Optimization of Diazepam Solubility with Auxiliary Substances and Thermo Analytical Investigation of Binary Systems

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Diazepam is one of the most frequently prescribed anxiolytics in psychotherapy. However, its solubility characteristics are very unfavorable. To improve these, different auxiliary substances like cyclodextrin derivatives and non-ionic macromolecular materials can be used. The binary systems between diazepam and auxiliary substances were prepared in four molar ratios, and by several methods (mixing, kneading, co-precipitation and spray drying) and the solubility and the dissolution rate were studied. All binary systems showed superior dissolution compared to pure diazepam. Differential scanning calorimetry and thermogravimetric analysis were used to characterize solid state interactions between diazepam and the auxiliary substances. Various kinetic parameters, as activation energy, virtual reaction order, pre-exponential factor were calculated. Taking in consideration the kinetic and thermodynamic parameters, the solubility results are in correlation with the stability order of the binary systems. The thermoanalytical investigation demonstrates that the thermic stability of diazepam is increased by the inclusion complexes. The thermoanalytical analysis sustains the hypothesis of formation of partial inclusion complexes between diazepam and cyclodextrins.

Keywords: diazepam, cyclodextrins, solubility, differential scanning calorimetry, thermogravimetric analysis

Diazepam (DZ) (7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2-one) (fig. 1) is an important member of the group of 1,4-benzodiazepine derivatives, which exerts anxiolytic, sedative, muscle-relaxant and anticonvulsant effects. However, its solubility characteristics are unfavorable as the solubility in water is 1.6228×10^{-4} mol·L^{-1} at room temperature [1]. DZ bioavailability, limited by its very poor water-solubility, could be improved by cyclodextrin complexation.

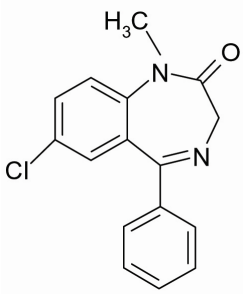

Fig. 1. Chemical structure of diazepam

Cyclodextrins (CDs) are water-soluble cyclic oligo-saccharides derived from starch, composed of α-1,4-linked d-glucopyranose units. As a result of their molecular structure, with hydrophilic exterior surface and hydrophobic cavity interior, CDs possess a unique ability to form inclusion complexes with many drugs. Between the native α-, β-, and γ-CDs which contain six, seven and respectively eight glucose units, the seven glucose unit containing β-CD with an inner cavity diameter of 6.0–6.5 Å and a depth of 7.9 Å is the most widely used, because of its excellent ability to incorporate hydrophobic aromatic rings [2,3]. Solubility enhancement and improved inclusion ability of the native β-CD can be achieved by derivatization such as methylation, hydroxyalkylation, sulfation or sulfoalkylation on the 2-, 3- and 6-hydroxyl groups. CDs characteristic cyclic structure and physico-chemical properties allow the inclusion of other guest molecules; this process is sometimes referred to as encapsulation [4].

CD complexation may improve some physicochemical properties of drugs, such as stability and water solubility and consequently also their bioavailability [5]. Due to these particular properties, CDs can encapsulate a variety of hydrophobic molecules or moieties inside their cavity through non-covalent interactions. The driving forces to form inclusion complexes are electrostatic, van der Waals and hydrophobic interactions as well as hydrogen bonding [6,7].

Thermoanalytical techniques (differential scanning calorimetry, thermogravimetry) are frequently used in the investigation of the thermal properties of CDs and their inclusion complexes [8,9].

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference material is measured as a function of temperature, as the two specimens are subject to identical temperature regimes in a heated or cooled environment at a controlled rate. DSC is a frequently preferred thermal analytical technique because of its ability to provide detailed information about both the physical and energetic properties of a substance.

An indication of the host-guest molecular interaction, provided by DSC analysis, is the disappearance of the melting endotherm of the guest in the DSC thermogram of the binary system, as well as the shifting of the melting peak of the guest. The melting enthalpy is an indication of the amount of guest not involved in the interaction with the CD [10].

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability

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and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated.

Comparative analysis of TGA curves of simple compounds, as well as binary systems, offers important recognition data of possible interactions and qualitative and quantitative elucidation of their mechanism.

Many processes of pharmaceutical interest such as complexation can be described in terms of changes of the Gibbs free energy. The standard free energy change is related to the activation enthalpy and entropy. Several driving forces have been proposed to be important for the specific affinity of ligand molecules (CDs) for the guest molecules (DZ) [11,12].

In previously published articles inclusion complexes of 13 benzodiazepines with 3 CDs (α-, β-, γ-CDs) in aqueous solution and in the solid phase were studied by solubility methods, spectroscopy (UV, IR), thermal analysis, and X-ray diffractometry, and their modes of interaction were assessed. The importance of the hydrophobicity of the guest molecule and the spatial relationship between host and guest molecules were clearly reflected in the stability constant magnitude of the inclusion complexes [13].

Inclusion complexation of DZ with different CD derivatives was used for the in the development of parenteral formulation; DZ solubility was enhanced linearly as a function of each studied CD concentration [14]. The same procedure was applied for the development of parenteral formulations with other benzodiazepine derivatives like lorazepam [15]. Complexes of nitrazepam with different CDs were prepared and characterized to study the effect of complexation on its dissolution rate; the complexes were characterized by DSC, Fourier-transform infrared, scanning electron microscopy and powder X-ray diffraction studies, indicating that a complex prepared by lyophilization had successful inclusion of the nitrazepam molecule into the CD cavity, which resulted in a marked improvement in the solubility and wettability of the substance [16].

An NMR spectroscopic study was published in order to evaluate the complexation of midazolam with β-CD, the results confirmed the formation of an inclusion complex in aqueous solutiom [17]. Complexes of clonazepam prepared with different CDs by various methods such as kneading, coevaporation, and physical mixing were characterized by Fourier transform infrared spectroscopy and DSC studies; the effect of complexation on the dissolution rate of clonazepam was studied [18].

The purpose of this work was the investigation of the solubility increasing effect of different CD-derivatives, polyethyleneglycol (PEG) 4000 and polyvinylpyrolidone (PVP); selection of the proper complex forming agent; and also to examine the interaction in solid state between DZ and CD derivatives and non-ionic hydrophilic macro-molecular materials respectively using thermoanalytical techniques.

Experimental part

Materials

DZ was kindly provided by Gedeon Richter Ltd. (Tîrgu Mureº, Romania), while the CDs were purchased from Cyclolab Ltd. (Budapest, Hungary): native β-, γ-CD and also the derivatized random-methylated-β-cyclodextrin (RAMEB) were used. Polyethylene glycol 4000 (PEG) was acquired from Rohm Pharma (Germany) and polyvinylpyrrolidone M=25000 (PVP) was provided by LobaChemie (Wien, Austria). All the chemical reagents were of analytical grade.

Preparation of the binary systems

We chose methods which provide high yield. DZ and CD were weighed in terms of different molar ratios (1:1, 2:1,2:3) based on the results of the phase solubility studies.

Physical mixtures (PM): the components were mixed in a mortar and sieved through a 100μm sieve.

Kneaded products (KP): physical mixtures of DZ and CDs derivatives were wetted in a ceramic mortar with a 50% v/v ethanol in water solution. The obtained paste was kneaded until the bulk of the solvent had evaporated. After drying at room temperature and then in the oven at 105°C, the KP were pulverized and sieved through a 100 μm sieve.

Spray-dried binary system (SD): the mixture of DZ and CD was dissolved with constant stirring using 50% v/v ethanol in water. This mixture of solutions was sonicated for 15 min. The resulted solution was subjected to spray drying (Niro Minor Atomizer apparatus, Denmark) using the following work conditions: temperature inlet 100ºC; temperature outlet 67ºC; rotational speed of 25000 / min, pressure: 3.6 atm. The product thus obtained was collected, packed in aluminum foil and stored in desiccator for further studies.

Co precipitation (CP): PEG respectively PVP was dissolved in 96%ethanol through heating. DZ was added to the solution, heating continuously the solution in order to evaporate the ethanol. The dried products were removed, pulverized, and passed through a 100 μm sieve.

Dissolution studies

In vitro dissolution studies of DZ, physical mixtures, kneaded products, spray-dried binary system and co-precipitation complexes were performed using a SR 8-PLUS Hanson-Research (USA) dissolution test station by adding the solid systems, equivalent to 10 mg of DZ, to 900 mL of water thermostated at 37 ± 0.5°C, and stirred at 100 rpm. At fixed time intervals, samples were withdrawn with a filter-syringe (0.45μm) and assayed spectro-photometrically (Shimadzu UV-1601, Japan) at 229 nm. The aliquot withdrawn at each time interval was replaced with the same volume of fresh dissolution medium. All the experiments were run in triplicate.

Differential scanning calorimetry (DSC)

The temperature and enthalpy measurements were performed using a Mettler Toledo DSC 823e Thermal Analysis system (Schwerzenbach, Switzerland). Approximately 1-2 mg of active material or binary systems placed in sealed aluminum pans was examined from 25 to 400 °C in a nitrogen atmosphere. The mass of empty pan and reference pan were taken into account for calculation of heat flow. The heating rate was 10°C/min.

From the DSC curves, the decomposition temperature (onset temperature), the transition temperature range (endset temperature), and the total calorimetric enthalpy change were calculated.

The relative degree of crystallinity (RDC) of DZ in the PM, KP and SD samples, as a percentage of the DZ mass fraction, was calculated according to the relation [19]:

$$DZ_{RDC} = 100 * \frac{\Delta H_{binary system}}{\Delta H_{uncomplexed DZ * c}}$$

where DZ_{RDC} is the uncomplexed guest %; $\Delta H_{binary\ system}$ is the normalized integral value for the product;

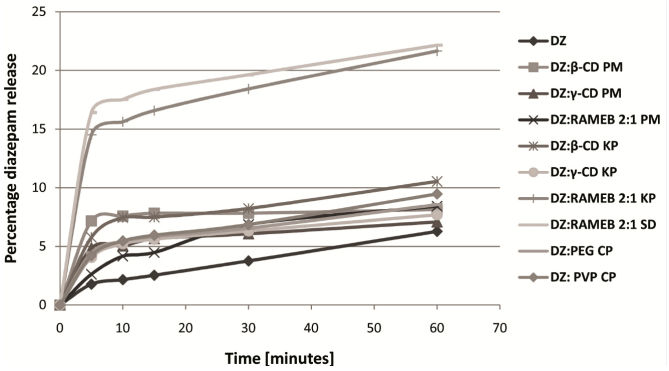

Fig. 2 Comparative dissolution profile of DZ and its products with auxiliary substances

$\Delta H_{uncomplexed\ DZ}$ is the normalized integral value for the active ingredient; and c is the percentage of active ingredient in the product.

Thermogravimetric analysis (TGA)

The thermogravimetric analysis was performed using a Mettler Toledo 851e equipment (Schwerzenbach, Switzerland) between 25-500 °C in a nitrogen atmosphere, with a heating rate of 5°C/min and the thermogravimetry (TG), differential thermogravimetry (DTG), and differential thermal analysis (DTA) curves were determined. The temperature (T) curve shows the linear increase of temperature during the process.

In the first step, the total kinetic parameters were calculated. The calculated kinetic parameters were the following: the reaction order (n), the activation energy (Ea) and the pre-exponential factor (A). The value of n (reaction order) is allocated by the Kissinger method [20] and it is the first kinetic parameter calculated by the computer:

$$n = 1.26S^{1/2}$$

where S is the form factor which presents the absolute value of the gradients of DTG curves in the points of min/max. The activation energy (Ea) is determined according to the natural logarithmic form of the Arrhenius-equation:

$$k(T) = Ae^{-Ea/RT}$$

which is widely used in the literature [21].

From the kinetic data and thermodynamic parameters were calculated: entropy, enthalpy and Gibbs free energy (activation free enthalpy).

The thermodynamic parameters: the Gibbs free energy (ΔG^*), the activation enthalpy (ΔH^*) and the activation entropy (ΔS^*), can be derived from the temperature dependence of the apparent stability constant of the CD complex [12].

The calculation of the activation entropy (ΔS^*) for the complex can be done by using the expression :

$$\Delta G^* = \Delta H^* - T\,\Delta S^*\ [12].$$

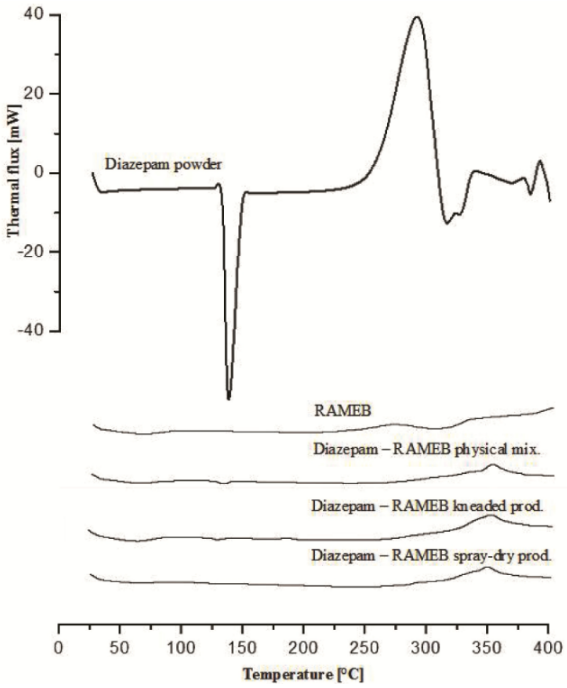

Fig. 3 DSC thermograms of DZ and its mixtures with RAMEB

Results and discussions

Dissolution studies

The increases in the dissolution characteristics depend on the nature of the CD derivative, the DZ concentration in the products and on the processing method. The various CD derivatives increased the solubility of DZ to different extents, RAMEB proving to be the most effective; the best results being obtained when using a DZ:RAMEB 2:1 spray-dried binary system. PEG 4000 and the PVP can be also useful in 3:7 molar ratio.

Differential scanning calorimetry

Differences in the thermal behaviour of DZ and DZ-CDs, DZ-PEG, DZ-PVP inclusion complexes were evident. As shown in figure 3 DZ exhibits a characteristic endothermic fusion peak at 133 °C and ΔH of 80 J/g, corresponding to the DZ melting point. The stability of DZ was not affected (no degradation observed) up to 240°C. Furthermore, β-, γ-CD and RAMEB show broad endothermic events in the range from 30 to 95 °C, which are related to the loss of adsorbed water, and small endo- or exo- effects at 210–325 °C due to thermal degradation. DSC thermograms of the physical mixture for DZ and β-, γ-CD show the existence of the endothermic peak of DZ indicating weak interaction between substances. The DZ peak in the physical mixture with RAMEB is reduced, indicating a more intense interaction of DZ with RAMEB. The DSC thermograms of freeze-dried complexes show disappearance of the diazepam endothermic peak at 133°C. This could be attributed to the formation of an amorphous solid

Sample	Tpeak (°C)	ΔH (J/g)	DZ_{RDC} (%)
DZ	133	-80	100.00
DZ - β PM 1:1	130	-50.2	62.75
DZ - β KP 1:1	129	-30.1	37.63
DZ - γ PM 2:3	130	-40	50.00
DZ - γ KP 2:3	129.1	-15.4	19.25
DZ - RAMEB PM 2:1	130.47	-20.32	25.40
DZ - RAMEB KP 2:1	129.10	-14.10	17.62
DZ - RAMEB SD 2:1	125.6	-4.20	5.25

Table 1
RESULTS OF DSC ANALYSIS OF DZ AND OF BINARY SYSTEMS WITH CD_s AND THE RDC OF DZ IN THE BINARY SYSTEMS

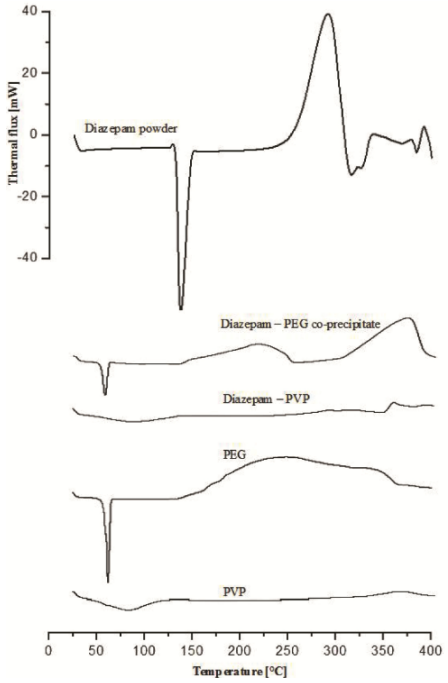

Fig. 4 DSC thermograms of DZ and its mixtures with PEG and PVP

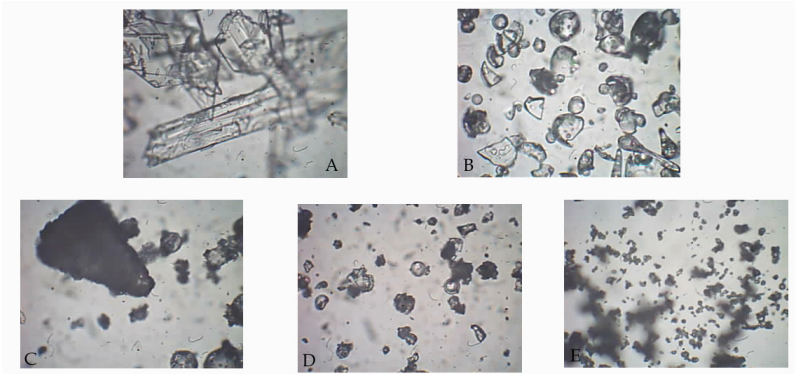

Fig. 5 Photomicrographs of DZ and its mixtures with RAMEB (A – DZ, B – RAMEB, C – DZ:RAMEB PM, D – DZ:RAMEB KP, E – DZ:RAMEB SP)

dispersion, molecular encapsulation of the drug into the CD cavity, or both.

The DSC thermograms of co-precipitate complexes show disappearance of the diazepam endothermic peak at 133°C (fig. 4).

The melting temperatures, the specific melting enthalpies and the RDC values determined from the DSC analysis of the binary systems are presented in table 1.

Shifts of the melting temperatures of the binary systems to smaller values, and decreases of the melting enthalpies

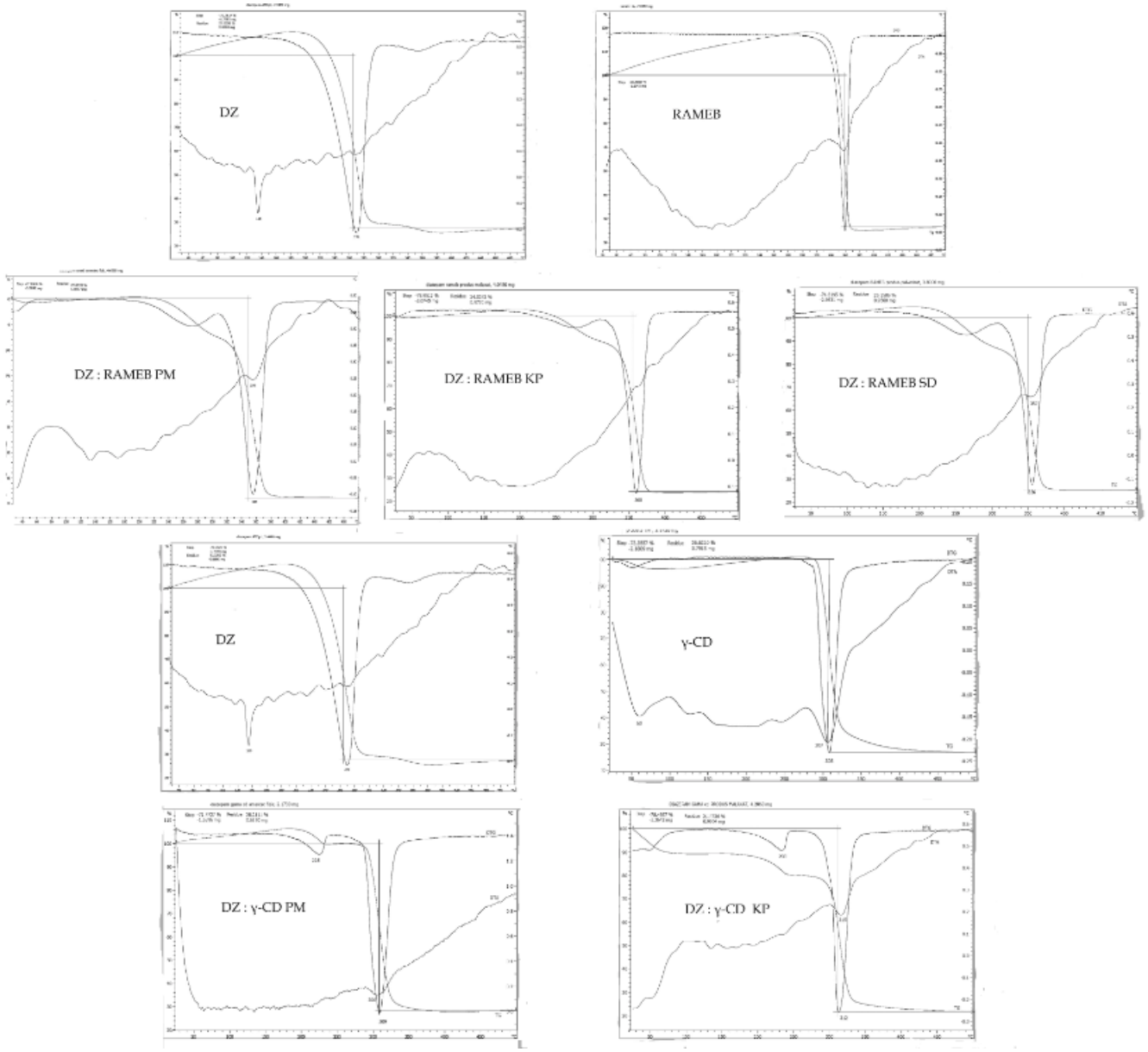

Fig. 6. Thermogravimetrical profile of Diazepam, RAMEB, γ-CD and their products

Table 2

No	Studied system	Virtual reaction order n	Activation energy E [kJ/mol]	Pre-exponential factor lg A	Activation enthalpy ΔH* [kJ/mol]	Activation entropy ΔS* [J/mol*K]	Gibbs free energy (activation free enthalpy ΔG* [kJ/mol]	Decomposition rate constant k [1/s]
1	Diazepam powder	1.7849	69814.74	5.738	-2407846.66	-143.46	-2365094.39	3.16694E-07
2	β-CD	1.2179	118898.85	9.731	-2338762.55	-66.97	-2338805.58	7.76848E-12
3	γ-CD	1.3857	157093.81	13.302	-2320567.59	1.43	-2320993.48	5.83588E-15
4	RAMEB	1.9326	266873.60	21.389	-2210787.80	156.34	-2257376.76	4.07812E-26
5	Diazepam - β-CD PM	0.9347	129986.12	11.200	-2347675.28	38.84	-2359249.60	2.60224E-12
6	Diazepam - β-CD KP	1.0590	157351.10	14.817	-2320310.30	30.44	-2329382.23	1.72048E-13
7	Diazepam - γ-CD PM	1.1801	173997.55	15.783	-2303663.85	48.96	-2318253.75	1.92508E-15
8	Diazepam - γ-CD KP	1.3652	212460.76	18.865	-2265200.64	107.99	-2297382.87	4.2106E-19
9	Diazepam - RAMEB PM	1.4537	199884.82	17.275	-2277776.58	77.53	-2300881.69	1.73199E-18
10	Diazepam - RAMEB KP	1.4837	203705.46	16.421	-2273955.94	61.16	-2292182.84	5.17926E-20
11	Diazepam - RAMEB SD	1.6164	268785.60	22.351	-2208875.80	174.76	-2260954.27	1.72579E-25
12	Diazepam - PEG CP	1.5622	179918.79	13.613	-2297742.61	7.38	-2299942.06	1.1911E-18
13	Diazepam - PVP CP	1.5262	186118.37	16.375	-2291543.03	60.29	-2309510.06	5.64259E-17
14	PEG	1.7692	147445.78	10.611	-2330215.62	-50.12	-2315281.29	5.83978E-16
15	PVP	1.4645	116931.38	7.792	-2360730.02	-104.12	-2329702.08	1.97584E-13

with decreasing of the DZ amount in the physical mixture is noticeable.

In the case of kneaded products a decrease of melting temperatures and melting enthalpies was observed. Also we noticed the low melting temperature and enthalpy in the case of RAMEB spray-dried binary system. These phenomena may be an index of the dispersion of the crystals of guest molecule in the matrix of CDs, and the formation of highly energetic crystals of DZ. DZ preserves a low degree of crystallinity in the KP and SD systems.

DZ, a crystalline substance, is formed by particles with shape and size; its 2:1 complexes show a significant change of morphology, presenting irregular particles, usually aggregated, which indicates an amorphous product resulted following complexation (fig. 5).

Thermogravimetry

TG, DTG and DTA curves of DZ and its coplexation products are presented on figure 6. The temperature (T) curve shows the linear increase of temperature during the process. Comparing the thermal curves of DZ, CDs, PEG, and PVP it exposed interactions between the active and the host molecule. The thermal curves evidenced the formation of inclusion complexes. The inclusion complexes symmetry of the DTA curve is strongly modified due to overlapping of several endothermic processes.

As shown in table 2 the kinetic and thermodynamic parameters of DZ with auxiliary substances thermic investigation, the activation energy of the inclusion complexes is higher than the one of the pure substance. The thermic behaviour depends both on the preparation method and the composition of the product.

Conclusions

The DSC sustains the hypothesis of partial inclusion complexes formation between DZ and CDs, suggested by the shifting of the melting peak of the guest molecule and the reduction of the entalpies values. The activation energy of the inclusion complexes is higher than the one of the pure substance one.

The thermic behaviour depends both on the preparation method and the composition of the analyzed product.

On the basis of activation energies and constants of the decomposing rate, the stability of DZ is influenced by the CD and the macromolecular substances as the following: DZ:RAMEB SD > DZ:RAMEB KP >DZ:γ KP> DZ:RAMEB PM> DZ:PEG CP>D:ZPVP CP> DZ.

The determined enthalpies for the interaction of DZ with CDs clearly prove the interaction was endothermic. The positive value for ÄS* can be explained in term of hydrophobic effect, involving the breakdown and removal of the structured water molecules inside CD cavity and around the hydrophobic substrate (DZ).

The thermoanalytical studies demonstrate the applicability of CDs and macromolecular substances for optimization of solubility of DZ and emphasize the fact of complex forming in the products.

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