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Study of β-blockers/β-cyclodextrins inclusion complex by NMR, DSC, X-ray and SEM investigation☆

R. Ficarra [b], P. Ficarra [a,*], M.R. Di Bella [a], D. Raneri [a], S. Tommasini [a], M.L. Calabrò [b], M.C. Gamberini [c], C. Rustichelli [c]

[a] *Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università di Messina, Vill. Annunziata 98168 Messina, Italy*
[b] *Facoltà di Farmacia, Università di Catanzaro, compl. Barbieri, 88021 Roccelletta di Borgia (CZ), Italy*
[c] *Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Modena e Reggio Emilia, Italy*

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Abstract

The formation of inclusion complexes between β-cyclodextrin with the two β-blockers, atenolol and celiprolol, have been studied in the aqueous environment and in the solid state by nuclear magnetic resonance (NMR) spectroscopy, X-ray, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) techniques. The magnitude of the chemical shifts of the interior and exterior β-cyclodextrin protons in the presence of each β-blocker indicated that these are included within the β-cyclodextrin cavity. In aqueous solution they form 1:1 complexes. In the solid state the formation of the β-cyclodextrin/atenolol (celiprolol) complexes is confirmed by X-ray, DSC and SEM, also employed to characterize pure substances and their physical mixtures.

Keywords: Atenolol; Celiprolol; β-Cyclodextrin; Nuclear magnetic resonance (NMR); Differential scanning calorimetry (DSC); X-ray powder diffractometry (XRD); Scanning electron microscopy (SEM)

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides with the ability to encapsulate entirely, or at least partially, into their hydrophobic cavity, a wide variety of guest molecules forming inclusion complexes [1].

The use of complexation of different drugs with CDs has been extensively studied in recent years with the aim of improving definite characteristics of pharmaceutical interest, such as solubility in aqueous media, dissolution rate, chemical stability and bioavailability [2].

This procedure has been evaluated for several series of pharmacologically active compounds. Attempts to compare the respective specific efficiencies of the free drug and the inclusion complex require a careful determination of the stoichiometry of the inclusion and the binding

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* Corresponding author. Fax: +39-90-6766407.
E-mail address: pficarra@pharma.unime.it (P. Ficarra)

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constants. In many cases different stoichiometries are reported for the same compound.

The β-blockers are characterized by a low solubility in aqueous gastric fluids and, hence, by a low dissolution rate [3] and interindividual variability in their bioavailability [4].

Nuclear magnetic resonance (NMR) techniques are already known as powerful tools for the characterization of inclusion complexes. Information provided by these methods includes: (i) confirmation of complex formation, (ii) calculation of the stoichiometry of the complex, and (iii) establishment of the geometry of the new chlatrate [5].

The aim of this work is to provide evidence that the β-blockers/β-CD inclusion complexes are formed and can be studied in the solid state as well as in aqueous solution, by using NMR, differential scanning calorimetry (DSC), X-ray and scanning electron microscopy (SEM) techniques.

2. Experimental

2.1. Materials

Racemic atenolol (AT) was supplied by Sigma-Aldrich Chemie (Germany), and racemic celiprolol hydrocloride (CE) was extracted by Selectol® (Rhone-Poulenc Rorer) tablets; its purity was confirmed by TLC. β-Cyclodextrin (β-CD) was purchased from Fluka Chemie (Switzerland).

All other materials were of analytical reagent grade. All solutions were prepared using distilled water and filtered by 0.22-μm Gelman Filters.

2.2. Apparatus

2.2.1. NMR

Proton NMR spectra were recorded at 25°C on a Varian Gemini 300 MHz spectrophotometer, in D_2O with 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS).

The chemical shifts are referred to DSS in D_2O used as external standard.

2.2.2. Differential scanning calorimetry (DSC)

The DSC curves of the different samples were recorded on a Perkin-Elmer DSC-4 differential scanning calorimeter calibrated with indium (3.21 mg, 99.99% pure, melting point 156.6°C) at heating rates of 10°C min^{-1}. The thermal behaviour was studied by heating 1–5 mg of samples in aluminium crimped pans under nitrogen gas flow over the temperature range 30–250°C.

Measurements were made in duplicate.

2.2.3. X-ray powder diffractometry (XRD)

Powder X-ray diffraction patterns were obtained with a Philips PW 1050/25 diffractometer system with Cu Kα radiation ($\lambda = 1.5418$ Å) over the interval $2–45°/2\theta$. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 20 mA; time constant, 4 s; angular speed 1° (2θ) min^{-1}; 1°, −0.1° and −1° slit; angular range $2° < 2\theta < 45°$.

2.2.4. Scanning electron microscopy (SEM)

The surface morphology of β-CD, β-blockers, their physical mixtures and their complexes were examined by Philips 500 scanning electron microscope. Each sample was fixed on a brass stub using double-sided tape and then gold coated in a vacuum by sputter coater Polaron E5100. Pictures were taken at an excitation voltage of 20 kV.

2.3. Preparation of samples

2.3.1. Preparation of the β-blockers/β-CD complexes

The AT or CE/β-CD complexes were prepared in distilled water using appropriate amounts of β-blocker and β-CD in a molar ratio of 1:1. The solution was shaken at room temperature for 24 h and then dried under reduced pressure.

2.3.2. Preparation of the physical mixture

The mode of preparation of the physical mixture was very simple. The calculated and exactly weighed (1:1 molar ratio) amounts of β-blockers and β-CD were pulverised in a ceramic mortar and carefully mixed.

3. Results and discussion

3.1. ^{1}H-NMR spectra

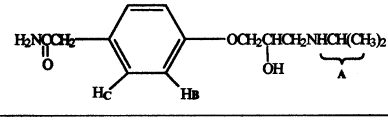
Insertion of a guest molecule into the hydrophobic cavity of a cyclodextrin results in modification of the NMR spectra of both the drug and the host molecule.

For NMR analysis two cases can occur:

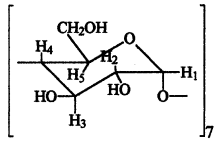
- the free and the complexed forms of a component originate separated signals on the NMR spectrum (i.e. a slow exchange exists between the free and bound states), or
- only shifts of the spectral lines are observed (fast exchange conditions) [6].

In this last case the observed value of $\Delta\delta$ may be used by a physical parameter related to the complex concentration, because it represents the chemical shift difference between the free and bound states.

Table 1
Chemical shifts (ppm) for the protons of atenolol and of β-cyclodextrin in the free state and in the pure complex (complex 1:1)

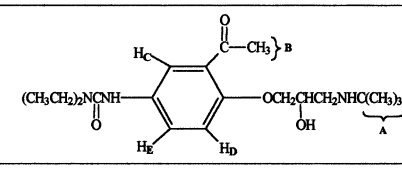


H Atenolol	δ_{free}	δ_c	Δδ (ppm)
H_A	1,182-1,161	1,069-1,048	0,113
H_B	7,281-7,252	7,135-7,108	0,131-0,144
H_C	7,015-6,987	6,867-6,838	0,148

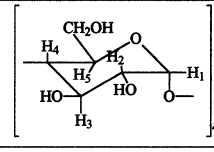


H β-CD	δ_{free}	δ_γ	Δδ (ppm)
H_1	4,946	4,925	0,021
H_2	3,522	3,505	0,017
H_3	3,830	3,782	0,048
H_4	3,448	3,429	0,019
H_5	3,726	3,654	0,072
H_6	3,758	3,726	0,032

Table 2
Chemical shifts (ppm) for the protons of celiprolol and of β-cyclodextrin in the free state and in the pure complex (complex 1:1)



H Celiprolol	δ_{free}	δ_c	Δδ (ppm)
H_A	1,420	1,264	0,156
H_B	2,663	2,519	0,144
H_C	7,591	7,448	0,143
H_D	7,437	7,290	0,147
H_E	7,122	6,984	0,138



H β-CD	δ_{free}	δ_c	Δδ (ppm)
H_1	4,946	4,915	0,031
H_2	3,522	3,493	0,029
H_3	3,830	3,780	0,050
H_4	3,448	3,415	0,033
H_5	3,726	3,678	0,048
H_6	3,758	3,719	0,039

The peak assignments of free AT and β-CD in the ^{1}H-NMR spectra are summarised in Table 1 and of free CE and β-CD in Table 2 where $\Delta\delta = \delta_{complex} - \delta_{free}$.

Assignment of all signals has previously been performed.

The spectra for AT (CE) in the presence of β-CD are compared with the spectra for the individual components and in both cases there are clear differences between the spectra with and without β-CD.

In our work only the H_3 and H_5 protons, located inside the cavity, and the H_6 proton, located on the cavity rim at the narrow end of the molecule, are appreciably shifted.

An upfield shift of the CD H_3 proton, joined to the appearance of the H_5 proton signal, which is overlapped with the H_6 signal, also indicating an upfield shift of these protons, as well as the minor modification observed for the H_1, H_2 and H_4 signals (Tables 1 and 2), indicates the entrance of

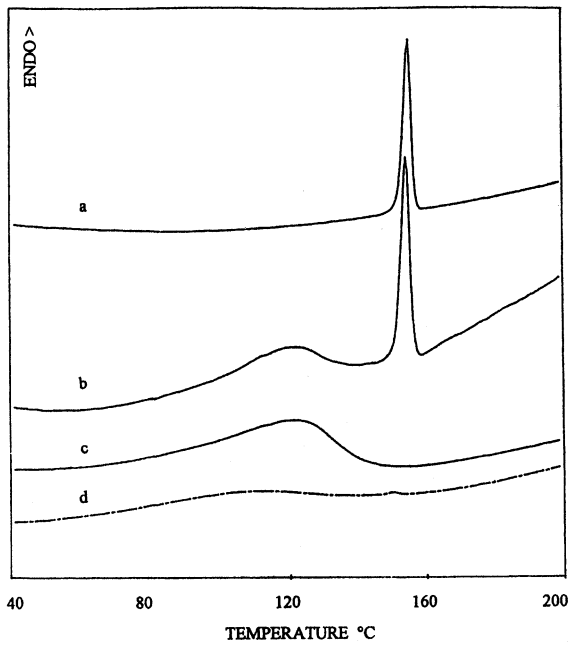


Fig. 1. DSC curves: (a) racemic atenolol, (b) equimolecular physical mixture of racemic atenolol and β-cyclodextrin, (c) β-cyclodextrin, (d) inclusion complex formed between β-cyclodextrin and racemic atenolol.

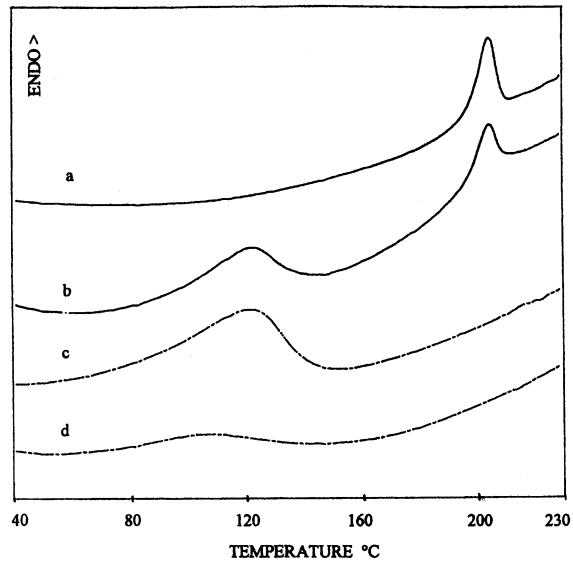


Fig. 2. DSC curves: (a) racemic celiprolol hydrochloride, (b) equimolecular physical mixture of racemic celiprolol and β-cyclodextrin, (c) β-cyclodextrin, (d) inclusion complex formed between β-cyclodextrin and racemic celiprolol.

group(s) of the drug molecule into the CD cavity and hence its complexation.

The mode of interaction of CD with the guest molecule can also be clarified. Upon complexation with the guest, methine protons (H_3 and H_5) located within the host β-CD cavity shift upfield because of the diamagnetic anisotropy of the included guest.

The order of the upfield shift is found to be for AT H_5 ($\Delta\delta$/ppm = −0.072) > H_3 (−0.048) > H_6 (−0.032) > H_1 (−0.021) ≅ H_4 (−0.019) ≅ H_2 (−0.017). The order of the upfield shifts is found to be for CE H_3 ($\Delta\delta$/ppm = −0.050) ≅ H_5 (−0.048) > H_6 (−0.039) > H_4 (−0.033) ≅ H_1 (−0.031) ≅ H_2 (−0.029). The shift in the proton located at the exterior of the torus (H_1, H_2 and H_4) is relatively small. Since the magnitudes of upfield shifts for H_3 and H_5 protons are very similar and they are too close in the complex for a separate NOE evaluation, the possible orientation of the β-CD ring remains uncertain, but it can be deduced that the molecule is included very deeply into the CD cavity.

3.2. DSC, XRD and SEM

The DSC curves of the racemic atenolol, the racemic celiprolol hydrochloride, the equimolecular physical mixture of atenolol (celiprolol) and β-cyclodextrin, with the same overall composition as the complex, the β-cyclodextrin, and the inclusion complexes between two β-blockers and β-cyclodextrin, are shown in Figs. 1 and 2. The differential scanning calorimetry curves of the raw materials (atenolol and β-cyclodextrin) compared with those obtained by coprecipitation confirm not only an interaction between the β-blockers and β-cyclodextrin, but also a real inclusion.

In fact, the formation of an inclusion complex was suggested by the absence of the melting endotherm of racemic atenolol at 152.68°C and of racemic celiprolol at 197.51°C in the DSC curves of the inclusion complexes (Figs. 1 and 2, curves d). The physical mixtures show, instead, two endothermic peaks, one for the β-CD (at 125.18°C) and one for the drug (atenolol at 152.68 and celiprolol at 197.51°C).

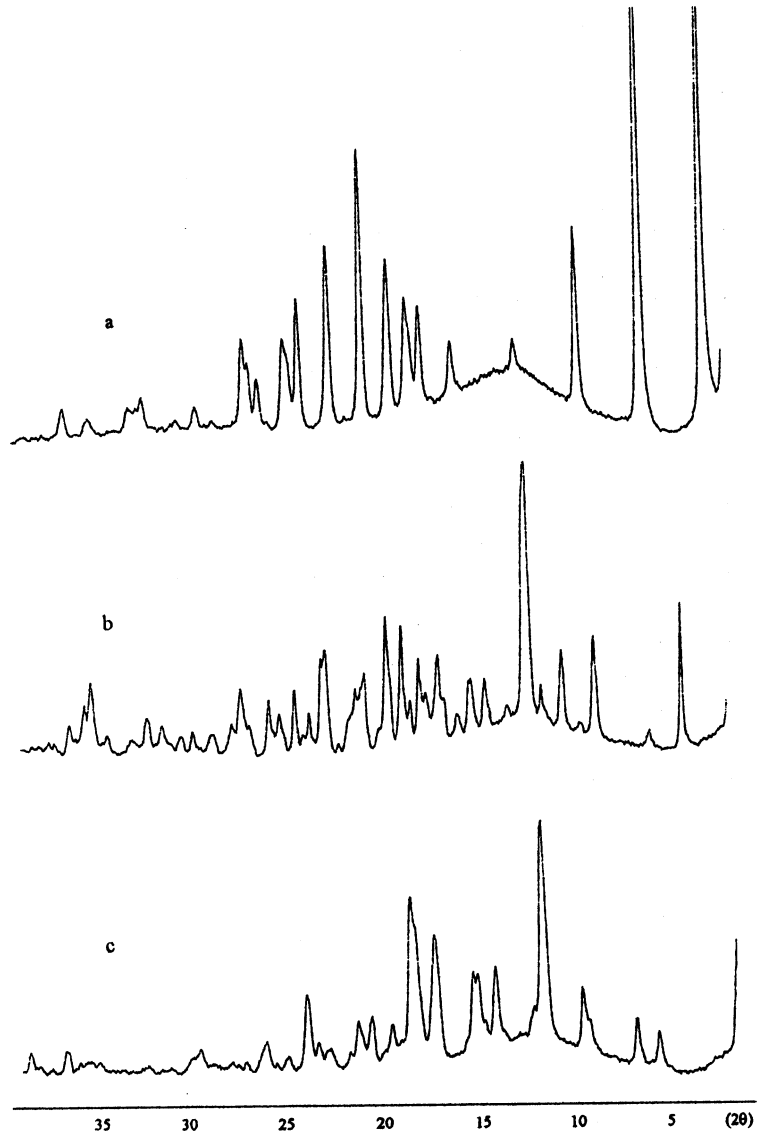


Fig. 3. X-ray diffraction profiles: (a) racemic atenolol, (b) β-cyclodextrin, (c) inclusion complex formed between β-cyclodextrin and racemic atenolol.

The powder X-ray patterns of the racemic atenolol, the β-cyclodextrin and the inclusion complex are shown in Fig. 3.

The X-ray diffractometry pattern of the physical mixture of atenolol and β-cyclodextrin is approximately the superposition of the patterns of the raw materials. On the other hand, the coprecipitate product has a completely different pattern in which it is no longer possible to distinguish the characteristic peaks of atenolol, thus confirming the existence of a new compound.

Characteristic high-intensity diffraction peaks were detected for each compound at: $2\theta = 2.2°$, 6.4°, 9.7°, 19.3°, 20.7°, 22.4° for racemic atenolol; $2\theta = 4.5°$, 9.0°, 12.6°, 18.9°, 19.7°, 22.7° for β-cyclodextrin; and $2\theta = 12.0°$, 15.7°, 17.6°, 18.7° for

the diastereomeric inclusion complex. The complete sets of the experimental values are listed in Table 3. Instead, the inclusion complex formed between β-cyclodextrin and racemic celiprolol hydrochloride was found to be amorphous (Fig. 4c). Thus the racemic atenolol and racemic celiprolol are completely included in the β-cyclodextrin to form a crystalline and an amorphous material, respectively, as also confirmed from SEM data (Figs. 5 and 6). Since each molecule of β-cyclodextrin can include in its cavity only one molecule of the racemic compounds, the 'inclusion complex' consists of an equimolecular mixture of the two diastereomeric complexes. In particular, the differences in the interplanar spacing, relative diffraction peak intensities and diffraction angles confirm that the inclusion complex for the atenolol possesses different crystalline structures.

4. Conclusions

All the data obtained from the NMR, DSC, X-ray and SEM studies showed that is possible to obtain the formation of an inclusion complex, in

Table 3
X-ray powder diffraction values

Atenolol			β-Cyclodextrin			Inclusion complex β-cyclodextrin-atenolol (1:1)			Celiprolol hydrochloride		
d (Å)	°2θ	I/I_0	d (Å)	°2θ	I/I_0	d (Å)	°2θ	I/I_0	d (Å)	°2θ	I/I_0
40.12	2.2	100	19.62	4.5	54	14.48	6.1	17	19.19	4.6	24
13.80	6.4	97	14.24	6.2	7	12.27	7.2	22	11.78	7.5	16
9.11	9.7	30	9.82	9.0	37	8.84	10.0	29	9.50	9.3	42
6.86	12.9	5	8.26	10.7	29	7.37	12.0	100	8.84	10.0	86
5.50	16.1	9	7.49	11.8	14	6.10	14.5	35	7.43	11.9	24
5.00	17.7	16	7.02	12.6	100	5.75	15.4	33	6.46	13.7	60
4.82	18.4	19	6.50	13.6	5	5.64	15.7	35	5.86	15.1	20
4.59	19.3	26	5.98	14.8	18	5.03	17.6	56	5.53	16.0	16
4.29	20.7	45	5.71	15.5	19	4.74	18.7	74	5.27	16.8	100
3.96	22.4	30	5.47	16.2	8	4.50	19.7	16	4.84	18.3	54
3.72	23.9	22	5.18	17.1	30	4.29	20.7	22	4.55	19.5	58
3.61	24.6	15	5.00	17.7	10	4.15	21.4	20	4.39	20.2	18
3.42	26.0	8	4.92	18.0	29	3.80	23.4	13	4.02	22.1	30
3.37	26.4	11	4.79	18.5	14	3.72	23.9	35	3.83	22.9	24
3.33	26.7	15	4.69	18.9	44	3.57	24.9	7	3.78	23.5	60
3.05	29.2	4	4.50	19.7	48	3.42	26.0	13	3.63	24.5	30
2.79	32.0	6	4.27	20.8	29	3.02	29.5	10			
2.58	34.7	3	3.91	22.7	37	2.47	36.3	10			
2.49	36.0	5	3.86	23.0	34	2.35	38.2	10			
			3.77	23.6	14						
			3.66	24.3	23						
			3.53	25.2	14						
			3.46	25.7	20						
			3.29	27.1	24						
			3.12	28.6	7						
			3.01	29.6	6						
			2.96	30.2	5						
			2.79	32.0	11						
			2.58	34.8	26						
			2.55	35.1	19						
			2.49	35.9	11						

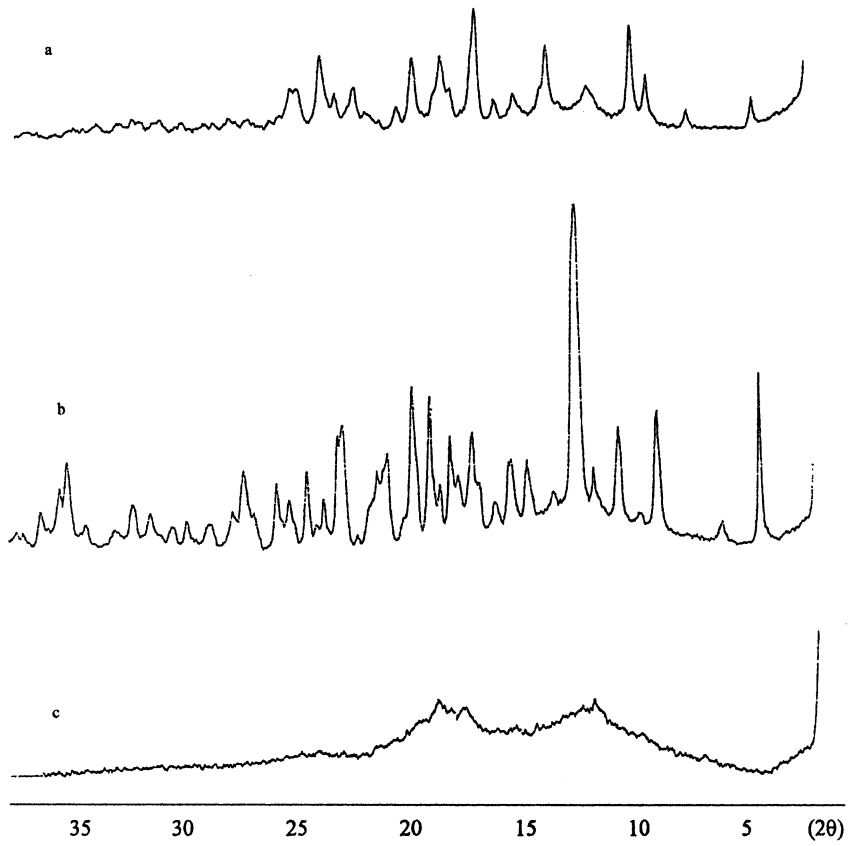


Fig. 4. X-ray diffraction profiles: (a) racemic celiprolol hydrochloride, (b) β-cyclodextrin, (c) inclusion complex formed between β-cyclodextrin and racemic celiprolol.

aqueous solution, between the cavity of β-CD and the β-blockers, atenolol and celiprolol. This behaviour supports the hypothesis of the formation of an inclusion complex that in a therapeutical formulation could improve the dissolution and subsequently the absorption of the drug.

Unfortunately the H_3 and H_5 shifts were too close in the complex for a separate NOE evaluation in the NMR study, and this clearly indicates a deep immersion in the cavity of the β-cyclodextrin. Since the magnitude of upfield shifts of both inner methine H_3 and H_5 protons is almost the same, the possible orientation of the β-CD ring remains uncertain, but much can be expected from the use of two-dimensional approaches.

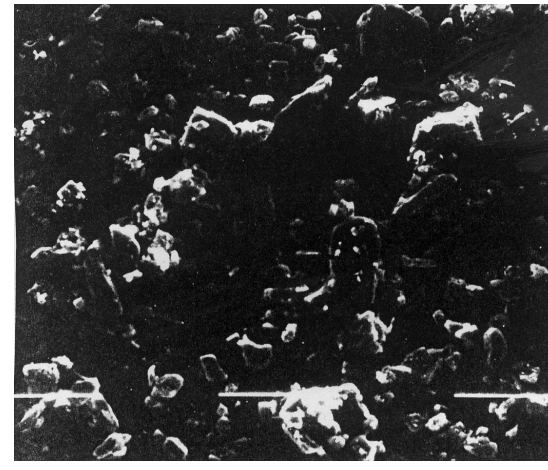


Fig. 5. SEM micrographs of β-cyclodextrin (bar, 100 μm).

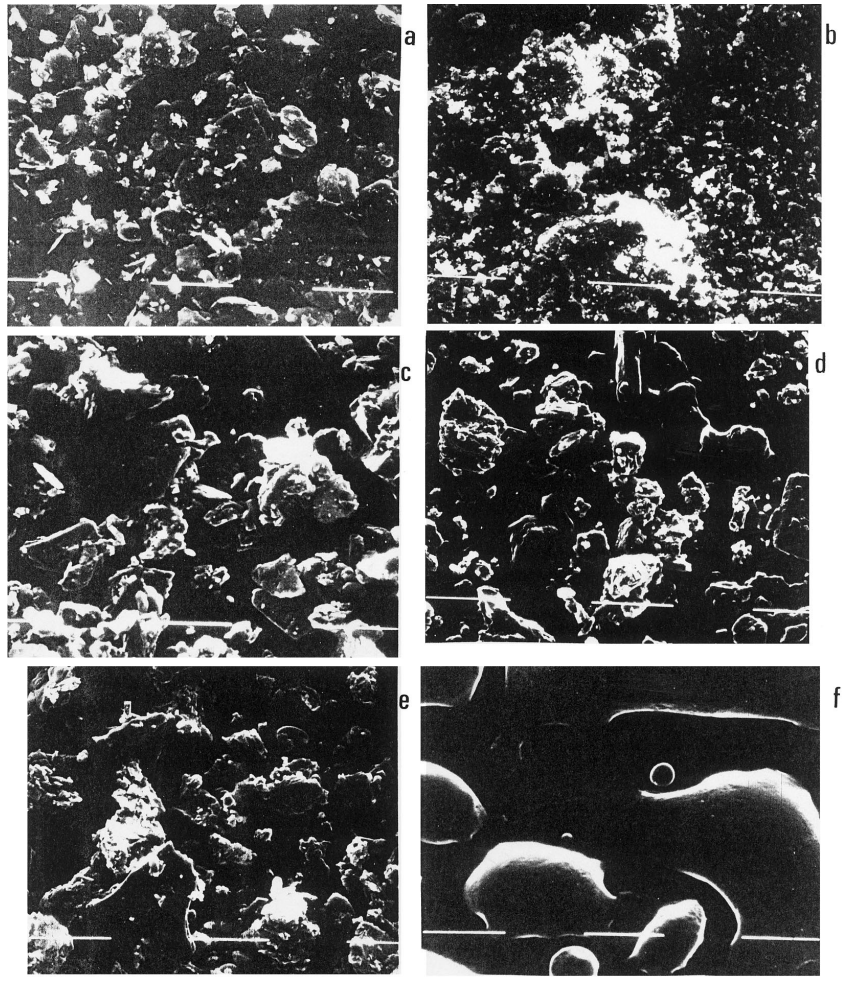


Fig. 6. SEM micrographs of pure substances and their physical mixtures and complexes (bar 100 μm); (a) pure atenolol, (b) pure celiprolol, (c) equimolecular physical mixture of racemic atenolol and β-cyclodextrin, (d) equimolecular physical mixture of racemic celiprolol and β-cyclodextrin, (e) inclusion complex formed between β-cyclodextrin and racemic atenolol, and (f) inclusion complex formed between β-cyclodextrin and racemic celiprolol.

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