the polymer support (Figure 5)

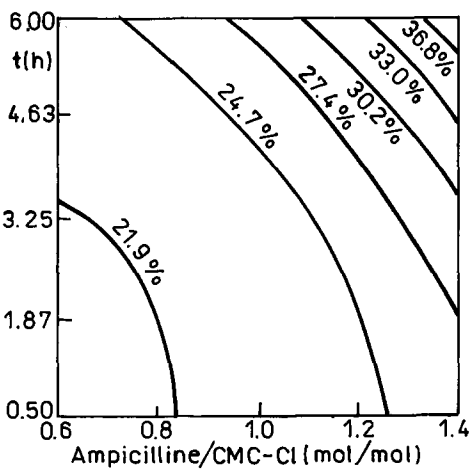


Figure 5. Constant level curves in the drug/support mole ratio - duration experimental plane. V_{DMSO} = 20 ml.

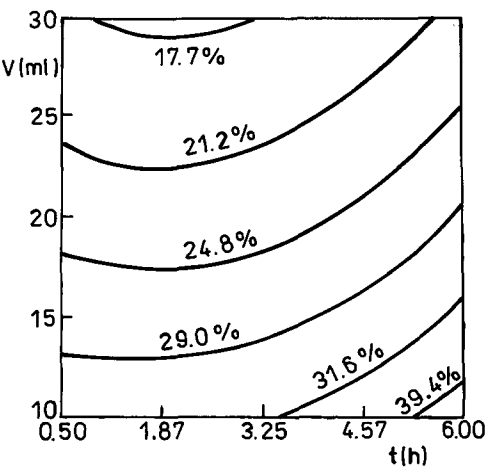


Figure 6. Constant level curves in the duration - solvent volume experimental plane. Ampicilline/CMC-Cl = 1 mol/mol.

Consequently, the conclusion may be drawn that the coupling efficiency is maximum with long reaction durations and small DMSO volumes (Figure 6). The following optimum coupling conditions for the ampicilline - CMC-Cl system are thus evident:

-ampicilline/CMC-Cl = 1.4 mol/mol;
-DMSO volume = 10 ml;
-Duration = 6 h.

A synthesis performed under these conditions resulted in a 42.3% coupling yield.

The kinetics of the drug release were studied by the alkali hydrolysis of the product (NaOH, 64 10^{-4} mol/l). The coupling product (0.3 g) was taken as a suspension in 100 ml solvent and hydrolysed at 37°C, under continuous stirring. The variation of the pH in time is illustrated in Figure 7.

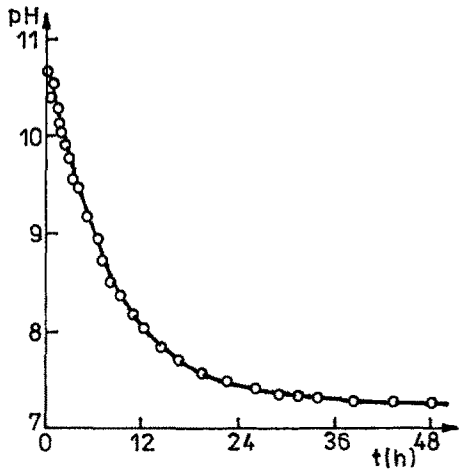


Figure 7 Variation of reaction pH for hydrolysis of ampicilline coupled on CMC-Cl.

The drug hydrolysis is noticed to proceed at a constant rate during the first 12 hours, its content in the hydrolysis product being of 92% with respect to the theoretical one after 24 hours. The sodium hydroxide consumption within the 24 - 48 h range might be explained by the hydrolysis of amide group in the drug leading to the acid. The solid product remaining after hydrolysis contained only nitrogen traces after washing and drying which did not permit the calculation of the remaining coupled drug. Hence the new drugs obtained by the ampicilline coupling on CMC-Cl release the antibiotic stepwise showing retard action. The performed tests showed their antimicrobial activity.

CONCLUSIONS

The ampicilline may be coupled on CMC-Cl by covalent bonds.

The efficiency of coupling reaction is influenced by the drug/support ratio, duration and solvent volume.

Maximum coupling yields are obtained with high drug/support ratios, long durations and small solvent colume.

The new obtained drugs show retard antimicrobial activity.

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