DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 16(2), 267-281 (1990)

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

BIOACTIVE POLYMERS 62. A NOVEL MACROMOLECULAR DRUG BASED ON 5-ACETYLAMINO-2-SULFAMOYL-1,3,4 THIODIAZOLE

Severian Dumitriu, Ostin C. Mungiu[x], Marcel Popa,
Maria Dumitriu and Cristina Dumitriu
Polytechnic Institute of Jassy, R-6600, Jassy, Romania
[x]Institute of Medicine and Pharmacy of Jassy, Romania

ABSTRACT

The condensation reaction of 5-acetylamino-2-sulfamoyl-1,3,4 thiodiazole (acetazolamide) on the poly(acrylic acid-co-styrene) copolymer was studied, with a view to obtaining a retard drug. Coupling was performed in a THF medium in the presence of dicyclohexylcarbodiimide as activator. The diuretic effect of the newly obtained drug was checked in rats.

INTRODUCTION

Drug retardation actually knows a wide development, as a consequence of the multiple pharmaceutical advantages which it exhibits. Generally, this process involves the utilization of biocompatible macromolecular supports, permitting drug coupling by

Copyright © 1990 by Marcel Dekker, Inc.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

ionic and covalent bonds[1-4]. Acetazolamide, used in glaucoma treatments, has certain disadvantages, referring to its rapid release off the human organism[5]. With a view to limiting such shortcomings, its coupling on various macromolecular supports is being studied. At the same time, the sobtainment of some retard systems with controlled release, made of pilocarpine included in xanthane gel (ophthalmic insert)[6], and acetazolamide coupled on natural and synthetic polymers, to be orally administered, respectively, is had in view.

The present paper is a study of acetazolamide coupling on an acrylic acid - styrene based copolymer.

Biomedical investigations have evidenced that the support is not toxic, while the reaction product has a prolongued diuretic effect.

EXPERIMENTAL

5-Acetylamino-2-sulfamoyl-1,3,4 thiodiazole (acetazolamide) coupling was performed on three different macromolecular supports, in order to select the one showing the highest reactivity. Two of them have a polysaccharidic nature (chlorocarbonyl methyl) cellulose (CMC-Cl) and xanthane (X), the formed being characterized by a substitution degree with -COCl groups, $DS_{COCl} = 0.37$, the latter one showing a carboxylic group content of $5.32\ 10^{-3}$ mol-COOH/g polymer.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

Another support used was a copolymer of the a acrylic acid with styrene, with a content of 16.9% acrylic acid. This was synthetized by copolymerization in solution (DMF solvent) at a 3/7 mole/mole ratio of comonomers (acrylic acid/styrene), at a temperature of 60°C and a reaction duration of 6 h, on using AIBN as initiator (c = 0.02 mole/l). The acrylic acid content of the copolymer (PAcA-S) was determined by titration with a solution of 0.1 N NaOH.

Acetazolamide (AcAA) coupling on CMC-Cl was performed in a heterogeneous system, by using DMF as reaction medium. In order to assure the necessary basic pH for the reaction development, small amounts of pyridine were added.

The coupling of AcAA on X was realized in THF, by using dicyclohexylcarbodiimide (DCI) as activator.

Drug coupling on PAcA-S was performed in a homogeneous medium, on using DMSO as solvent. The reaction activation was realized by using DCI, at a temperature of 10 ± 1°C.

For a systematic study of the coupling reaction of AcAA on PAcA-S, THF was used as solvent (homogeneous system), and DCI as activator, respectively. To establish the dependence of coupling drug amount, on the reaction parameters (AcAA/PAcA-S ratio, time and THF volume), the experments were performed according

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

Table 1

Variables codification

Codded variables Real variable	-1.682	-1	0	1	1.682
AcAA/PAcA-S (mole/mole-COOH), x_1	1.10	1.35	1.65	1.95	2.20
Time (h), x_2	12.0	21.7	36.0	50.3	60.0
Volume of THF (ml), x_3	20.0	28.1	40.0	51.9	60.0

to a centrate, rotatable, composed, second order degree experimental design.

Variables codification and their variation limits are presented in the Table 1.

By processing the experimental results on a SINCLAIR - SPECTRUM - Z personal computer, the coefficinets of the regression equation correlating the AcAA coupled amount on the reaction parameters was obtained. It has the form:

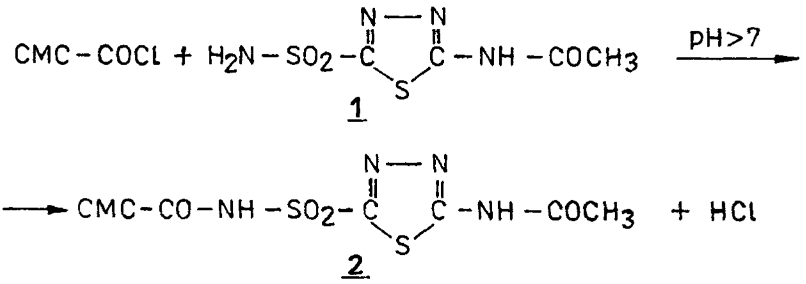
$$\% \text{ AcAA} = 14.333 + 3.766\, x_1 + 3.7\, x_2 + 5.173\, x_3 - \\ - 0.64\, x_1^2 - 0,821\, x_2^2 + 0.374\, x_3^2 + 0.224 \\ x_1 x_2 + 0.309\, x_1 x_3 + 1.299\, x_2 x_3$$

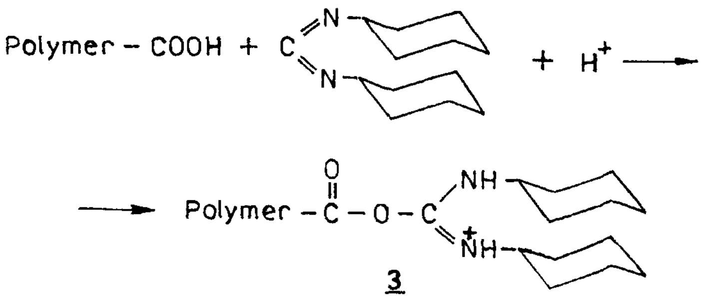
Analysis of the drug content in the coupling products was performed by the Kjeldhal method (nitrogen dosage).

UV Spectral analysis of the reaction products was performed in THF solution, on a UNICAM spectrophotometer. IR Spectra have been recorded on a PERKIN-ELMER spectrophotometer.

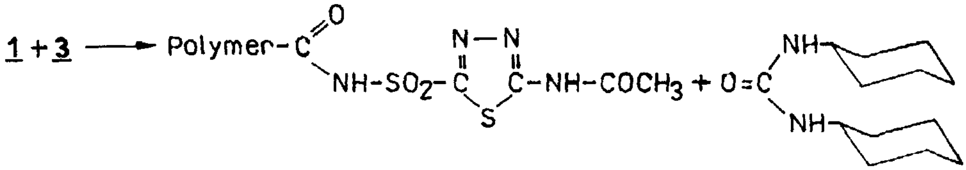
RESULTS AND DISCUSSION

The AcAA coupling reaction is based on the direct condensation of the sulphonamidic group from drug with the carboxylic group of the support (or of the chloroanhydrous one in the case of CMC-Cl). When using X or PAcA-S as macromolecular supports, the reaction is activated by DCI.





Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.



Polymer = xanthane, poly(acrylic acid-co-styrene)

On analysing the results presented in Table 2, one can observe that the efficiency of the coupling reaction is maximum when using PAcA-S as support in THF. Good results are also to be obtained when using DMSO as solvent (sample 5), on assuming that, by increasing the reaction duration over 24 h, the AcAA ratio in the coupling products is comparable with that obtained when using THF as solvent.

Table 2

Influence of the Nature of the Macromolecular Support and of Solvent upon Coupling's Efficiency

Sample	Polymer	Solvent	Duration (h)	%AcAA in the coupling products
1	CMC-Cl	DMF	4	4.92
2	X (Keltrol^{R})	THF	46	6.35
3	X (Keltrol^{R})	DMSO	46	5.55
4	PAcA-S	THF	46	13.50
5	PAcA-S	DMSO	24	8.80

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

The explanation for such results, better as compared with those obtained when using polysaccharidic supports, lies in the utilization of the homogeneous medium in which the reaction proceeds, and which favourizes the majority of the functional groups of the support. One can also observe that THF, too, may constitute the suitable solvent for the reaction occurrence, its presence inducing the chemical bonding of some higher amounts of drug. Starting from these observations, the systematic study of the coupling reaction of AcAA on PAcA-S - in THF solution - has been undertaken, with a view to establishing the influence factors as well as the way in which they influence the process.

Preliminary experiments led us to the conclusion that the highest influence upon coupling is played by the drug/support molar ratio, THF volume and the reaction duration. Their action upon the reaction, expressed by the variation of the AcAA amount chemically bound in the coupling products, is shown in Figure 1.

One can thus observe that the coupling's efficiency increases with the drug/support molar ratio (cuve 1). On working in the 1.1 - 2.2 mole AcAA/mole -COOH domain, the drug content in the coupling products increases continuously, tending to a constant value at the superior limit of the interval. In the case of

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

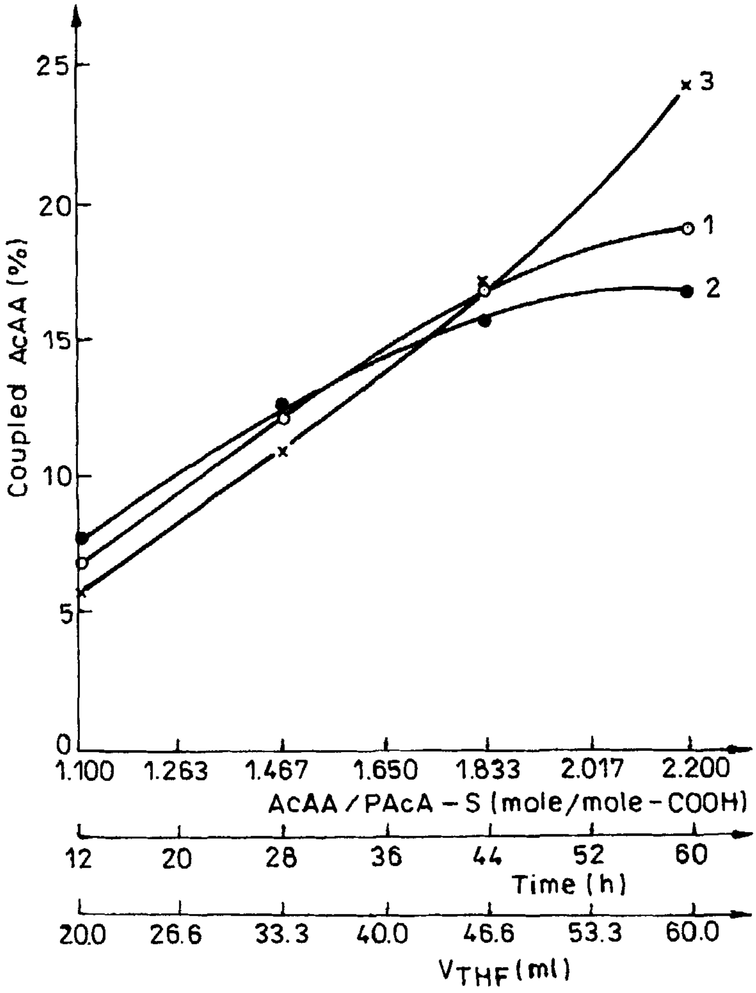


FIGURE 1. Variation of the AcAA amount coupled according to the reaction parameters. 1 - AcAA/PAcA-S ratio (mole/mole-COOH) (V = 40 ml THF; t = 36 h); 2 - Duration (V = 40 ml THF; AcAA/PAcA-S ratio = 1.6 mole/mole-COOH); 3 - Solvent volume(AcAA/PAcA-S ratio = 1.6 mole/mole)

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

coupling all functional groups of the support, the content of the drug in the reaction products should attain a value of 39.2%, which means that the maxiumum yield of reaction, attained in this case, is of approximately 57%. Knowing that the increase of the AcAA/PAcA-S ratio over a value of 2 mole/mole-COOH does not induce significant increases of the amount of coupled drug, the above - mentioned value may be thus considered as optimal.

Duration influences highly the coupling process (curve 2). On working on the 12 - 60 h time interval, one can observe a continuous increase of the percent of coupled drug, up to approximately 50 h, thus indicating as optimal this duration of the reaction.

The solvent volume represents another factor of importance (curve 3), influencing even stronger the reaction, as compared with the other two above mentioned parameters. Increase of the THF volume induces the continuous increase, with no tendency of stabilization to a constant value in the experimental field taken for study. The maximum coupling yield reaches a value of 70%, when working with AcAA/PAcA-S = 1.6 mole/mole -COOH ratio and a duration of 50 h. The explanation of such an influence is based on the interactions manifested between the carboxylic groups of the copolymer, determining its various conformations - dependent on

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

the concentration. RMN Spectroscopy, applied to the synthesized copolymer evidenced the presence of triades, in a 1.31% (AcA-AcA-AcA), as weel as that of the AcA-AcA-S type (21%), which thus attests the existence of several neighbouring carboxylic groups. On disolving the copolymer in small amounts of THF, by the interaction of these neighbouring groups, as well as by intermolecular interactions, quite complicated chain conformations are to be obtained. The reactivity of the functional groups is reduced, as a consequence of the low access of the activator and of the drug to the carboxylic groups. The coupling yield decreases, yet increasing with increasing the dilution, when macromolecules acquire large conformations, releasing the -COOH groups from their interactions.

One can thus appreciate that the efficiency of AcAA coupling on PAcA-S is maximum when taking the maximum values of the studied parameters.

Spectral characterization of a reaction product, synthesized under conditions of optimum, shows clearly the chemical bonding of the drug to the macromolecular support.

Thus, the UV absorption spectra (recorded in THF) (Figure 2) evidence, besides the absorption bands at 261, 263 and 271 nm, which are characteristic to the polymer, a band at 267 nm, too, due to the tiazo ring from the AcAA molecule.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

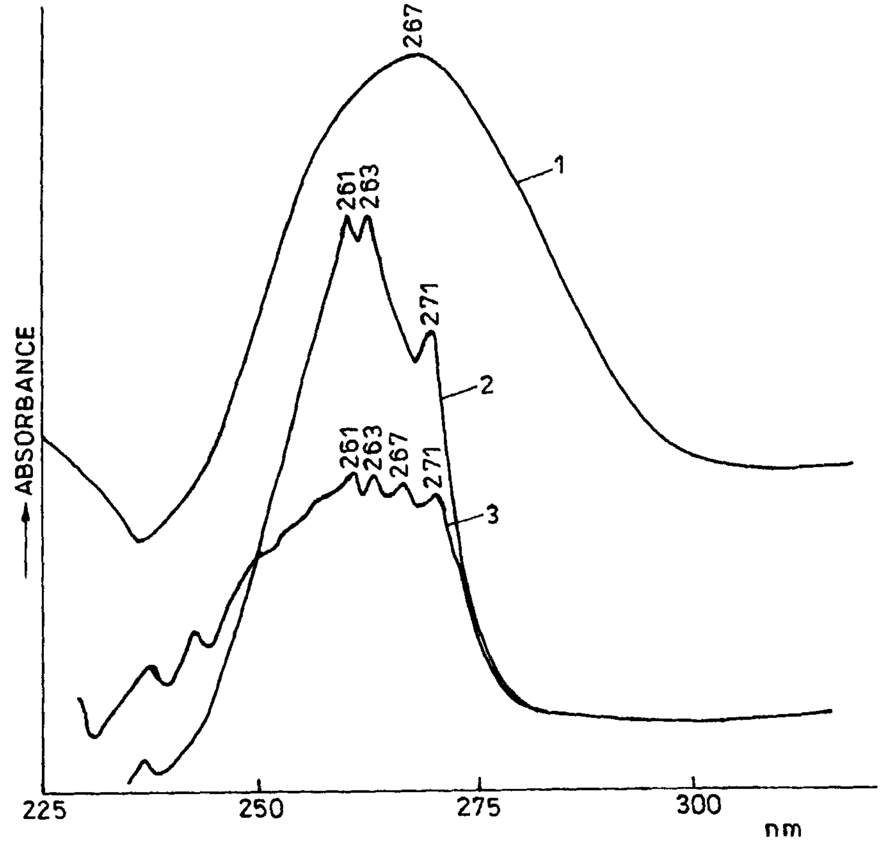


FIGURE 2. UV Absorption spectra. 1 - AcAA; 2 - PAcA-S; 3 - Coupling product.

At the same time, the general form of the spectrum of the coupling product is similar to that corresponding to the free drug.

IR Spectrum (Figure 3) shows the bands characteristic to the secondary amide groups, at 1540 and 1670 cm^{-1} wave numbers. The acetylic groups absorbes at 1360 cm^{-1}. For the R-SO$_2$-N$<$ group, two absorption bands,

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

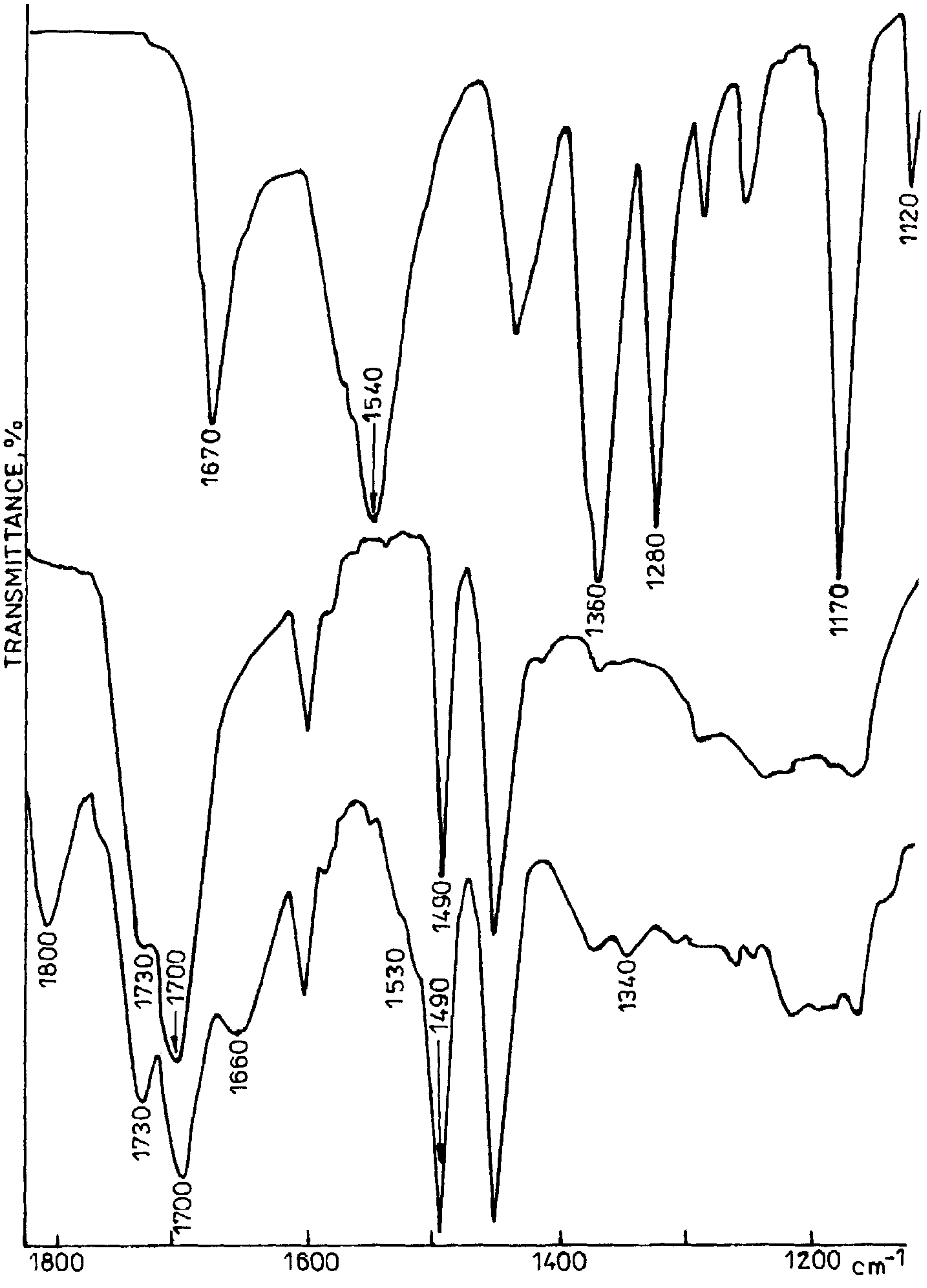


FIGURE 3. IR Spectra. 1 - AcAA; 2 - PAcA-S; 3- Coupling product.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

at 1170 cm^{-1} and 1320 cm^{-1} - corresponding to the vibrations of SO_2 - are evidenced. The presence of the aromatic nucleus is manifested in the spectrum by the absorption bands at 1490, 1600 cm^{-1}, while, in the 1700 - 1730 cm^{-1} field, the abosrption of the COOH group from the polymer appears. In the spectrum of the coupled product, there are to be met all these absorption bands, along with some specific only to the drug: 1340 cm^{-1} attributed to CH_3-CO; 1530 and 1660 cm^{-1} attributed to -NH-CO, as well as 1800 cm^{-1} attributed to a new group, of the R-CONH-SO_2 type, formed by coupling. All data obtained by spectral analyses evidence thus the coupling of AcAA on PAcA-S by covalent bonding.

The diuretic effect of retarded acetazolamide - as compared with that the normal acetazolamide - was tested on rats. Classical procedures were applied[7], using devices of urine contention and collecting. Male and female rats, forming batches of 6 individuals each (3 males and 3 females) 100 ± 10 g in weight, have been given no food 24 h before the administration of the drug. The substances to be investigated have been administered per os, using a probe, in 5 ml physiological salt, so that all animals should be given the same amount of active substance (10 mg/Kg). The standard batch was administered physiological salt, exclusively.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

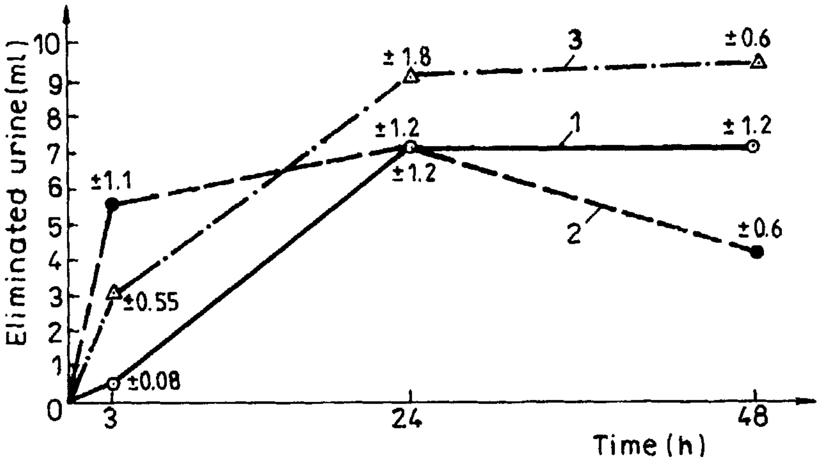


FIGURE 4. Time variation of urine amount eliminated by rats. 1 - wittness; 2 - to which a acetazolamide was administered; 3 - to which retard acetazolamide was administered.

The amounts of urine eliminated after 3, 24 and 48 h were measured, the results obtained being listed in the Figure 4; they evidence a strong diuretical action of the retarded product.

CONCLUSIONS

The optimal conditions for the reaction of acetazolamide with the PAcA-S copolymer were established (the AcAA/PAcA-S ratio = 1.6 mole/mole-COOH; V_{THF} =

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/27/12
For personal use only.

40 ml; time = 36 h). The structure of the retard drug has been analyzed by IR and UV spectroscopy. The diuretic action of the retard acetazolamide was maintained at a constant value, ranging between 24 - 48 h.

REFERENCES

1. S. Dumitriu and M. Popa, J. Bioact. Compat. Polym., 1, 285 (1986)
2. L. B. Wingard, Biochem. Pharm., 32, 2647 (1983)
3. M. F. Refojo, H. S. Lin and F. L. Leong, J. Bioengineer, 2, 437 (1978)
4. Cr. Simionescu and S. Dumitriu in Biomedical and Pharmacological Applications, E. Chiellini and P. Giusti eds., Plenum Press, N. Y. and London(1983), p. 115
5. x ^{x} x Farmacopeea Română, Ed. IX-a, Editura Medicală, Bucureşti, (1976), p. 38
6. S. Dumitriu, P. Vancea, D. Costin and M. Popa, Clinical Materials, 2, 141 (1987)
7. V. Bobulescu and O. C. Mungiu, Farmacologie, I. M. F. Iaşi, (1980), p. 76