Benzodiazepines are the most often prescribed drugs [1] belonging to the most numerous group of continuously developed anxiolytic therapeutic substances. They are characterised by relatively low toxicity and weaker drug-dependence properties so that their withdrawal syndrome is milder than that of the classical sleep-inducing and sedative drugs [2]. Therapeutic substances based on 1,4-benzodiazepin-2-one derivatives are very slightly soluble or practically insoluble in water and they can be obtained only in the solid state form. From the biopharmaceutical point of view it is a drawback as in the liquid form the bioavailability of drugs is much greater and their pharmacological effect is thus much sooner [3,4].

The solubility of benzodiazepines can be enhanced by different means, e.g. by addition of solubilisers, which has been confirmed by the diazepam solubility study in the presence of propylene glycol, benzyl alcohol, benzoic acid, sodium benzoate, Tween 80, Cremophore EL, sodium lauryl sulphate and glycerophol [5,6]. The solubility of lorazepam, oxazepam, prazepam and temazepam has been improved in a similar way [7], while the solubility of diazepam, clonazepam, lorazepam and chlordiazepam has been improved in the presence of ethanol, propylene glycol and PEG 200 as co-solvents [8]. The presence of some other auxiliary substances such as gelatine [9] or lactose [10] has been also shown to improve the solubility of diazepam. Another approach aimed at improvement of the solubility of many 1,4-benzodiazepin-2-one derivatives, like oxazepam [11], diazepam [12], midazolam [13], alprazolam [14] is based on preparation of inclusion complexes with cyclodextrins.

Moreover, it has been shown that preparation of solid dispersions also increases the solubility of some benzodiazepines. Using PEG 6000 or Gelita Collgel as support of solid dispersions, an increase in the solubility and the rate of dissolution have been reported for oxazepam [15-17]. Similar effects have been observed when using Macrogol 6000 and introduction of Brij 35 to the system [18]. Attempts have been made at formulation of solid dispersions of oxazepam with D-mannitol [9]. When diazepam was used in solid dispersion in amylodextrin an increase in the solubility of the active substance in gastric juice and intestinal juice has been noted [19]. From among different Macrogols the best improvement in solubility has been obtained for PEG 4000 [20] and PEG 6000 [21]. For temazepam similar results have been reported for solid dispersions with PEG 6000 and PVP K30 [22,23].

The aim of this study was to improve the solubility of 1,4-benzodiazepin-2-one derivatives differing by substituents at positions 1, 3 and 7, in solid dispersions with PEG 6000, to enhance the parameters of the active substance release and absorption, hence its bioavailability.

**EXPERIMENTAL**

**Reagents and substances studied**

Diazepam (series G11001, Glaxo Smith Kline), nitrazepam (series G11001, Glaxo Smith Kline), oxazepam (series G06001, Glaxo Smith Kline), PEG 6000 (Merck).
Solid dispersions preparation

The solid dispersions were obtained by the melting method. The active substance was added to PEG 6000 molten in water bath and the mixture was stirred till solidification. The product was stored for 24 h in a dessicator, then the mass was refined and homogenized by sieving to the mesh size of 0.49 mm and 0.20 mm. The solid dispersions contained 10, 20, 30 and 40% of the active substance.

Physical mixtures of 1,4-benzodiazepin-2-one derivatives studied and the support PEG 6000 containing 10, 20, 30 and 40% of the active component were obtained by sieving through the mesh size of 0.49 mm and 0.20 mm.

Identification of solid dispersions obtained

X-ray diffraction study

The solid dispersions, physical mixtures and pure therapeutically active compounds were subject to X-ray diffraction study by the powder method, using the monochromatic Cu Kα (λ=1,5418 Å) radiation with Ni filter. The diffraction patterns were recorded in the angular range 2θ < 40°.

Differential scanning calorimetry

The obtained solid dispersions, physical mixtures and pure therapeutically active substances were analyzed also by the differential scanning calorimetry (DSC). Portions of 2 mg of the substance studied and the standard (Al2O3) were placed in the heated head of the DSC apparatus. The measurements were carried out in the range from 20 to 450°C, at the rate of temperature increase 10°C/min in nitrogen atmosphere.

Determination of the contents of diazepam, nitrazepam and oxazepam in solid dispersion with PEG 6000 by the spectrophotometric method

The UV spectra of the 1,4-benzodiazepin-2-one derivatives in the 0.1 mol/dm3 HCl solution in substance and in the presence of PEG 6000 were recorded in the range of 200-400 nm. No absorbance was observed for PEG 6000 in the HCl solution at the analytical wavelengths characteristic of the active compounds studied (diazepam 240 nm, nitrazepam 280 nm, oxazepam 240 nm). Therefore, the method is specific and the presence of the support does not affect the results of determinations. The concentration of the 1,4-benzodiazepin-2-one derivatives in 0.1 mol/dm3 HCl solution was determined by use of a Cary 118 Varian UV-vis spectrophotometer.

The accuracy and precision of the spectrophotometric method of determination of the compounds studied were also established. The overall percent recovery of diazepam, nitrazepam and oxazepam in the presence of PEG 6000 were 102,7%, 101,8% and 102,4% with relative standard deviations of 1,49%, 0,85% and 0,75%, respectively.

Determination of solubility

The weighted portions of the therapeutically active substance, its physical mixtures with the support and solid dispersions were placed in flasks of 50 cm3 in capacity, and 15 cm3 of distilled water was added to each flask. The contents were shaken at ambient temperature for 24 h. The residual precipitate was centrifuged, dissolved in 0.1 mol/dm3 HCl and subjected to determination of the compounds analyzed by the spectrophotometric method at the wavelength characteristic of each particular compound. The results are collected in Table 1.

DISCUSSION

The results proved the presence of crystals of the derivatives studied in all solid dispersion samples (Figure 1). The diffraction peaks characteristic of particular active compounds studied were clearly visible although their intensity was lower than in the diffraction patterns of the corresponding physical mixtures. The above observations indicate that in the process of formation of solid dispersions by the melting method a solid state solution of the therapeutically active substance in PEG 6000 is obtained.

The results were interpreted on the basis of a comparison of thermograms of solid dispersions, physical mixtures and pure components of the dispersions and the mixtures. The thermogram of PEG 6000 revealed two endothermic peaks: one at about 62°C related to the melting of this substance and the other at 406°C related to its decomposition. The DSC curves of diazepam and oxazepam show a single endothermic peak each, at 134°C and 260°C, respectively, corresponding to the melting point of these compounds. The DSC curve of nitrazepam has two peaks: the endothermic one at about 230°C related to the melting of this compound and the exothermic one at about 287°C related to its decomposition. The DSC curves of the physical mixtures containing 10, 20 and 30% of diazepam show two endothermic peaks at about 62°C and 406°C, assigned to PEG 6000, whereas the curve of the physical mixture containing 40% of diazepam reveals an additional endothermic peak at about 134°C characteristic of diazepam. The DSC curves of the solid dispersions of diazepam with PEG 6000 are similar. The DSC curve of the solid dispersion
containing 40% of diazepam reveals two endothermic peaks at about 62°C and 406°C assigned to PEG and the endothermic peak at about 134°C assigned to the melting of diazepam, but the latter peak is lower than the corresponding one in the thermogram of the physical mixture of the same components at the same concentrations. The DSC curves of the physical mixtures and solid dispersions of nitrazepam with PEG 6000 containing 10, 20, 30 and 40% of nitrazepam show the endothermic peaks at about 62°C and 406°C assigned to the melting and decomposition of the support and the exothermic peak at about 287°C characteristic of nitrazepam. The exothermic peak at ~287°C is more intensive in the DSC curves of the physical mixtures than that of the solid dispersions of the same compositions. The DSC curves of the physical mixtures and solid dispersions of oxazepam and PEG 6000 containing 20,

Figure 1. X-ray diffraction patterns: a - PEG 6000, b - diazepam, c - physical mixture 10%, d - solid dispersion 10%, e - physical mixture 20%, f - solid dispersion 20%, g - physical mixture 30%, h - solid dispersion 30%, i - physical mixture 40%, j - solid dispersion 40%.
30 and 40% of oxazepam reveal three endothermic peaks at 62°C and 406°C assigned to the support and at about 206°C assigned to oxazepam. With increasing content of oxazepam both in the physical mixture and solid dispersion, the endothermic peak at about 206°C corresponding to the melting of the active substance becomes more intensive.

This peak was also greater for the physical mixtures than those for solid dispersions of the same composition. The thermograms of the physical mixture and solid dispersion containing only 10% of oxazepam show only the endothermic peaks related to the presence of PEG 6000, at about 62°C and 406°C.

The melting method was also used by other authors to obtain solid dispersion of oxazepam with PEG 6000 (15-18). Based on our results it was proved that this method is very useful to prepare solid dispersions for other derivatives of 1,4-benzodiazepin-2-one: diazepam and nitrazepam.

Solid dispersion influence on the solubility of the investigated compounds. Solubility of selected 1,4-benzodiazepine-2-one derivatives increases in presence of PEG 6000. Further increase of solubility is caused by making solid dispersions with this carrier (Table 1).

### CONCLUSIONS

1. The analyzed 1,4-benzodiazepine-2-one derivatives partly dissolve in PEG during preparations of solid dispersions.
2. Solubility reached with solid dispersions was higher than that of physical mixtures for each proportion of PEG in the samples.

### REFERENCES