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International Journal of Pharmaceutics 187 (1999) 199–207

international journal of pharmaceutics

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Some physicochemical properties of 7-oxoacyl-L-alanyl-D-isoglutamines

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Received 22 September 1998; received in revised form 25 May 1999; accepted 25 May 1999

Abstract

N-(7-Oxoacyl)-L-alanyl-D-isoglutamines are substances with similar effects on immune system as *N*-acetylmuramyldipeptide (MDP). They were synthesized to eliminate some side effects of MDP: pyrogenicity, leucopenia, hypertension and fast elimination from the body. The aim of our work was to determine some physicochemical properties of a series of *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines, that have zero to six methylene groups between terminal methyl and 7-oxo group. The following properties were examined: water solubility, lipophilicity, thermal behaviour and true density. The results were compared and it was established that the lipophilic parameter determined by high-performance liquid chromatography is increasing by the increase of alkyl chain while the water solubility is decreasing at the same time. In both cases the substance with the longest chain (*N*-(7-tetrakanoyl)-L-alanyl-D-isoglutamine) is an exception. The lipophilic parameter of this substance is lower and water solubility higher than with the substances with three, four and five methylene groups between terminal methyl and 7-oxo group. These results can be explained by the twisted conformation of *N*-(7-tetrakanoyl)-L-alanyl-D-isoglutamine molecule in water solution. By means of thermal analysis it was discovered that the melting point is decreasing with the increase of alkyl chain. From the true density and melting enthalpy measurements it is evident that with the increase of alkyl chain the arrangement of molecules in solid state is increasing up to the molecule with four methylene groups between terminal methyl and 7-oxo group. Substances with longer chains have lower true density and melting enthalpy because of the different arrangements of the molecules. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Desmuramyldipeptide; Lipophilicity; 7-Oxoacyl-D-isoglutamines solubility; Thermal behaviour; True density

1. Introduction

An intensive search for immunostimulating drugs is in process. One of the leading compound is *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), which is the smallest immunomodulating glucopeptide. Replacement of *N*-acetylmuramyl moiety with 7-oxoacyl residue retains the immunomodula-

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PII: S0378-5173(99)00192-1

tive effect but, depending on its length, influences physicochemical properties. Thermal properties, solubility, partition coeffi-cient, etc., are important and deciding for partitioning through biological membranes, biological response (Sollner, 1993; Sollner et al., 1996) and for drug formulation. For the compounds that have a common parent structure and differ only in alkyl chain length, a linear change in physicochemical properties is expected. When not so, their behaviour is said to be exceptional. The goal of the scientists is to be able to predict breaks in physicochemical properties and to find explanations for their appearance.

Numerous studies of physicochemical properties for compounds, with common cyclic parent structure that differ only by length of alkyl side chain, have been done. Linear dependence in physicochemical behaviour of these substances was observed through alkyl chain length to 5 ± 1 carbon atoms (Yalkowsky et al., 1972; Forster et al., 1991). Interesting observations on how the chain length can affect properties such as solubility, wettability, partition and biological response have been reported by Buckton et al. (1991).

Less work has been done with aliphatic molecules. A linear decrease of hydrocarbons solubility by increase of chain length to eight carbon atoms was reported by Amidon et al. (1975). For nonbranched alcohols a nonlinear dependence of solubility decrease by increase of alkyl chain was observed (Amidon et al., 1975). The aim of this contribution was to correlate some physicochemical properties of homolog series of 7-oxoacyl-D-isoglutamines (Fig. 1) with the alkyl chain length.

2. Materials and methods

Six different *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines (Fig. 1) were synthesised at the Faculty of Pharmacy in Ljubljana. (Sollner, 1993). LK-450, LK-452 and LK-453 were crystallised from acetone and LK409, LK-451, LK-404 and LK-405 from methanol with addition of diethylether. All used chemicals were of pharmaceutical or analytical grade.

2.1. Solubility determination

The solubility of the homologs was determined in the purified water at 24 ± 0.5°C. Excess amount of the sample was added to 2 ml of water and subsequently agitated in a shaker for 24 h. The suspension was then filtered through a 0.20-μm membrane filter to remove undissolved particles. The adsorption of substances by the membrane was found to be negligible. After proper dilution with methanol the supernatant was submitted to HPLC analysis. Each solubility result is an average of at least three parallel measurements. The standard deviation was less than 5%.

2.2. Thermal analysis

A differential scanning calorimeter (DSC Perkin-Elmer DSC-4 Norwalk, CT, USA) was used to evaluate the transition temperatures and enthalpies. Indium was used as the standard and samples in amount of 2–5 mg in Al-pans were

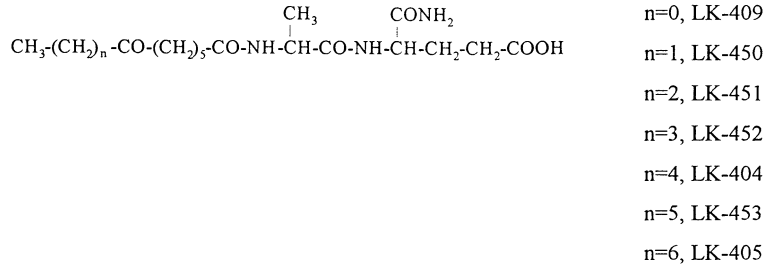


Fig. 1. Chemical structure of the investigated *N*-acyl-L-alanyl-D-isoglutamines.

heated under nitrogen atmosphere (40 ml/min). Empty Al-pans were used as a reference. Determi-nations were made at least in duplicate. A 1°C/min heating rate was used for all determinations. The standard deviation of temperature is inside of ± 0.5°C and for melting enthalpy $\pm$ 5%.

2.3. Lipophilicity calculation

Logharithmic values of the partition coefficient ($\log P$) were calculated according to the 'hydrophobic fragment constants' method of Rekker and Mannhold (1992). For the molecules where electronegative centers in the structure were separated with one methylene group, four magic constants were added. For aliphatic chains longer than two methylene groups, two magic constants were added (Plečnik, 1994).

2.4. Lipophilicity measurement

The $\log k_w$ values as hydrophobic parameters were determined by means of HPLC, consisted of Knauer pump 64, Knauer injector 0258 with 20-μl loop and LKB variable wavelength detector at 210 nm. Barspec Data System software was used for peak registration and calculation of the retention times. A Nucleosil C_{18} column, 5 μm, 120×4.0, was used as stationary phase. A methanol–phosphate buffer (pH 3.0) mobile phase was utilized at a flow rate of 1 ml/min and at 24 ± 1°C. The capacity factor was calculated as $k' = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times of the sample and potassium iodide, respectively, for different volume fraction of methanol in the mobile phase. Each chromatographic run was repeated at least five times. $\log k_w$ was calculated using the linear extrapolation technique.

2.5. True density determination

Accurate weighted samples were put into an AccuPyc 1330 helium pycnometer (Mycromeritics, USA). The measurement temperature was 24 ± 1°C. The density value is an average of at least three determinations.

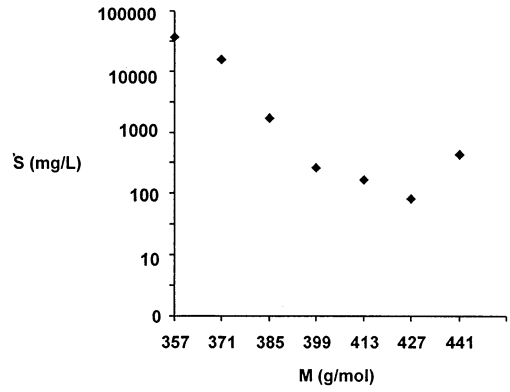


Fig. 2. Correlation between compound solubility and number of carbon atoms in the chain.

3. Results and discussion

The physicochemical properties of 7-oxoacyl-D-isoglutamines determined in the experiments are listed in Table 1. The water solubility of 7-oxoacyl-D-isoglutamines shows a falling trend by increase of chain length (Fig. 2) until LK-453 with $n = 5$. Surprisingly, the solubility of LK-405 ($n = 6$) is higher than the solubility of LK-452, LK-404 and LK-453 ($n = 3$, 4 and 5). For the systems that produce ideal solutions there is a correlation between the melting behaviour and solubility. For such systems one can predict the rise of solubility from decrease of melting point by lengthening of a side chain (Buckton et al., 1991). The basis for this is that, in order to dissolve, molecules must be removed from the crystal lattice (Mayer and Rowland, 1984). In the three-step solubility model, where solute/solute and solvent/solvent bonds are broken and solute/solvent bonds are formed, the melting points do not necessarily successfully predict the solubility. In our case the melting point decreased by lengthening of side chain parallel with the water solubility decrease until LK-404 (Fig. 2) (Srčič et al., 1996). These results are similar to those of Forster et al. (1991) who investigated *p*-hydroxybenzoates. Accommodation of solute in the solvent is most probably the main factor that influences solubility. The exceptional behaviour of LK-405 ($n = 6$) can be explained by a greater mobility of chain and/or its hydrophobic interaction with hydrophobic part of

Table 1
Some physicochemical properties of 7-oxoacyl-L-alanyl-D-isoglutamines

Compound label	n	M (g/mol)	Solubility (g/l)	Solubility (mol/l)	Calculated log P	Log K_w	T_m (K)	ΔH_m (kJ/mol)	ρ (g/cm^3)
LK-409	0	357	>37 500	>105	−1.117	1.47	432.8	46	1.29
LK-450	1	371	15 940	43	−0.160	2.03	448.9	56	–[a]
LK-451	2	385	1700	4.4	0.359	2.30	443.9	46	1.40
LK-452	3	399	262	0.66	0.878	2.62	426.8	52	1.39
LK-404	4	413	168	0.41	1.397	3.03	431.6	70	1.51
LK-453	5	427	86	0.20	1.916	3.45	427.6	43	1.46
LK-405	6	441	438	0.99	2.345	2.32	418.6	23	1.42

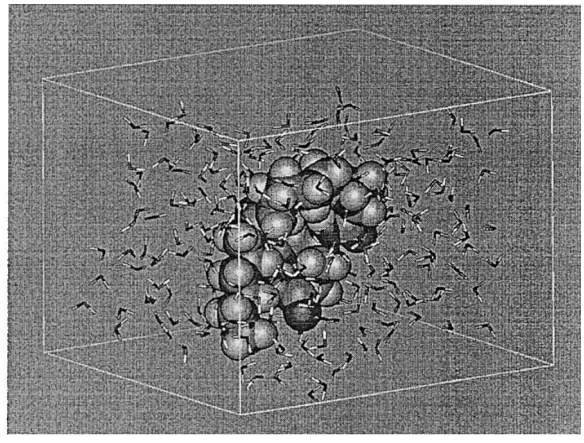
[a] Not determined.

the neighbouring LK-405 molecules. A possible explanation for the solubility being higher than expected could be also the formation of micelles. This result is similar to measurements of some other authors, who reported about the break of aqueous solubility at a certain length of carbon chain (Amidon et al., 1975; Mayer and Rowland, 1984; Forster et al., 1991). As reported by Forster et al. (1991) shorter chains have much less freedom of movement than larger ones which can twist and accommodate in the solute by the rotation of hydrophobic functional groups in the way that they are oriented to the water molecules with the smallest possible lipophilic surface.

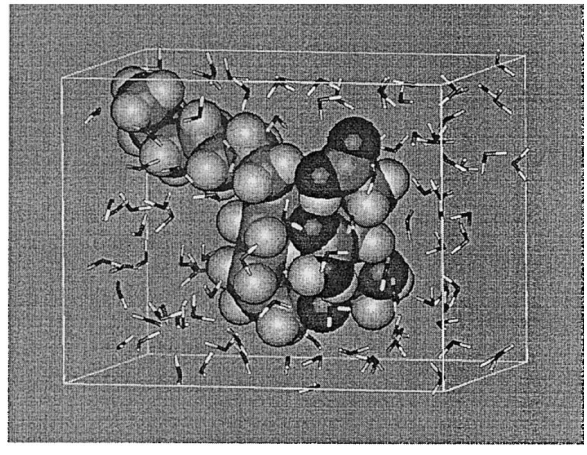
In order to elucidate the conformational change a computer conformational analysis of compounds with five and six methylene groups was carried out. The Monte Carlo approach was used (Šolmajer et al., 1999) and essential difference has been established between the stable conformers (Fig. 3). It seems that the opened conformation structure of molecule with six methylene groups enables the hydrophobic interaction with the other molecules of solute (increase of solubility), which is not the case with the more closed structure consisted of five methylene groups.

For the compounds with a common parent structure that differ only by side alkyl chain length, a linear change in lipophilicity is expected. Such a trend accords also with Rekker's mathematical model that predicts a rise of lipophilicity (log P) by lengthening of the side chain by a value of 0.519 for each methylene group (Table 1, Fig. 4). Taking in mind pK_a values of some analogues that were determined previously (LK-409, pK_a = 4.88; LK-451, pK_a = 4.93; LK-404, pK_a = 5.28; LK-405, pK_a = 5.49) (Plečnik, 1994) capacity factors (log K_w) were determined at water phase, pH 3.0. Log K_w values show the same trend as the Rekker model up to the molecule LK-453. LK-405 is not the most lipophilic as could be expected from its chain length and Rekker's simulation. This result is in good correlation with the unexpectedly high solubility of LK-405. For this reason it is assumed that orientation of the alkyl chain of the molecule in the solvent and potential association with other lipophilic chain (lipophilicity masking) is a good explanation for LK-405 lipophilicity decrease and solubility increase in comparison with shorter homologs. Higher solubility and lower lipophilicity of LK-405 could be the reason for its different partitioning through the biological barriers and consequently high immunorestoration activity which was reported by Sollner et al. (1996).

Fig. 5 represents the dependence of the true density on the alkyl chain length. By lengthening of the chain the true density increase is observed to the point, as we think, where closed structure of the molecule exists. Any further increase of



A



B

Fig. 3. Energy minimized structure of LK-453 (A) and LK-405 (B) found by the Monte Carlo procedure.

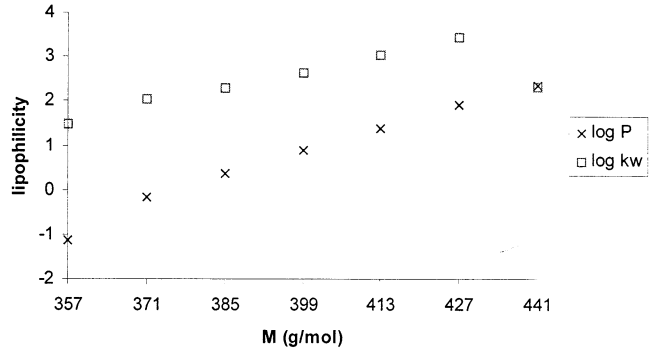


Fig. 4. Dependence of calculated log P and measured log K_w values from length of the molecule.

chain length causes a twist of the molecule side alkyl chain that influences the crystal packaging and, in our case, the decrease of true density. The highest density of LK-404 is in correlation with its highest value of melting enthalpy (Table 1) and the narrowest peak among all measured compounds (Fig. 6). Higher thermal conductivity of LK-404 allows a greater heat flux and most rapid melting process among all the tested LK substances (Hemminger and Höhne, 1984). The melting enthalpy of LK-405 is the lowest among all the homologs which additionally suggests its exceptional behavior in water. The decrease of melting enthalpy by increase of chain length from $n=4$ to $n=6$ corresponds with the decrease of melting point.

It is assumed that solid properties can also be a reflection of two different crystallization procedures that were used. Substances with even number n were crystallized from methanol with addition of diethylether and with odd number n from acetone. How the liquid from which the solid is precipitated can influence the thermal properties of the solid is seen from DSC curves (Fig. 6), where polymorphs were detected at most compounds that were precipitated from methanol with ether added (LK-409, LK-451, LK-405; Srčič et al., 1996). The exceptional behaviour of LK-404 where no polymorph was detected is in good correlation with its high true density what could be a reflection of most ordered structure of molecules in the solid state, in comparison to other investigated substances. Influence of the solvent used for precipitation, on thermal behaviour was investigated with LK-452 and LK-453. The samples originally crystallized from acetone were recrystallized from methanol with addition of ether. For both samples polymorphs were detected (shown in Fig. 7 for LK-453).

4. Conclusions

Physicochemical properties of the homolog series of N-(7-oxoacyl)-L-alanyl-D-isoglutamines that were determined in the liquid and solid phase show breaks in their trends. As a result of the substantial conformational structure change, LK-405 has higher solubility and lower lipophilicity than expected. Also, melting enthalpy and true density reveal a decrease as the length of the

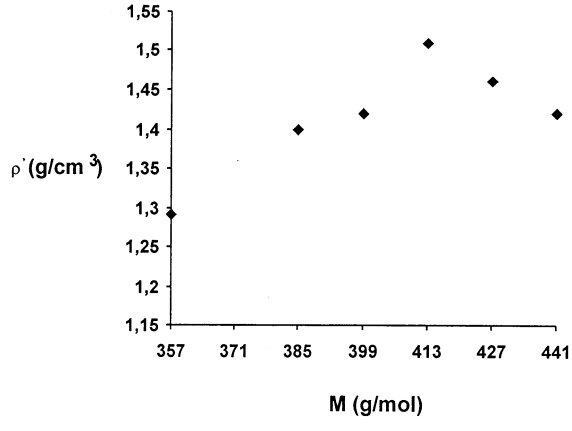


Fig. 5. Correlation between true density and length of the molecule.

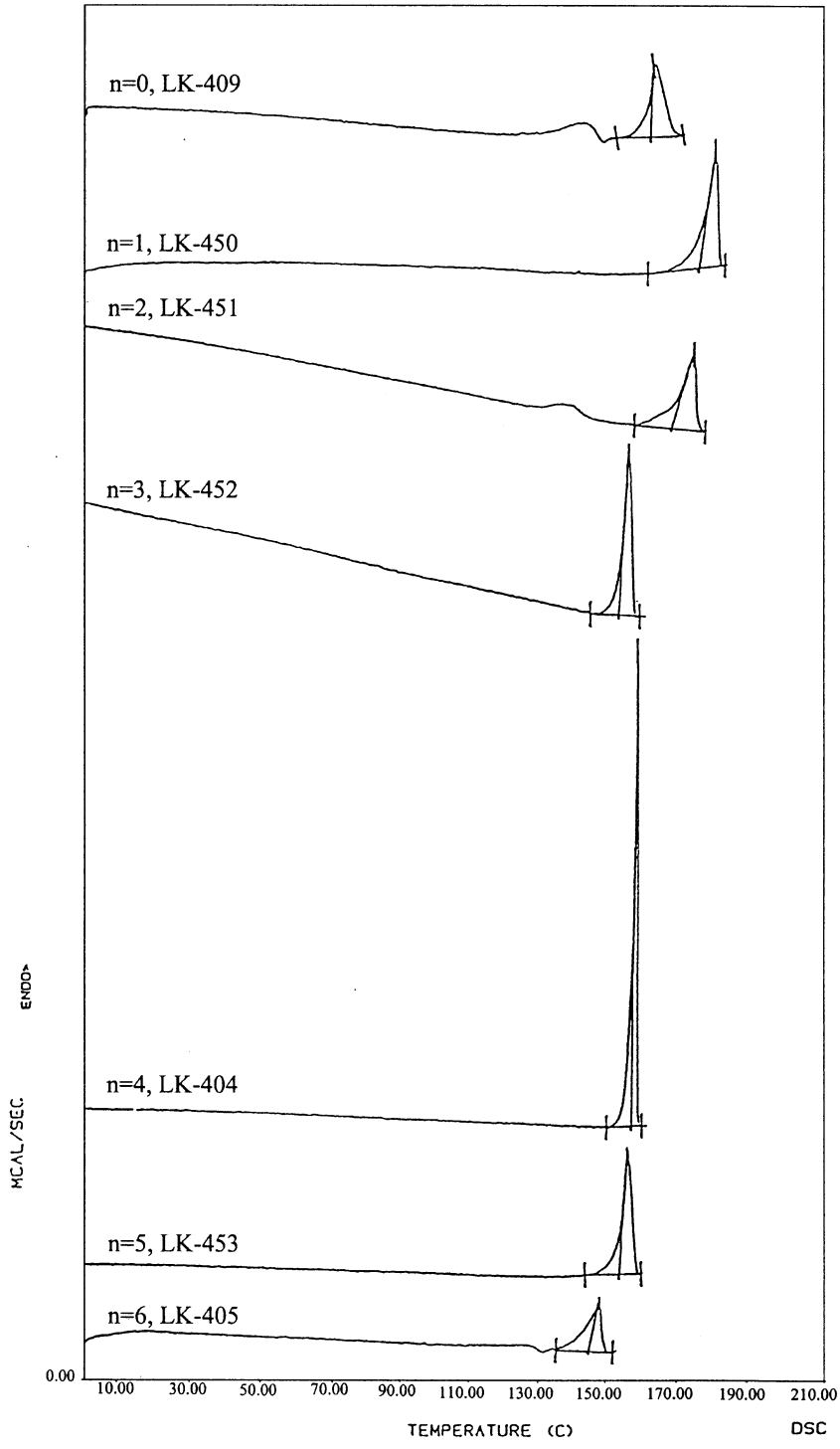


Fig. 6. Thermograms of investigated *N*-acyl-L-alanyl-D-isoglutamines.

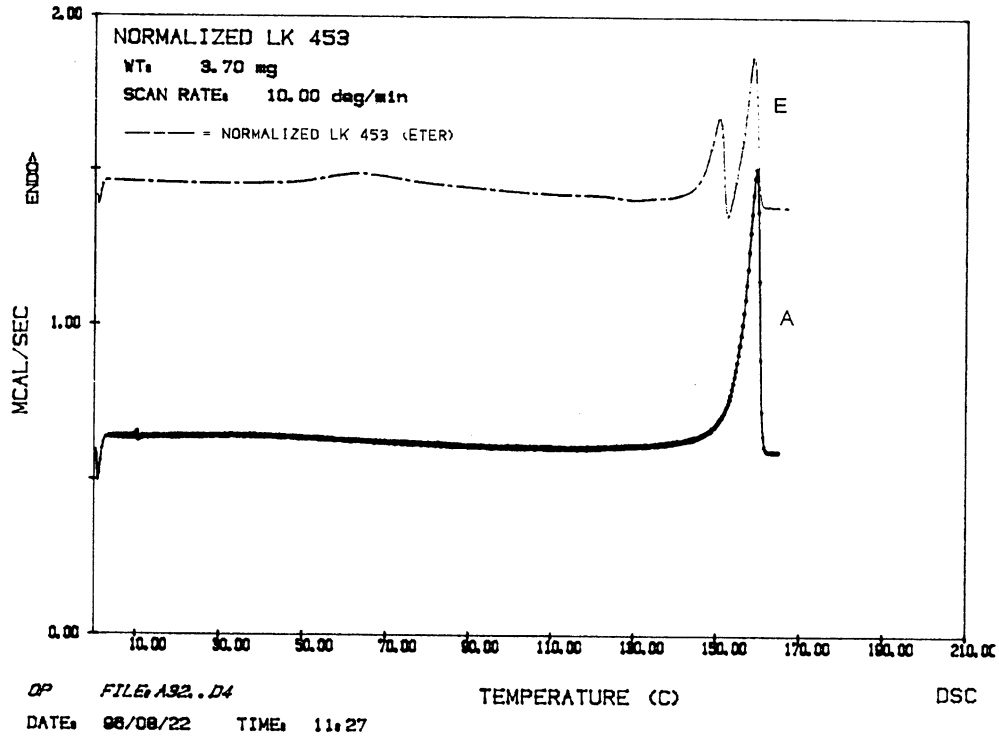


Fig. 7. Thermograms of LK-453 crystallized from acetone (A) and from methanol with addition of diethylether (E).

molecule increases. All the results should be considered in a quantitative structure–activity relationship, and will influence the decision of which compound will be used for further investigation.

Acknowledgements

We are grateful to Associate Professor Dr Marija Sollner and to Professor Dr Slavko Pecăr of the Faculty of Pharmacy, Ljubljana, and to Pharmaceutical Company Lek,d.d. Ljubljana for providing the substances used in this work. The useful collaboration of Professor T. Šolmajer is also acknowledged.

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