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PREPARATION AND EVALUATION OF EUDRAGIT E MICROSPHERES CONTAINING BACAMPICILLIN

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ABSTRACT

Microspheres with bacampicillin were prepared by the solvent evaporation method using systems methanol, acetone and methyl acetate / liquid paraffin and Eudragit E as polymer. Sieve analysis showed that the particle size of the microspheres follows log - normal distribution with average size of 123, 206 and 300 μm, respectively. Scanning electron microscopy was used to prove that all chosen systems provided the particles of regular spherical shape without aggregation.

HPLC method was developed for testing drug content, drug stability and dissolution. The results of HPLC analysis showed the exisistence of degradation

products of bacampicillin in microspheres prepared by the use of all three solvents. The degree of degradation was the lowest in the case of methyl acetate. The experimental values of dissolution profiles fit well to 0. order and combined 0. and $t^{1/2}$ order. The comparison of dissolution profiles of microspheres and bacampicillin itself shows that microspheres produce retard effect and therefore bacampicillin is not expected to be released in saliva after peroral administration of microspheres.

INTRODUCTION

Microencapsulation is a useful technique for coating particles. In the pharmaceutical field microencapsulation is used for the aims as follows: production of sustained release and gastroresistent dosage forms, reduction of odor and volatility, to disguise the unpleasant taste, to prevent incompatibilities etc. Most convenient physico-chemical methods for preparation of microspheres are coacervation and solvent evaporation method. Both are relatively simple to perform in laboratory conditions. In literature several core and coating materials are used. When applying solvent evaporation method the following systems of solvents are most widely used:

methylene chloride/water (1) and acetone/liquid paraffin (2,3). Some other works report methods of preparation based on different physical processes, for example dehidration of gelatin microspheres by isopropanol in water/mineral oil system (4) instead of evaporation of the solvent.

In this work bacampicillin hydrochloride was incorporated in microspheres by solvent evaporation method in order to cover its unpleasant taste. Because of its high water solubility (209 g/l (5)) the microencapsulation procedure by the evaporation process in an oil phase has been chosen. The systems used were methanol, acetone and methyl acetate / liquid paraffin. Eudragit E which is a copolymer, cationic in character, based on dimethylaminoethyl methacrylate and neutral methacrylic acid esters, was used as polymeric material. According to its chemical structure, Eudragit E is soluble in solutions to pH 5 whereas above pH 5 it becomes insoluble and swells. It was also reported that Eudragit E film coatings are resistant to saliva, which means that any unpleasant tastes are reliably masked on administration.(6)

Additionaly drug stability, physical and biopharmaceutical tests were carried out to characterize final products prepared in three different systems.

MATERIALS

Bacampicillin hydrochloride was supplied by Lek Ljubljana, Yugoslavia (quality corresponds to USP XXI) and Eudragit E was a product of Rhöm Pharma, Darmstadt, FRG. Other reagents were all of analytical grade.

METHODS

Preparation of Microspheres

Eudragit E was dissolved completly in the solvent (methanol, acetone or methyl acetate) and magnesium stearate was added. Bacampicillin was dispersed separately in the same solvent previously cooled to 5°C and this dispersion was added to Eudragit solution. The obtained mixture was stirred at 5°C over 10 minutes and then poured slowly with stirring into cold liquid paraffin (5°C). The emulsion was heated to 40°C and stirred 250 rpm until the solvent was removed completly by evaporation. Then 30 ml of n-hexane was added to suspension of microspheres. After few minutes microspheres were separated by filtration, washed twice with n-hexane and dried at room temperature under reduced pressure over night.

The preparation procedure is represented in Figure 1 and Table 1.

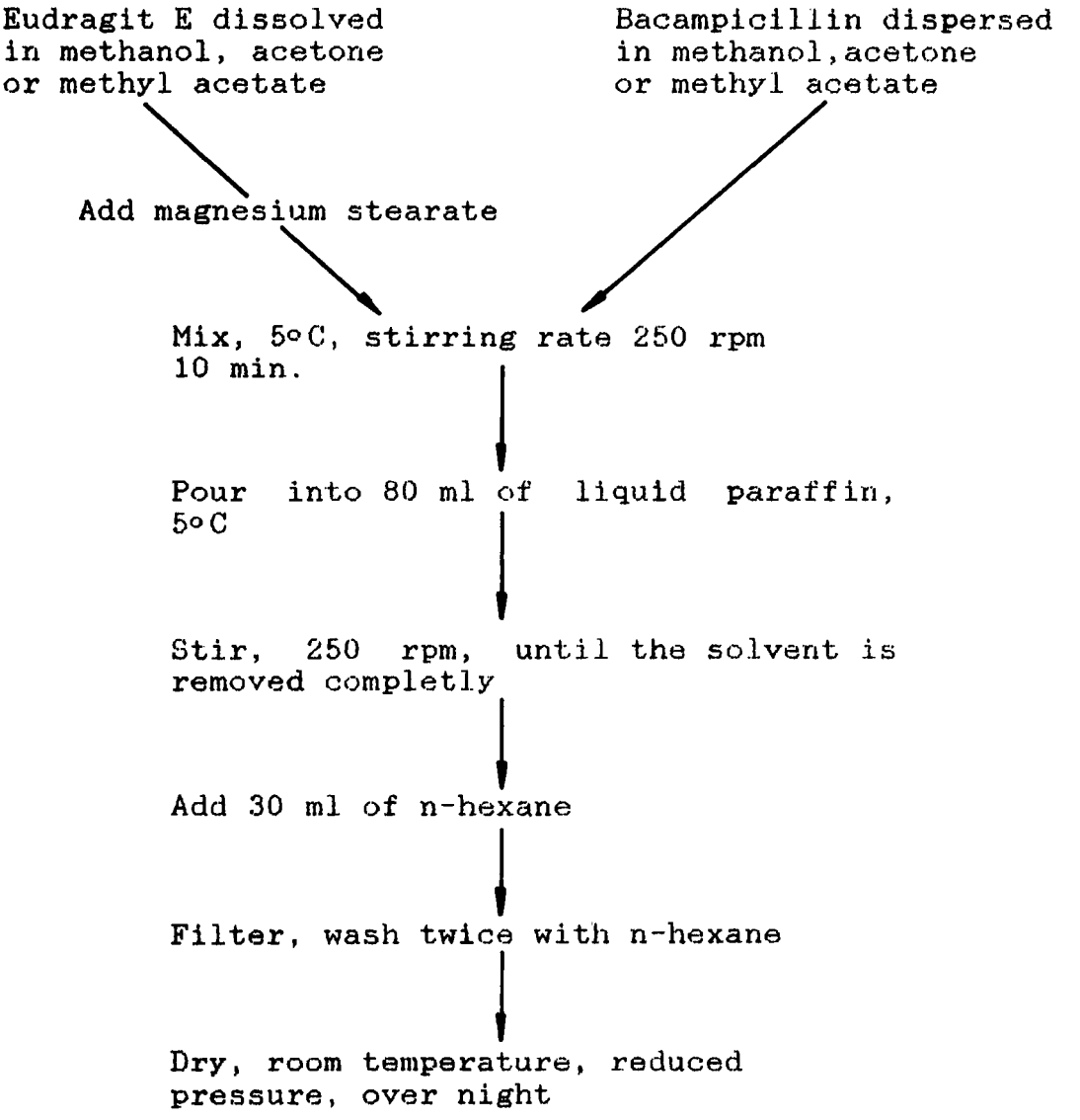

FIGURE 1.

Microspheres Preparation Scheme

TABLE 1.

Prescription for Preparation of Microspheres with Bacampicillin.

	Methanol	Acetone	Methyl acetate
Liquid paraffin (ml)	80	80	80
Bacampicillin (g)	1.6	1.6	1.6
Eudragit E (g)	1.6	3.2	3.2
Mg stearate (g)	0.16	0.20	0.32
Solvent (ml)	9.6	19.2	20.8
Boiling point (°C)	65	56	57
Time (h)*	4	1.5	1.25

*Time needed for complete evaporation of the solvent.

Electron Microscopy

The surface characteristics were examined by means of a scanning electron microscope. The microspheres were coated with C + Au/Pd using Vacuum evaporator (Joel). Samples obtained were examined with a scanning electron microscope (Joel) at accelerating voltage 10 kV using secondary electron technique. Tilt was 45° and working distance 12 mm.

Sieve analysis

Particle size distribution was determined by sieve analysis. Apparatus Vibrations-Prüfsiebmaschine Thyr 2,

GDR was used. We have chosen the screens with the following mesh sizes : 400, 315, 250, 200, 160, 125, 100, 80 and 63 μm to perform subsequently chi-square test for log-normal distribution of particles. We used chi-square statistics, which is calculated as follows:

$$X^2_E = \Sigma ((O - E)^2 . E^{-1}) \quad (1)$$

where O is observed weight of individual fraction and E is expected weight of the same fraction, calculated from accomodated normal distribution. X^2_E values were compared with tabulated chi-square values (X^2_f) for defined degrees of freedom.

We used 5g samples and sifting time 10 minutes. The procedure was carried out twice for each product.

High Performance Liquid Chromatography

HPLC analyses were taken using a system constructed from LC-pump T414, injector Rheodyne 7125 fitted with a 20 μl loop, Uvicon 735 LC detector with variable wavelenght and sensitivity of 0.04 AUFS and recorder Kontron 330. The column used was PLRP-S, 5 μm, 125*6 mm i.d., the mobile phase consisted of acetonitrile and 0.01 M phosphate buffer pH=6.5 (50/50), flow rate was 0.6 ml/min, and the column effluent was detected at 220 nm. The retention time of intact bacampicillin was 10 min.

The method was developed from that described by Ellström and Nyqvist (7).

Drug Content Determination

Microspheres were pulverized and dispersed in distilled water. The mixture was shaked vigorously over 20 minutes and filtered. The samples were analysed by HPLC.

Dissolution Studies

The apparatus was the same as described in USP XXI under Apparatus 2, i.e. apparatus with paddle stirring element (ERWEKA DT-D, FRG). The test was carried out under the following conditions: 350 mg of microspheres were dispersed in 1 l of phosphate buffer pH = 6.5, rotation speed of the paddle was 100 rpm, room temperature. The 4 ml samples were drawn at 0, 2, 4, 6, 8, 10, 15, 20, 30 , 45 and 60 minutes and filtered through filter paper to remove solid particles. The test was realized also for unencapsulated bacampicillin. All the samples were analysed by HPLC. Experiments were carried out four times for methyl acetate microspheres and twice for substance.

RESULTS AND DISCUSSION

Preparation of Microspheres

Microspheres loaded with bacampicillin were prepared using three different systems. Criterion for the selection of the solvent was the value of

dielectric constant. It's already known that solvents with dielectric constant between 10 and 40 exhibit poor compatibility with liquid paraffin and that the system of this solvent / liquid paraffin was applicable to the microencapsulation of drugs (8). Methanol (ε = 32.6) and acetone (ε = 20.7) fall within the range, while methyl acetate has lower dielectric constant (ε = 6.7) and is therefore partly miscible with liquid paraffin. As a consequence flocculation of the emulsive drops containing methyl acetate mixture occurs. This phenomena was prevented by partial saturation of liquid paraffin with methyl acetate (1 ml methyl acetate / 10 ml liquid paraffin) before the microencapsulation procedure.

The volumes of the solvents and the ratios between bacampicillin and Eudragit were optimized according to the desired characteristics of the dispersion (of bacampicillin in the solvent). The times needed for complete evaporation of the solvents depend first of all on the boiling point and less on the volumes of the solvents (Table 1). Magnesium stearate was used to prevent flocculation and aggregation of microspheres in all studied systems and to enable the isolation of final products. Addition of n-hexane in final stage of microencapsulation contributes to the hardness of microspheres and accelerates sedimentation. The yield

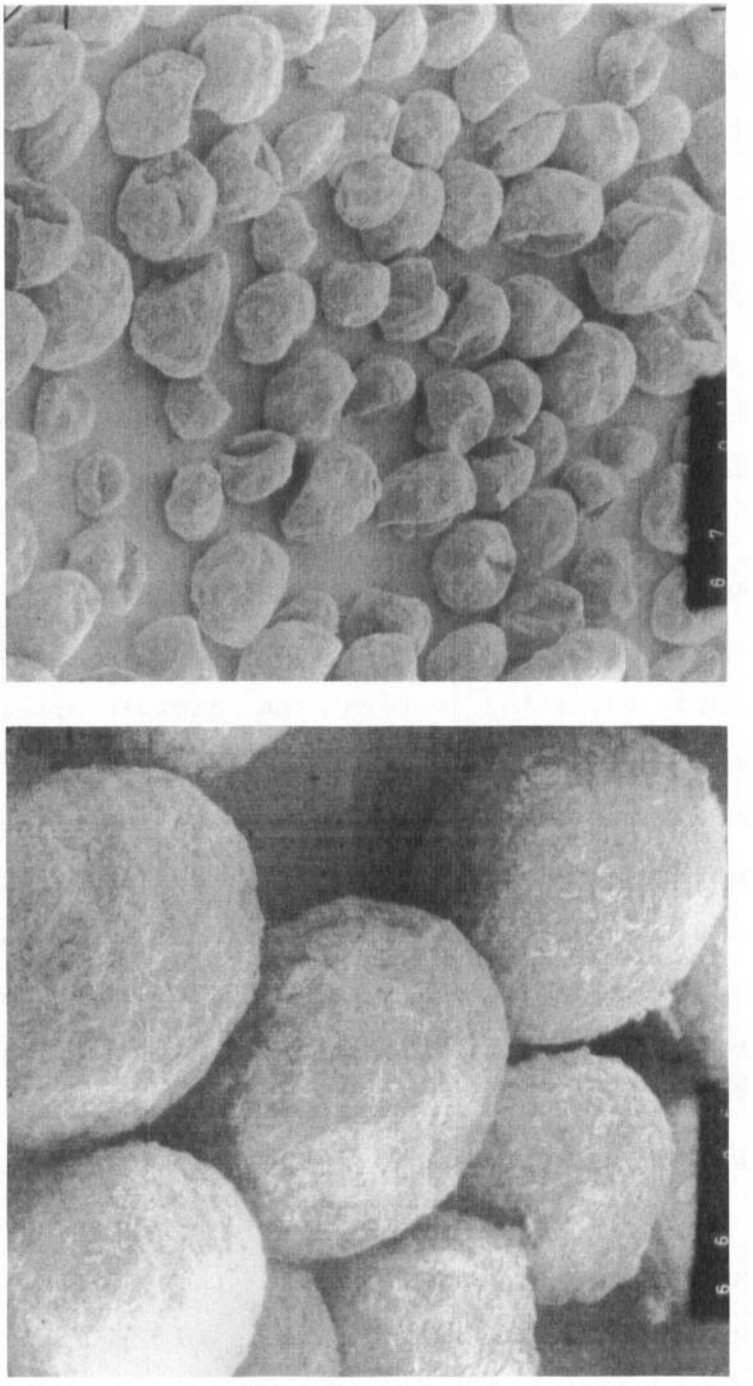

FIGURE 2.

Scanning Electron Micrographs of Microspheres Containing Bacampicillin. Solvents: A. Methanol, B. Acetone and C. Methyl acetate. Magnification: 190 x.

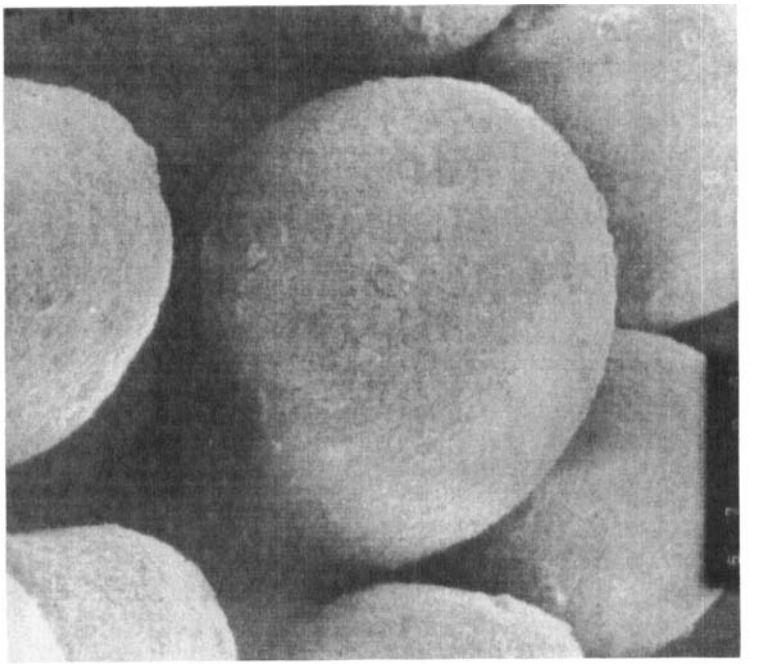

FIGURE 2C.

of the procedure regarding material balance was 89 % when using methanol, 91 % for acetone and 95 % for methyl acetate. The final products were free flowing powders.

Electron Microscopy

Regarding the results of scanning electron microscopy which was used for observation of the shape of microspheres, all chosen systems provided the particles of regular spherical shape without aggregation, as shown in Figure 2.

Sieve Analysis

The results of sieve analysis show that the average size of microspheres was 123 μm, 206 μm and 300 μm if prepared in the systems with methanol, acetone and methyl acetate, respectively. We suppose that the smallest average particle size of methanolic microspheres arises from the fact that bacampicillin is soluble much better in methanol than in acetone and methyl acetate. Consequently, the system Eudragit E / bacampicillin / solvent was real solution in the case of methanol while the other two systems were suspensions. Additionaly, sieve analysis showed that the particle sizes of microspheres follows log - normal distribution in all three systems. The results of sieve analysis are given in Figure 3 and Table 2.

Evaluation of Bacampicillin Stability

Bacampicillin is known to be quite unstable substance due to its chemical structure (9,10). Having in mind experimental conditions (organic solvents, elevated temperature) we had expected degradation of the molecule during the process of preparation, what was subsequently verified by the use of HPLC analytical method. The chromatograms (Figure 4 A,B,C,D) show the presence of degradation products for all three systems. As expected most unknown peaks appeared in the case of methanol and least in the case of methyl acetate. When

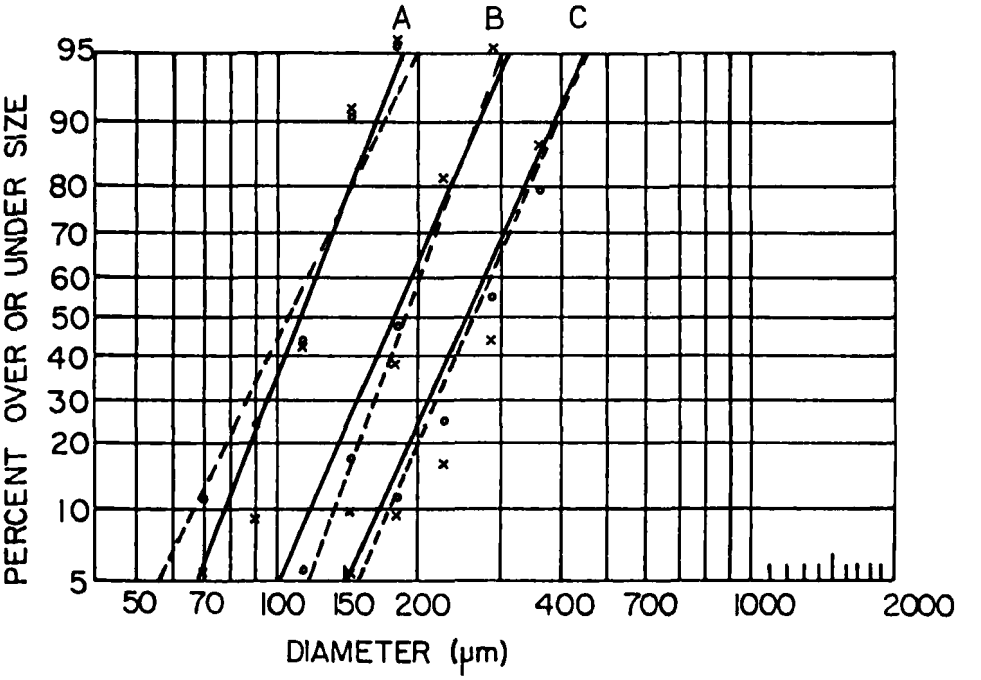

FIGURE 3.

Plotting of Particle Size Distribution Data for Microspheres Containing Bacampicillin. Solvents: A. Methanol, B. Acetone and C. Methyl acetate.
Experiment 1: ° Experimental Values
—— Values of Accomodated Normal Distribution
Experiment 2: × Experimental Values
- - - - Values of Accomodated Normal Distribution

TABLE 2.

Results of Chi-square Test (X^2_E) for Log-normal Distribution of Microspheres Prepared with Different Solvents.
X^2_t = 9.49 , NS = Non Significant Differences.

Experiment	Methanol	Acetone	Methyl acetate
I.	0.83 (NS)	0.16 (NS)	0.25 (NS)
II.	1.86 (NS)	0.42 (NS)	0.88 (NS)

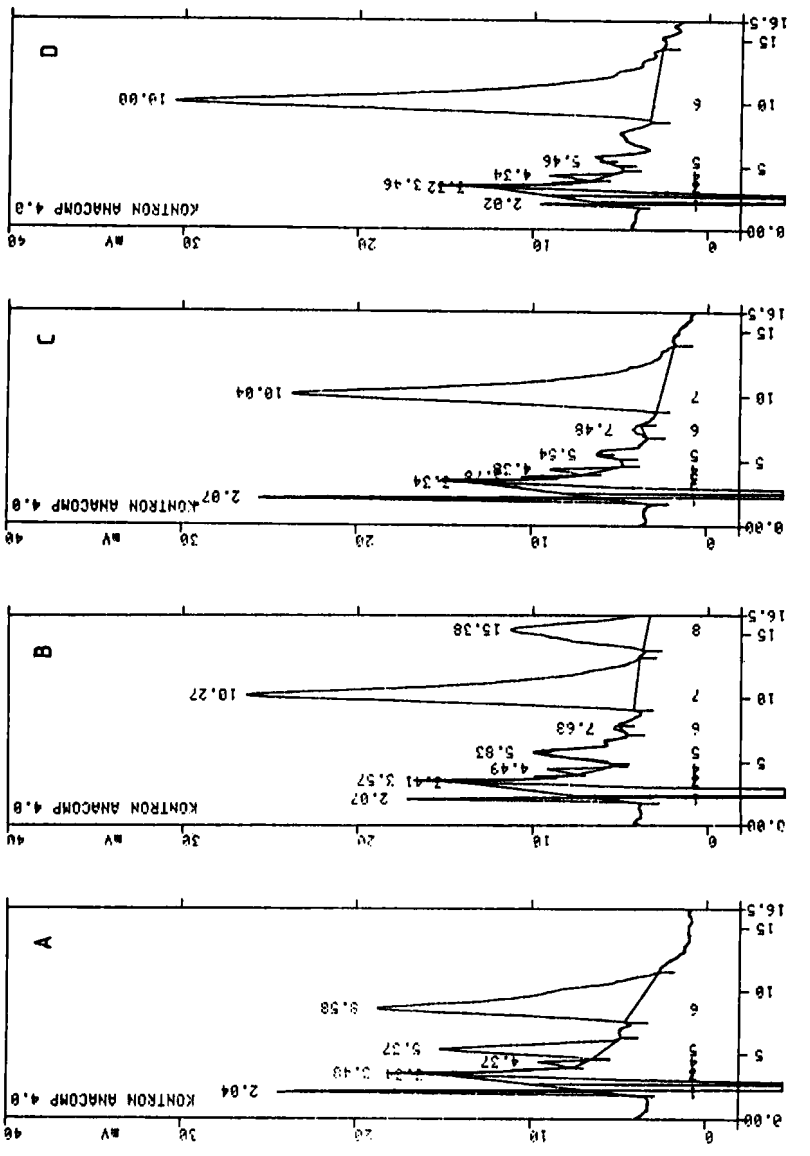

FIGURE 4. Chromatograms of Bacampicillin and its Degradation Products Isolated from Microspheres. Solvents: A. Methanol, B. Acetone and C. Methyl acetate. D. Standard Solution of Bacampicillin in Water.

developing the microencapsulation procedure using methyl acetate the stability aspect was taken into consideration separatly and the degree of degradation was minimized. In the case of methanol microspheres where the time is the longest, the peak belonging to bacampicillin is covered by one of the degradation products.

Therefore all subsequent control tests (drug contents and dissolution) were carried out with methyl acetate microspheres.

Drug Content Determination

Bacampicillin content in methyl acetate microspheres was 21 % (w/w). The difference between incorporated and determined quantity may be attributed mostly to degradation of bacampicillin and/or its adsorption on Eudragit and less to material loss during the procedure of microencapsulation.

Dissolution Studies

The results of dissolution tests are given in Figure 5 and Table 3. The experimental values in Figure 5 represent aritmetic mean of four parallel experiments. Coefficient of variation didn't exceed 10 % within the whole interval of observation. We attempted to describe the dissolution profile by a model function. As seen from Table 3, the experimental points fit well to 0.order and combined 0. and $t^{1/2}$

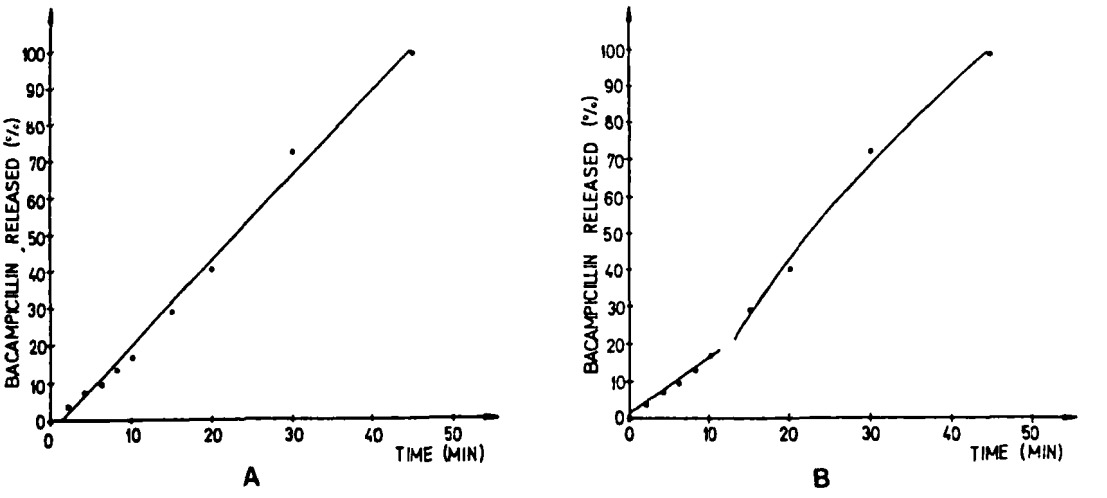

FIGURE 5.

Dissolution Profiles of Bacampicillin. Points - Experimental Values, Curve - Model Response.
A. 0. Order, B. Biphasic 0. and $t^{1/2}$ order

TABLE 3.

Correlation Coefficients for Linear Relatioship of Zero-Order, $t^{1/2}$-Order and Biphasic Kinetics.

Experiment	0.order (0-95%)*	$t^{1/2}$order (0-95%)	0.order (0-20%)	$t^{1/2}$order (20-95%)
I.	0.996	0.972	0.995	0.997
II.	0.997	0.974	0.989	0.999
III.	0.990	0.970	0.994	0.982
IV.	0.996	0.979	0.996	0.998
average**	0.995	0.971	0.998	0.996

*The values in paranthesses mean % released bacampicillin.
**The values of correlation coefficients where average values of % released bacampicillin were fitted to model function.

order in contrary to $t^{1/2}$ order which gives unsatisfactory fit. Biphasic release profile might support the hypothesis that the core of the capsule represents the matrix system of a dispersed drug and around the core there is a polymeric membrane (11). Solvent evaporation method which was used in our work namely gives matrix type microcapsules. As bacampicillin is unsoluble in methyl acetate it can be possible that Eudragit, which is soluble in methyl acetate, sorrounds matrix cores containing particles of bacampicillin dispersed in Eudragit.

Dissolution test with unencapsulated bacampicillin indicates that hundred percent release of bacampicillin is practicaly instantaneus.

Finally, the comparison of dissolution profiles of microspheres and bacampicillin itself shows that bacampicillin is not expected to be released in saliva after peroral administration of microspheres.

CONCLUSIONS

Eudragit E microspheres loaded with bacampicillin can be easily prepared by the use of solvent evaporation method. The procedure gives satisfactory results for all three chosen systems when considering size distribution and shapes of microspheres. The different steps of process of microencapsulation

produce the degradation of bacampicillin particulary in the case of methanol. Varying the conditions of preparation the degree of degradation was minimized when using methyl acetate. The dissolution experiment showed that microencapsulation retards the release of bacampicillin and therefore it is not expected to be released in saliva after peroral administration of microspheres.

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