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PREPARATION AND EVALUATION OF ETHYLCELLULOSE MICROCAPSULES WITH BACAMPICILLIN

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ABSTRACT

Ethylcelluloses of different types were used to microencapsulate bacampicillin. Polymer deposition from cyclohexane was performed by temperature change. As coacervation - inducing agent different polyisobutylenes (Oppanol B - 200, B - 100, B - 50, B - 3) were used. Fine products with slower drug release were obtained. Average diameters of prepared microcapsules were determined with sieve analysis and it was shown that the particle size of the microcapsules follows log - normal distribution. Scanning electron microscopy was used to examine the shape and the surface characteristics of the microcapsules. HPLC method was developed for testing drug content and its dissolution. Drug content in the microcapsules was in all cases over 80% regarding the

amount added. Dissolution of bacampicillin from microcapsules was retarded comparing to the dissolution of bacampicillin itself. The experimental values of dissolution were fitted with different model kinetics. To describe the dissolution profiles we suggested the combined zero and first order kinetics. The bitter taste was quite satisfactory disguised in all prepared microcapsules.

INTRODUCTION

Microencapsulation is a well known method with many possible applications in the field of pharmacy: production of sustained release and gastroresistant dosage forms, reduction of odor and volatility, disguise of the unpleasant taste, prevention of incompatibilities etc. Many different coating materials and processes of preparation are used. In our case the coacervation method using ethylcellulose coating by polymer deposition from cyclohexane by temperature change was performed. Different copolymeres can be used as coacervation - inducing agent, for example polyethylene and polyisobutylenes with different molecular weight (1,2,3,4).

In present work bacampicillin hydrochloride was incorporated in microcapsules by coacervation method using ethylcellulose as wall forming polymer in order to cover its unpleasant taste. The influence of

different types of polyisobutilene(PIB) as coacervation inducing agent on the procedure of microencapsulation was observed. For the preparation ethylcelluloses with distinct physicochemical properties in different wall to core mass ratios were used. The products obtained were isolated in terms of negligible agglomeration and evaluated with different physical and biopharmaceutical tests in order to characterize final products.

MATERIALS

Bacampicillin hydrochloride was supplied by Lek, Pharmaceutical and Chemical Works, Ljubljana, Yugoslavia (quality corresponds to USP XXl). Ethylcelluloses of different types: N - 100, N - 50, N - 22 and N - 7 having an ethoxyl content 47,5 to 49,0% were the products of Hercules incorporated, U.S.A. Polyisobutylenes(PIB), Oppanol B - 3, B - 50, B - 100 and B - 200 were the products of B.A.S.F., West Germany. Other reagents were all of analytical grade.

METHODS

Preparation of Microcapsules

1g of ethylcellulose and 50ml cyclohexane in 100ml beaker with reflux funnel was heated on the water bath to 80 - 81°C (the boiling point of cyclohexane) and stirred at the speed of 350rpm. After boiling for ten to twenty minutes the bacampicillin hydrochloride previously suspended in hot cyclohexane was added. Soon

after that the reaction mixture was slowly cooled (the rate of cooling was about 2°C/5min.). When the temperature of the system fell to about 70°C, the saturated solution of PIB in cyclohexane was added and the reaction mixture was further slowly cooled to 60°C. The content of the reaction vessel was then poured in 100ml of cold cyclohexane(T = 5°C) and stirred. After that the product was filtered and washed with cold n - heptane in the sense to avoid the agglomeration.

Electron Microscopy

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

Sieve Analysis

Particle size distribution was determined by sieve analysis. Apparatus Vibrations - Prufsiebmaschine Thyr 2, GDR was used. The sieves with following mesh sizes were chosen: 800, 500, 315, 200, 125 and 80 μm to perform subsequently chi - square test for log - normal distribution of particles. We used chi - square statistics, which is calculated as follows:

$$X_E^2 = \sum ((O - E)^2 \cdot E^{-1}) \qquad (1)$$

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_t) for defined degrees of freedom.

The samples in amount of 2.5g were shaken for 20 minutes.

High Performance Liquid Chromatography (HPLC)

HPLC analysis were taken using a system constructed from LC - pump T 414, injector Rheodyne 7125 fitted with a 20 µl loop, Uvicon 735 LC detector with variable wavelength and sensitivity of 0.04 Aufs and recorder Kontron 330. The colomn used was PLRP - S, 5 µm, 125 * 4.6 mm i.d., the mobile phase consisted of acetonitrile and 0.01 M phosphate buffer pH = 8 (60/40); flow rate was 0.7 ml/min. and the effluent was detected at 254 nm. The retention time of intact bacampicillin was 5 min.

The method was developed from that described by Ellström and Nyqvist(5).

Drug Content Determination

The microcapsules were pulverized and weighed sample was dispersed in 50 ml of distilled water. The mixture was shaken vigorously for 30 minutes and filtered. The content of bacampicillin was determined by HPLC analysis.

Dissolution Studies

Rotating paddle method was used for determination of dissolution rate. The apparatus used was the same as described in USP XXl under Apparatus 2 (Erweka DT - D, FRG).

The weighed amount of microcapsules (which contains approximately 150 mg of the drug) was dispersed in one litre of distilled water at room temperature. Rotation speed of the paddle was 100 rpm. The 2 ml samples were drawn at different time intervals and filtered to remove solid particles. The test was carried out also for unencapsulated bacampicillin. All the samples were analysed by HPLC.

RESULTS AND DISCUSSION

The preparation of microcapsules was firstly performed using ethylcellulose N - 100 and different types and concentrations of polyisobutylene (Oppanol B - 200, B - 100, B - 50, B - 3) as coacervation inducing agent. Because of greater viscosity of PIBs with higher molecular weight lower concentrations were used. The best results were obtained in the case of Oppanol B - 50 as copolymer (Table 1).

PIB lowers the solubility of ethylcellulose in cyclohexane. The aggregation of microcapsules decreases by increasing molecular weight of PIB and so PIB of low molecular weight doesn 't act as a protective colloid.

TABLE 1.

Microscopic and Macroscopic Observation of the Products Obtained at the Experiments Using Different PIBs. The Mass Ratio of Ethylcellulose N - 100 and Bacampicillin is 1 : 1.

PIB	PIB Concentration (g PIB/g of React. Mixture)	Product Obtained
Oppanol B-3	15/100	Shapeless, Nonregularly Coated, Large Particles
Oppanol B-50	8/100	Spheric, Completely and Regularly Coated Particles
Oppanol B-100	4/100	"Gelatinous" Mixture, Shapeless Particles
Oppanol B-200	2/100	Crystals and Precipitates, Uncoated Particles

When PIB of high molecular weight is used in the proces of microencapsulation, smaller coacervate droplets are formed and larger coacervate volume is produced but only 50% of added ethylcellulose is utilized for the wall formation even at 20°C. Using PIB of low molecular weight all the ethylcellulose is used for wall formation below 50°C (3,6,7).

The above mentioned statements could offer the explanation for the results presented in Table 1. When PIB of low molecular weight is used the agglomerates can appear as a consequence of larger coacervate droplet size and low viscosity of continuous phase. If PIB of high molecular weight is used a lot of

ethylcellulose is not utilized for wall formation and remains precipitated in the solution.

Further experiments were carried out with Oppanol B-50 at the concentration of 8 g PIB 50/100 g of reaction mixture. Different ethylcelluloses with distinct physicochemical properties were used (Table 2). The core/wall mass ratio was varying too. The results obtained by microscopic control are presented in Table 2.

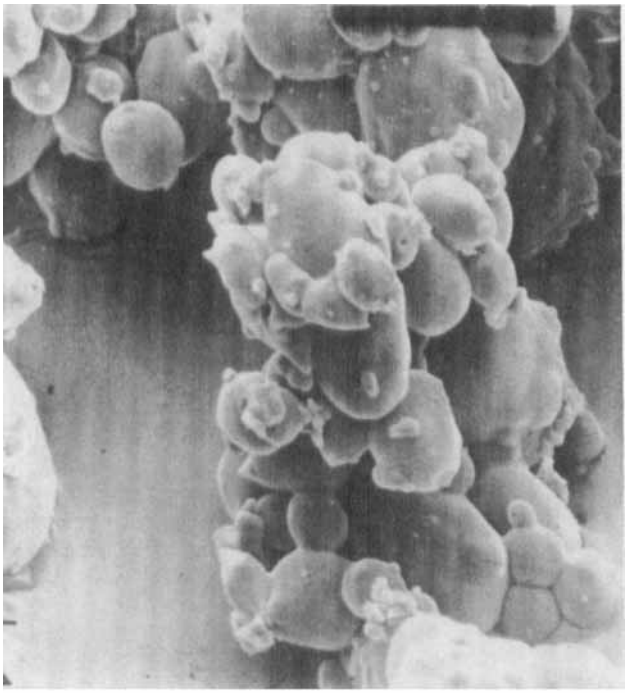
Microscopic control during the procedure of the preparation showed that the formation of the microcapsule film was ended at 60°C (core particles were coated with EC). Slow cooling under 60°C is therefore unnecessary. The isolation of microcapsules was carried out in terms of negligible agglomeration. We tried to avoid the agglomeration with washing the filtered products with different solvents cooled to 5°C. The solvents used were cyclohexane, petroleum ether and n-heptane. They are very lipophilic and poses very low dielectric constants. Nonagglomerated product as free flowing powder was obtained with n-heptane whereas some agglomerates could appear at products obtained by the separation with pretroleum ether or cyclohexane. The yield was always greater than 80 %. Scanning electron micrographs of samples and bacampicillin crystals are presented in Fig.1

TABLE 2.

Microcapsules Prepared with Different Ethylcelluloses(EC) and at Different Core to Wall Mass Ratios Using Oppanol B - 50 at Concentration 8g PIB/100g Reaction Mixture. Microscopic Observations are Presented.

The Signature of the Sample	The Type of EC Used	Mass Ratio Core/EC	Microscopic Observation in Ciclohexane
A_1	N - 100	1:1	Spheric,Coated Particles,Partialy Agglomerated
B_1	N - 50	1:1	Spheric,Coated Particles,no Agglomeration
B_2	N - 50	2:1	Spheric,Coated Particles,Some EC Agglomerated
B_3	N - 50	1:1.5	Spheric,Coated Particles,no Agglomeration
C_1	N - 22	1:1	Nonregular Shapes, Coated Particles, no Aglomeration
C_2	N - 22	2:1	Nonregular Shapes, Coated Particles, Partialy Agglomerated Product
C_3	N - 22	1:1.5	Nonregular Shapes, Coated Particles, no Agglomeration
D	N - 7	1:1	Nonregular Shapes, Coated Particles, Partialy Agglomerated.

A



B

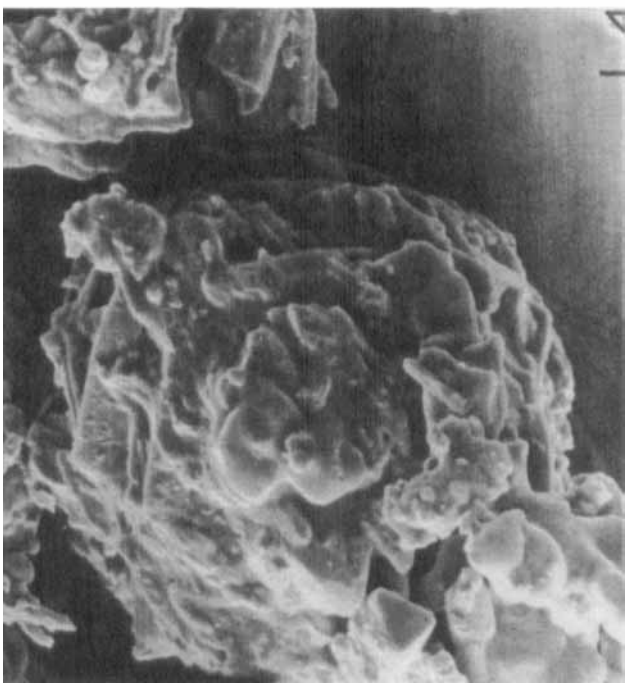
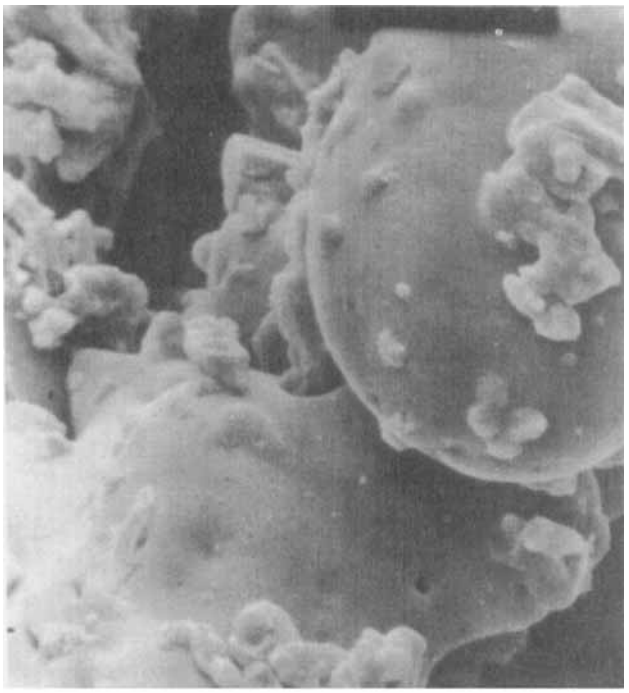


FIGURE 1.

Scanning electron micrographs of some microcapsules prepared and of bacampicillin crystals.
A. sample B_3 (magnification: 630x), B. sample C_2 (magnification 630x), C. sample C_3 (magnification 1900x) and D. bacampicillin crystals (magnification 630x).

C



D

FIGURE 1 continued

We can see that EC and different core/wall mass ratios have influenced the shape of microcapsules. Mostly spherical shaped microcapsules with smooth surface were obtained using EC N - 50 as coating material with core to wall ratio 1:1.5 (Fig. 1A) whereas the others are more irregular shaped. All the microcapsules presented are partialy agglomerated products of the size about 100-200 μm. There are also small particles adsorbed on the surface of the microcapsules. These can be smaller microcapsules or even the crystals of bacampicillin as the consequence of slight solubility of the drug in hot cyclohexane (bacampicillin is practically insoluble in cold cyclohexane).

The results of sieve analysis are shown in Tables 3 and 4. Four representative samples are presented.

TABLE 3.

Average Diameters of Microcapsules. The Logharitms with Standard Deviations and Antilogharitms of Average Values are Given.

MICROCAPSULES	AVERAGE DIAMETER		
	log d +	log s	d(μm)
B_3	2.337	0.359	217
B_2	2.396	0.377	249
C_3	2.419	0.255	262
C_2	2.549	0.352	354

The microcapsules obtained with EC N - 50 and core/wall mass ratio 1:1.5 have the smalest average particle size. Microcapsules with EC N - 22 with core/wall mass ratio 2:1 exhibit the highest value for average diameter. The results show that with ethylcellulose of lower viscosity bigger particles were obtained. It can be also noticed that using ethylcellulose of the same viscosity, microcapsules containing more bacampicillin poses higher values for average diameter. Additionaly, sieve analysis showed that the particle sizes of microcapsules follow log - normal distribution in all systems. The results are given in Table 4.

TABLE 4.

Results of Chi - square Tests for Log - normal Distribution of Microcapsules Prepared with Different Ethylcelluloses.
X^2_t = 9.49, NS = Non Significant Differences.

Sample	Calculated Value for X^2_c
B_2	0.656(NS)
B_3	0.519(NS)
C_2	0.397(NS)
C_3	0.689(NS)

The values of bacampicillin content are shown in Table 5

TABLE 5.

Content of Bacampicillin in Different Microcapsules.

Microcapsules (Sample)	Determined % of the Drug (W/W)	Expected Values in %(W/W)
A_1	45.3	50.0
B_1	43.1	50.0
B_3	31.9	40.0
B_2	66.7	66.7
C_1	48.4	50.0
C_3	39.6	40.0
C_2	65.0	66.7
D	48.0	50.0

Because of bacampicillin unstability (8,9) we expected degradation of the molecule during the process of preparation at elevated temperatures. The results (Table 5) showed us that determined values are relatively high, so we suppose that degradation doesn't occur. The chromatograms of bacampicillin in microcapsules (Fig.2A) show no other peak than that of bacampicillin in water solution (Fig.2B).

We can assume that bacampicillin is stable under preparation conditions. The results of dissolution tests are given in Table (6) and Fig.(3).

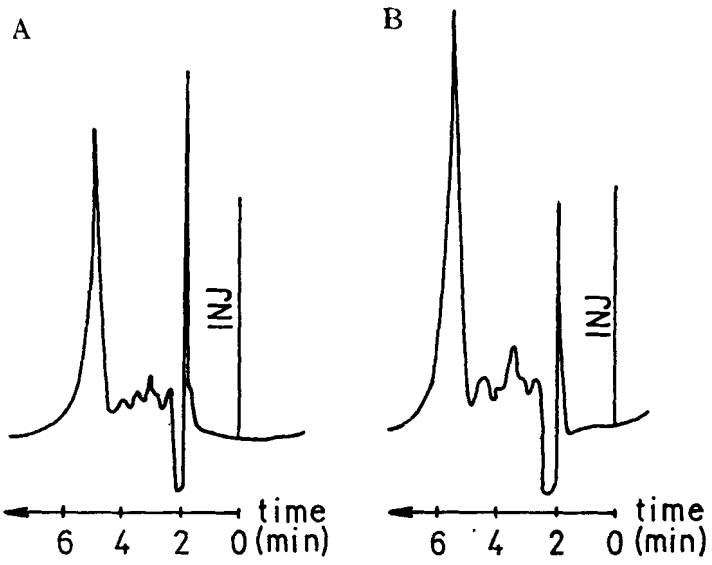


FIGURE 2.

Chromatograms of Bacampicillin Isolated from Microcapsules (A) and Standard Solution of Bacampicillin (Concentration is 0.2mg/ml) in Water(B).

TABLE 6.
Water dissolution of bacampicillin microcapsules samples containing approximately 150 mg of bacampicillin.

	conc. (mg/l)							
time(min.)	A_1	B_1	B_2	B_3	C_1	C_2	C_3	D
0	0	0	0	0	0	0	0	0
2	54	30	/	22	18	/	78	/
5	88	26	130	29	40	82	111	30
8	/	37	/	37	61	/	117	/
10	111	/	134	/	/	113	/	66
12	/	54	/	49	88	/	132	/
15	119	63	139	54	99	123	137	73
20	130	83	140	70	/	132	/	97
30	/	/	142	/	119	/	139	112
40	/	97	144	75	/	144	/	121
60	133	119	147	82	157	151	144	123
90	/	131	157	92	/	/	/	149

/ = the concentration was not determined

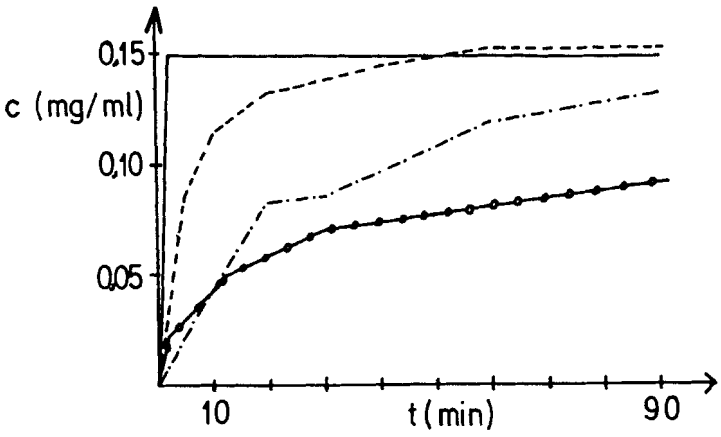


FIGURE 3.

The Dissolution Profiles of Bacampicillin itself and from Microcapsules. Presented are some Representative Samples.
Legend: ——— Dissolution of Bacampicillin itself
-·-·-·- Dissolution of the Sample B_1, 300.00 mg were Weighed
-o-o-o- Dissolution of the Sample B_3, 375.30 mg were Weighed
----- Dissolution of the Sample C_2, 462.30 mg were Weighed.

Dissolution test with bacampicillin indicates that hundred percent release of bacampicillin is practicaly instantaneus(Fig.3). Comparing the dissolution profiles we can firmly say that all the microcapsules prepared retarded the drug liberation. The best results from the point of retardation gave the samples A_1, B_1 and B_3(Table 6, Fig.3). The slowest liberation rate exhibit the sample B_3; even after 90 minutes the substance is not released completely. The differences in liberation rate are attributed to the physicochemical properties

of ethylcellulose used. Dependance of the liberation rate on viscosity or molecular weight of ethylcellulose has already been studied (10,11). Our results obtained are in good agreement with the data reported. Drug release decreases with increasing molecular weight till the minimum and after that point, when molecular weight of ethylcellulose is approximately 13 * 10^4 (depending on molecular weight of PIB used), it begins to increase with increasing molecular weight of ethylcellulose(12). On the other hand molecular weight is in good, almost linear correlation with viscosity(11).

We attempted to describe the dissolution profile by a model function. Many kinetics (zero order, first order, square - root, Hixon - Crowell cube root kinetics and combinations) were used for evaluation of the drug release from film - coated microcapsules (8,13,14,15,16,17). We performed all kinetics for prepared microcapsules with linear regression method(Table 7).

As we have already mentioned the combined release kinetics from microcapsules can also appear(8,13,14,15). So we suggested the biphasic release mechanism to describe the removal of a water soluble drug (bacampicillin) from ethylcellulose microcapsules. We assumed that the microcapsules we prepared are film - coated. Therefore we supposeded that in the beginning, when the concentration of the

TABLE 7.

Correlation Coefficients for Linear Relationship of Zero Order, First Order, Higuchi and Hixon - Crowell Kinetics.

	Samples							
Kinetics	A_1	B_1	B_2	B_3	C_1	C_2	C_3	D
0.order	0.885	0.914	0.523	0.882	0.914	0.849	0.741	0.818
1.order	0.989	0.989	0.900	0.962	0.987	0.992	0.903	0.986
$\sqrt{t}$ order	0.980	0.984	0.733	0.979	0.987	0.972	0.900	0.955
$\sqrt[3]{t}$ order	0.969	0.990	0.761	0.941	0.993	0.963	0.847	0.967

drug is relatively high, the release rate was time independent (zero order release) and afterwards, when the concentration of the drug in the core fell, the release followed first order kinetics(13). Determined constants and correlation coefficients for combined zero order and first order kinetics are presented in Table 8.

In most cases the results in Table 8 exhibit higher values for the correlation coefficients than those in Table 7. We think that combined release is more appropriate although all the values for the correlation coefficients are not close to one and it seems, that the release patterns from ethylcellulose microcapsules exhibit kinetics which can not be simulated by any model properly.

TABLE 8.

The Values of Release Constants (Zero and First order) with Correlation Coefficients.

Microcapsules	0.order $k_0 * 10^2$	r_0	1.order $k_1 * 10$	r_1
A_1	1.71	0.970	1.17	0.986
B_1	0.41	0.997	0.48	0.977
B_2	1.34	0.879	0.33	0.958
B_3	0.31	0.951	0.11	0.990
C_1	0.73	0.998	0.85	0.981
C_2	1.13	0.967	0.94	0.959
C_3	1.56	1.000	0.30	0.971
D	1.31	0.998	0.78	0.982

On the basis of the results obtained we can conclude that the liberation rate varies among the prepared microcapsules dependent on the viscosity of the ethylcellulose used and on the mass core to wall ratio. The bitter taste of bacampicillin in all microcapsules was quite satisfactory disguised, but the sample B_3 exhibits the best properties regarding the bitter taste. This is in good accordance with the results obtained at the dissolution tests, the slowest liberation rate was determined at the same sample.

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