Physico-Chemical Analysis of Binary Complexes of Furosemide and Randomly Methylated β-Cyclodextrin

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Furosemide is a benzoic acid derivative with a powerful diuretic activity used in the treatment of edemas and hypertension. Binary complexes of furosemide and randomly methylated β-cyclodextrin (RAMEB) were obtained by specific methods (physical mixtures, kneading, ultrasonication) in molar ratio of 1:1 and 1:2 and then analyzed by NMR spectroscopy, thermal analysis and in vitro membrane diffusion. The analysis of the inclusion complexes proved that RAMEB is capable of including the diuretic in its cavity, the final product having a better in vitro bioavailability. These results can be the first step in obtaining the new improved pharmaceutical preparations.

Keywords: furosemide, RAMEB, NMR spectroscopy, thermal analysis

Furosemide is a benzoic acid derivative [1] with a powerful diuretic activity used in the treatment of edemas and hypertension [2]. Its solubility in water being very low [3] it leads to a poor bioavailability, which can be improved by association with cyclodextrins [4]. Cyclodextrins are toroidal shape oligosaccharides with a cavity that can accommodate a large number of pharmaceuticals [5].

In previous papers [6, 7] we obtained binary and ternary complexes of furosemide with randomly methylated β-cyclodextrin (RAMEB) and polyvinylpyrrolidone (PVP) by specific methods (physical mixture, kneading, ultrasonication) in molar ratio of 1:1 and 1:2. The binary and ternary products were analyzed by in vitro dissolution tests, differential scanning calorimetry and X-ray diffraction.

In the present study the analysis of the binary complexes were continued by thermogravimetric analysis, NMR spectroscopy and in vitro membrane diffusion. The objective was to prove that the final products were real inclusion complexes.

Experimental part

Furosemide, 4-chloro-2[(furan-2-ylmethyl)amino]-5-sulphamoylbenzoic acid [8] (fig. 1) [10], was received as a gift from SC Terapia SA (Cluj-Napoca); RAMEB was purchased from Cyclolab R&D Ltd.(Budapest, Hungary).

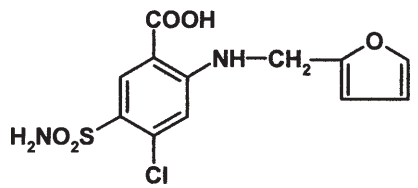

Fig. 1. Chemical structure of furosemide

Preparation of inclusion complexes

Products were prepared in 1:1 and 1:2 guest : host molar ratios. Three methods were applied in the preparation of the inclusion complexes:

-Physical mixture (PM): simple powder mixing using a mortar and a pestle, resulting the physical mixture of the two compounds;

-Kneaded product (KP): the physical mixtures were kneaded with 50% ethanolic solution until the bulk of solvent evaporated and then dried in the oven at 105ºC;

-Ultrasonication (US): the physical mixtures were dissolved in 50% ethanol, placed in the ultrasonic apparatus for 1 hour, dried and pulverized.

NMR spectroscopy

Solid state ^{13}C NMR spectra were recorded at 100 MHz ^{13}C Larmor frequency with a Bruker AVANCE-400 spectrometer. All NMR experiments were performed at room temperature. The sample was center-packed in zirconia rotors to minimize the effect of rf field inhomogeneity. Standard cross-polarization magic-angle-spinning (CP/MAS) [9, 10] experiments were performed at a spinning frequency ν_R = 8 kHz, using a ^1H 90^0 pulse length of 3.6 μs. The ^{13}C NMR spectra were acquired under two-pulse phase-modulated (TPPM) ^1H decoupling at 70 kHz by averaging 512 scans with a recycle delay of 3 s.

The CP transfer was optimized for the first Hartmann – Hahn matching condition ($\nu_{1C} = \nu_{1H} - \nu_R$), where the rf fields on the ^1H and ^{13}C channels have been calibrated to 50 and 42 kHz, respectively and the CP contact time was set to 1 ms for all the experiments. Carbon-13 chemical shifts are expressed in parts per million (ppm) and calibrated with respect to tetramethylsilane as described in the literature [11].

In vitro membrane diffusion

In vitro membrane diffusion tests were carried out with a Sartorius diffusion tester (Goettingen, Germany). 100 ml simulated gastric medium (SGM) containing 100 mg FS or the appropriate amount of binary complex were allowed to transfer through an artificial membrane to 100 mL simulated plasmatic medium (SPM), at 39°C for 150 minutes. Samples of 5 mL were taken at 30 min intervals from each fluid. The concentration of FS in both fluids was determined at 232 nm, with an Unicam UV/VIS spectrophotometer.

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Thermogravimetric and differential thermal analysis

The thermal analysis was accomplished by using the Derivatograph-C (MOM, Budapest, Hungary). The heating rate was 5°C/min and the temperature interval was between 25-300°C.

Results and discussions

NMR spectroscopy

In the ^{13}C CP-MAS NMR spectrum of furosemide the following chemical shifts (δ) were noticed: 36.8 ppm, 103 ppm, 108 ppm, 114 ppm, 124.4 ppm, 133 ppm, 139 ppm, 142 ppm, 148 ppm, 151 ppm and 170 ppm. In the carbon spectrum of RAMEB are present just four major peaks at: 57.2 ppm, 70.1 ppm, 80.5 ppm and 99.4 ppm. For furosemide complexes with RAMEB US 1:2 the following shifts (δ) were noticed in the ^{13}C spectrum: 16.3 ppm, 29.5 ppm, 40.9 ppm, 57 ppm, 70 ppm, 80.4 ppm, 99.1 ppm and 173.8 ppm (fig. 2).

After the insertion of furosemide into the cavity of RAMEB one can observe the the appearance of four new peaks at 16.3 ppm, 29.5 ppm, 40.9 ppm and 173.8 ppm.

The appearance of the new peaks which are not characteristic for the guest molecule as well as the shift down field of the cyclodextrin peaks proves there has been an interaction between the two substances suggesting the formation of an inclusion complex.

In vitro membrane diffusion

Membrane diffusion of FS was influenced by its inclusion in the cavity of RAMEB. By comparing the membrane diffusion of the 1:1 and 1:2 ratios, it is obvious that the optimum diffusion is achieved when using the 1:2 complex and the best preparation method is ultrasonication (fig. 3-5).

Thermogravimetric and differential thermal analysis

The data from thermogravimetric (TG, DTG) and differential thermal analysis (DTA) (fig.6 a-h) confirm the results obtained earlier by DSC.

Similarly to the calorimetric curves, thermogravimetric and differential thermal analysis show the appearance of an exothermic process of decomposition, with a loss of mass, around 220°C. For the binary complexes of furosemide with RAMEB the exothermic peak which indicates the decomposition of furosemide disappear and one can see a loss of mass around 260°C; at this temperature the decomposition of the entire sample takes place. The disappearance of the exothermic peak of furosemide proves that an inclusion complex was formed between the two substances.

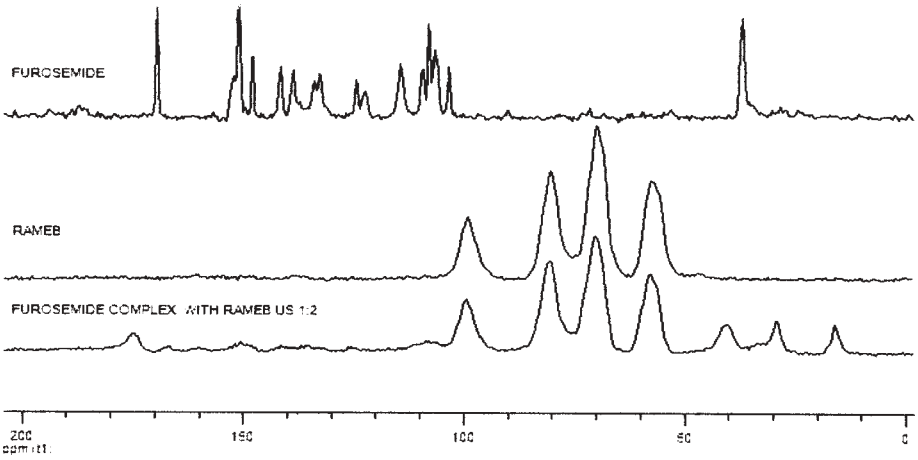


Fig. 2. NMR spectra of furosemide, RAMEB and their US 1:2 complex

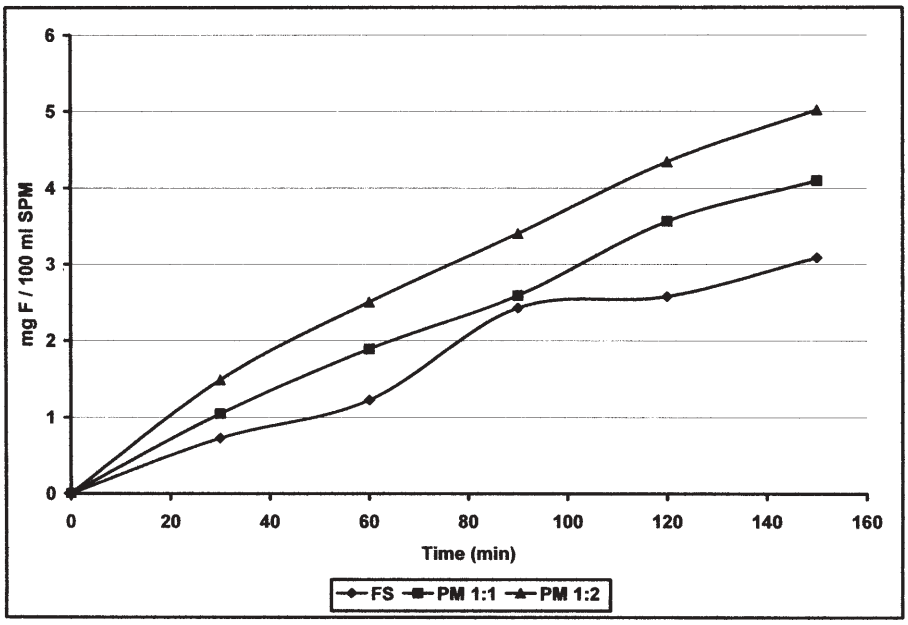


Fig. 3. In vitro diffusion of furosemide from PM products

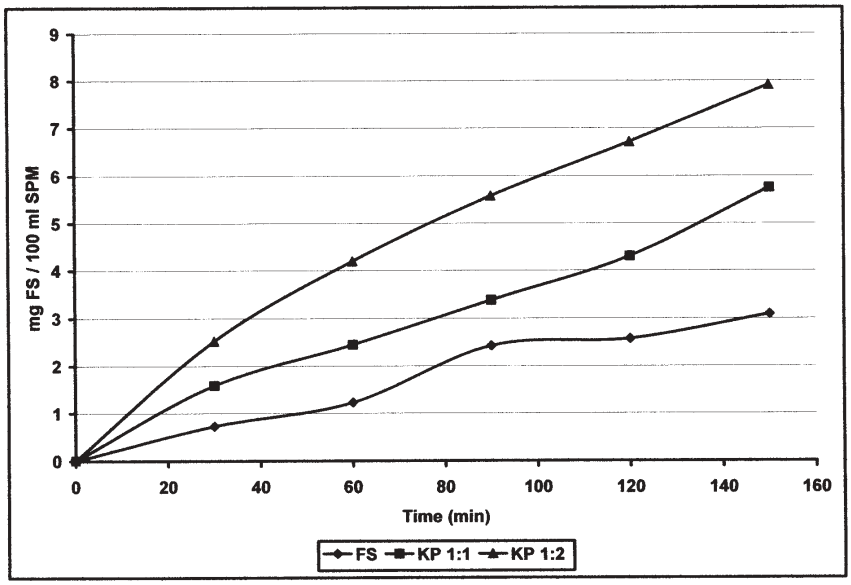


Fig. 4. In vitro diffusion of furosemide from KP products

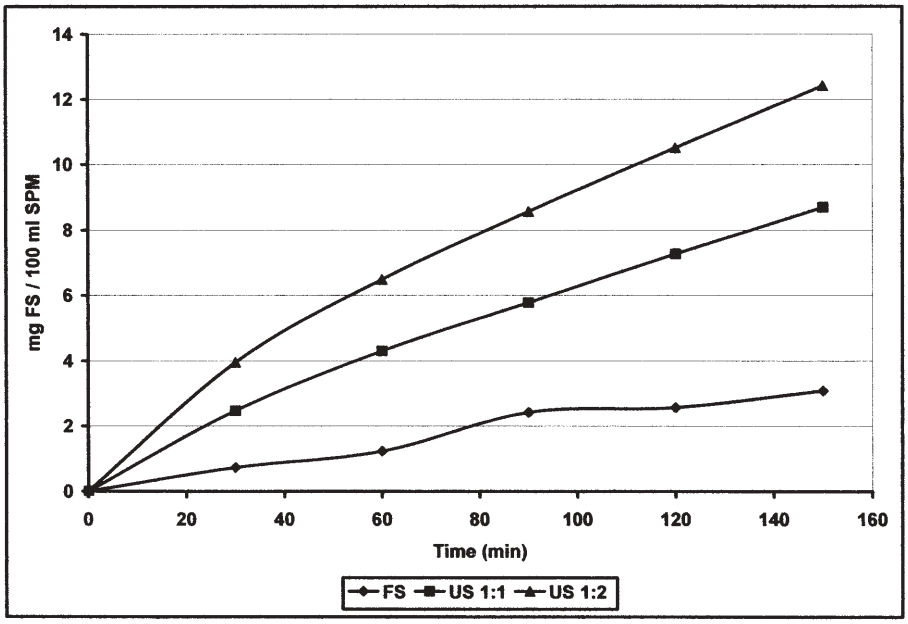
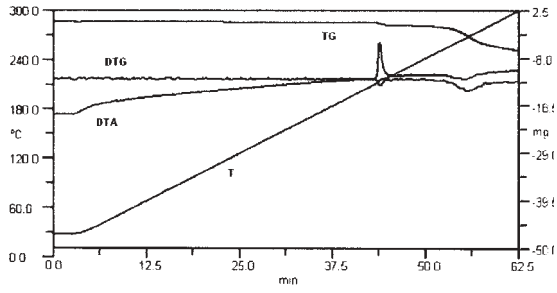
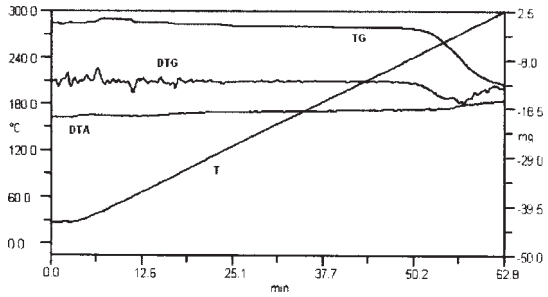


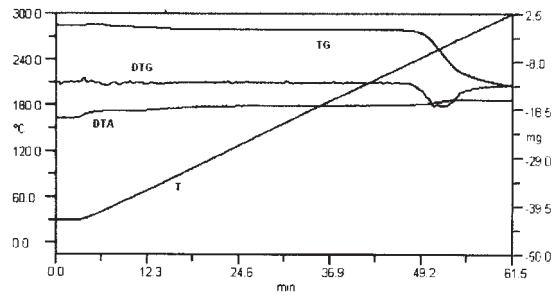
Fig. 5. In vitro diffusion of furosemide from US products



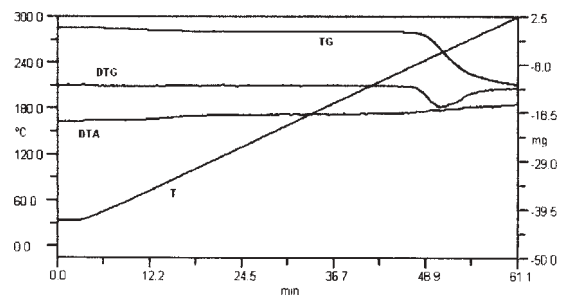
a. FS



c. FS + RAMEB PM 1:2

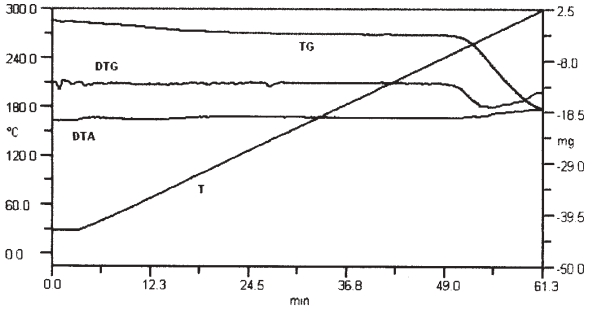


b. FS + RAMEB PM 1:1

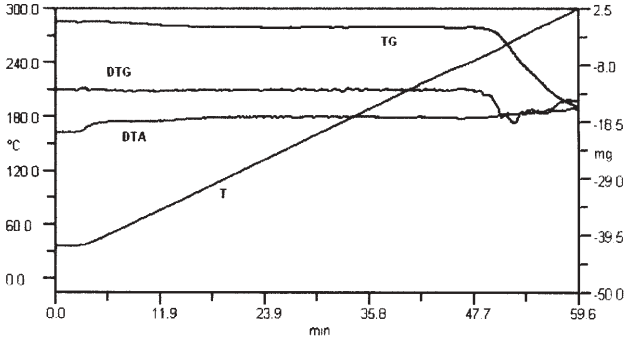


d. FS + RAMEB KP 1:1

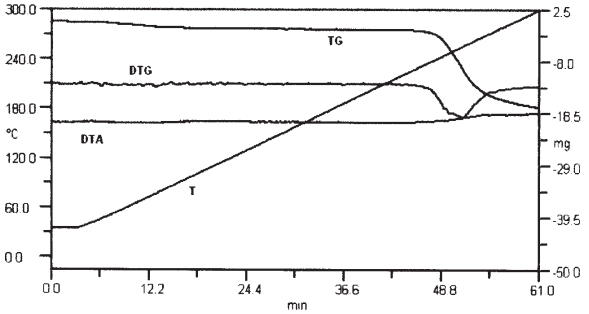
Fig. 6a-d. Derivatograms of furosemide and its binary products with RAMEB



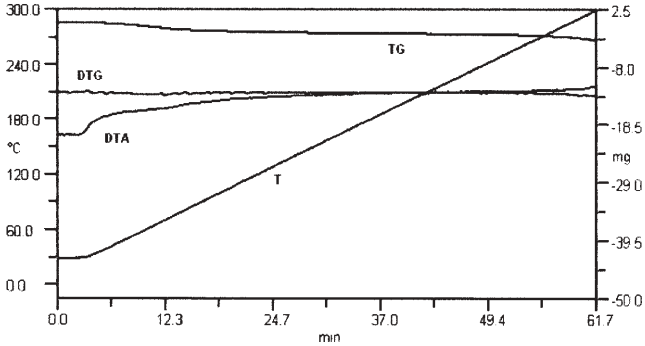
e. FS + RAMEB KP 1:2



g. FS + RAMEB US 1:2



f. FS + RAMEB US 1:1



h. RAMEB

Fig. 6 e-h. Derivatograms of furosemide and its binary products with RAMEB

Conclusions

NMR spectroscopy and thermal analysis show the appearance of an interaction between furosemide and RAMEB that can be the formation of a real inclusion complex. These results confirm earlier results concerning binary and ternary complexes of furosemide and RAMEB.

In vitro diffusion tests show an increase of the active substance diffusion through a semipermeable membrane, the best results being obtained by ultrasonication method in molar ratio of 1:2.

In conclusion, there is a possibility for obtaining future products with furosemide and RAMEB, with a better in vitro and in vivo bioavailability.

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