Solid-phase polycondensation of aspartic acid
1. Kinetics of the process as evidenced by TGA and DSC data

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The TGA and DSC methods were used to investigate the process of thermostimulated polycondensation of aspartic acid, which was found to follow two parallel pathways. This apparently is due to the existence of two spatial regions, where the chemical transformation can take place. The first region ("potential") already exists before the reaction begins, whereas the second ("dynamic") is formed during the reaction progress. An autoaccelerated reaction is the first step in each of the pathways. The autoacceleration is likely to result from the reaction of the starting monomer with the reaction products. Some kinetic parameters were evaluated from experimental data. The rate constants of autocatalytic reactions in the two regions differ more than by an order of magnitude. This is the reason for very complex shape of the TGA and DSC isotherms. The kinetic curves in the differential forms have two maxima. The effective activation energy values for the reaction in the reaction regions considerably differ. According to the TGA and DSC data, they are 31.6/37.5 and 46.6/43.6 kcal mol^{-1}, respectively, for the approximate analytical and accurate computer methods of the experimental data processing. Excessively high values obtained for polycondensation suggest that the reaction proceeds in the solid phase.

Key words: aspartic acid, polycondensation, thermogravimetry, differential scanning calorimetry.

A large body of studies was done on polycondensation of amino acids. Polymers of this class of compounds are of significant importance in both chemical engineering (for example, in production of polyamides) and chemistry and biochemistry. In the present work, L-aspartic acid (Asp) has been chosen as an object for the study. The process of its polycondensation has been studied in details.[1] However, in the works published priority is given to the different methods of polycondensation and effects of the reaction conditions on the composition and properties of the products.[2] Based on the results of such studies, conclusions on the reaction mechanism are drawn. The works on kinetics are as yet scarce in mechanistic investigations. Such attempts have been undertaken only comparatively recently.[3—5] An assumption[3—5] has been made on a two-step process: polyaspartic acid is formed first, than, after dehydration of polyaspartic acid, polysuccinimide (PSI).

The purpose of the present work is to study kinetics of thermal polycondensation of aspartic acid in the solid phase. Since study of kinetics of the solid-phase chemical processes requires understanding structural changes, this reaction has been studied by X-ray scattering in our preceding works.[6,7]

Experimental

The object for the study of kinetics of thermal polycondensation in the temperature range 170—228 °C is L-aspartic acid (Asp) of pharmaceutical pure grade (Panreac, Spain).

The thermally stimulated transformation of Asp to PSI is accompanied by water liberation (a volatile side product under the reaction conditions), therefore, thermogravimetry (TGA) was used for the kinetic studies of the reaction on a Perkin—Elmer TGA-7 instrument (in a flow of air or argon).

Measurements by differential scanning calorimetry (DSC) were performed on a Perkin—Elmer DSC-7 instrument. An isothermic DSC-thermogram was recorded until a solid-phase transformation process of Asp to PSI was complete. For the correction of the instrument base-line drift in the course of the long-time experiments, each sample was heated for the second time under identical conditions after the reaction completion. Then, the second DSC-thermogram, related only to the change in the specific heat of the sample in a crucible and the DSC baseline drift in the course of entire experiment, was subtracted from the first DSC-thermogram, reflecting process of the solid-phase transformation of Asp to PSI. The measurements were made in the open system in air. No allowance for the weight loss was made, which should not have considerably affected the shape and time needed to reach the first maximum value of the reac-

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tion rate, since the weight loss by this moment does not exceed 15%.

IR spectra were recorded on a Perkin—Elmer 1720X IR Fourier-spectrometer and Bruker Vertex-70 spectrometer in KBr pellets using transmission and reflection—absorption regimes.[8] Raman spectra were recorded on a Bruker SENTERRA Raman microscope using laser excitation (785 nm) without preliminary preparation of the samples for analysis.

Results and Discussion

The TGA method successfully applied earlier[3] was used for the kinetic studies of the Asp polycondensation. However, in contrast to the study when measurements were made in the dynamic regime,[3] in the present work the temperature was fixed for each experiment.

The loss of Asp in weight with time (the TGA isotherms) at 170—228 °C in the flow of argon is shown in Fig. 1. The kinetic curves have a complex shape. It is well seen that at moderate temperatures 203 and 208 °C, the rate of the weight loss initially increases, then decreases, after that again increases and decreases. Thus, the polycondensation rate as a function of time reaches two maxima. Apparently, this phenomenon should be observed within entire temperature range studied. However, when the temperatures are low the experimental time is too short for the rate to reach the second maximum. At high temperatures, however, the positions of both maxima are so close in time that it is impossible to observe their separation. The presence of two maxima on the curves describing the weight loss versus time for the Asp polycondensation, at first glance, agrees with the hypothesis on a two-step process.[3] However, assuming that in our system two sequential reactions take place (polycondensation and dehydration of the polyacid formed), it can be expected that at the initial step Asp is consumed followed by the consumption of the intermediate product. In this case, the rate constant of the first reaction (polycondensation) can be much higher than the rate constant of the second reaction (dehydration of polyacid). Such a sequence is very improbable, since the solid-phase reaction conditions are unfavorable for the bimolecular process, in which preliminary approach of the reacting groups is required, that is hindered in the solid phase.

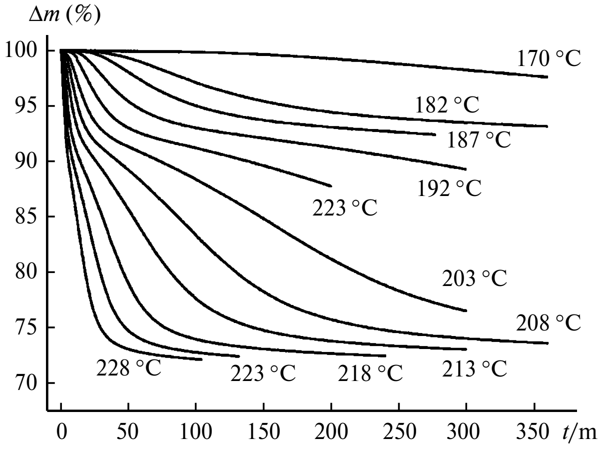

Fig. 1. The loss of Asp in weight as suggested by the isothermic TGA registered at different temperatures.

The data on the isothermic TGA (see Fig. 1) show that the both steps of the process proceed with autoacceleration. This contradicts a generally accepted scheme of a simple transformation of Asp to polyaspartic acid (pAsp), which then in the course of similar simple reaction eliminates another water molecule to be converted to polysuccinimide (PSI):

$$\text{Asp} \longrightarrow \text{pAsp} + H_2O \longrightarrow \text{PSI} + 2\ H_2O.$$

However, the simple reactions under consideration cannot proceed with autoacceleration, since as the starting compound is consumed, the rate would decrease from the very beginning of the heating.

None other products except for water are liberated from Asp under these conditions. Thus, water is the initial and the final product of the reaction. The highest value of the Asp weight loss during its transformation to PSI at isothermic TGA is 27%. This agrees with such a stoichiometry when each molecule of the starting Asp liberates two molecules of H_2O.

Analysis of full vibrational spectra showed[5] that the final solid product of the reaction under isothermic conditions at various temperatures is PSI. It is identified from the IR absorption bands at 1793, 1731, 1715, 1391, 1210, and 1164 cm^{-1}, as well as from the Raman spectrum lines at 1798, 1170, 788, 698, and 637 cm^{-1}. In addition, we found that the IR spectra of the products of the solid-phase transformation of Asp to PSI, recorded at different steps of the process in the flow of argon and in air, are virtually identical. It can be concluded that the gaseous atmosphere used for the polycondensation of Asp apparently has no noticeable effect on the kinetics and, probably, mechanism of this process.

The DSC (curve *1*) and TGA (curve *2*) thermograms of Asp at 208 °C are shown in Fig. 2. It is seen that the curves *1* and *2* are very close in shape: the rate of change of the parameter measured has two maximum values separated in time. The inset shows derivatives of these curves. In the differential form, both peaks of the thermograms can be separated using the Frazer—Suzuki standard function. The first maxima of the curves in the inset virtually coincide along the whole length. Thus, the time of evolution of a part of thermal flow consumed in the course of the reaction coincides with the time when liberation of water is registered. The change in the thermal flow is registered without time delay, whereas, the weight loss is recorded at the moment, when water already leaves the reaction zone. Therefore, the coincidence in time of the TGA and DSC kinetic curves is an evidence

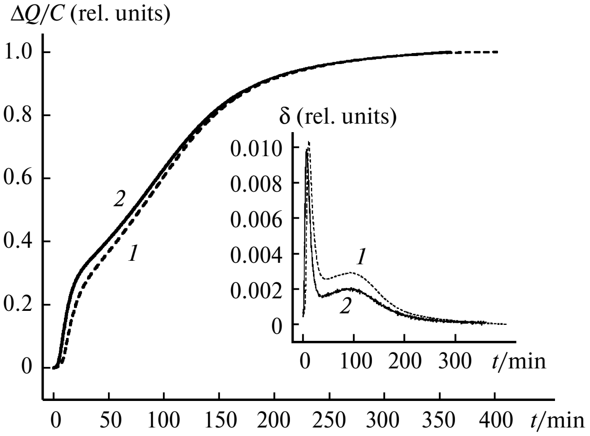

Fig. 2. The DSC in air (*1*) and TGA in argon (*2*) isotherms of Asp at 208 °C; in the inset derivatives of the DSC and TGA curves are shown; Δ*Q* is the fraction of the evolved heat, *C* is the extent of transformation.

that the time of liberation of water is negligible. In another words, liberation of water registered by the TGA as the weight loss reflects a true kinetics of the polycondensation.

It can be suggested that the autoacceleration results from the reaction proceeding in the solid phase. Acceleration can be caused by the growth of the reaction nuclei[9] or the gradual growth of the interface between the crystalline (the starting compound) and amorphous (the product) phases.[10] The experiments at 169—192 °C showed that at the very beginning of the reaction, the process of water liberation proceeds with retardation, rather than with autoacceleration. The TGA isotherm of Asp measured at 169 °C in the flow of Ar (curve *1*), and the isotherm derivative (curve *2*) are shown in Fig. 3. It is seen that the rate of water liberation initially decreases. Kinetics of this process is described by the first-order rate law. An initial decrease in the rate of water liberation in the course of polycondensation continues up to the temperature of 192 °C, though, the extent of transformation by this reaction is very low and does not exceed 0.7% of the total water liberation.

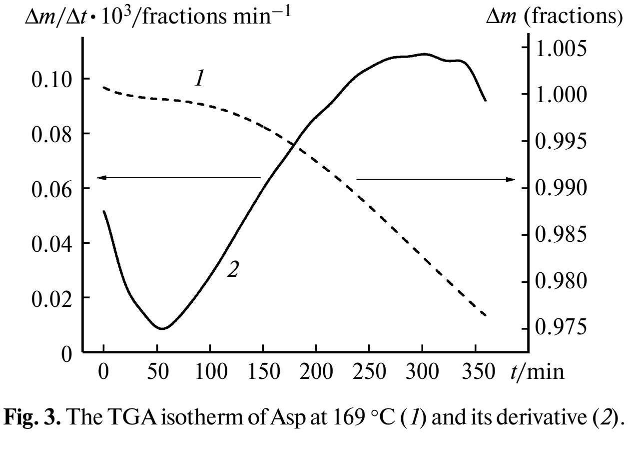

Fig. 3. The TGA isotherm of Asp at 169 °C (*1*) and its derivative (*2*).

The models accepted in the present time explain autoacceleration in the solid-phase reactions either by the growth of nuclei (the Avrami—Erofeev equation), or by the growth of the interface between the crystalline and amorphous phases. These models leave no room for the reaction retardation, since amount of the nuclei should increase with time, and with increase in the extent of transformation, the reaction surface area should increase. Therefore, for the explanation of our experimental results the autoacceleration model is more suitable, it suggests that the reaction accelerates upon the action of the product arising from Asp, *i.e.*, the model of autocatalysis. The rate of the Asp polycondensation initially reaches the maximum value, and then decreases (see Figs 1 and 2). Such rate changes contradict the "solid-phase" models. If it is assumed that the formation of nuclei or the development of the interface between the crystalline and amorphous phases is a rate determining step of the process, the polycondensation process, starting in the autoacceleration regime, is expected to proceed until complete exhaustion of the monomer. In our case, a sharp drop in the rate is observed long before Asp is consumed.

Now we can try to describe the reaction using kinetic equation for autocatalytic reactions.[11] In deriving this equation, it is suggested that either a small amount of autocatalyzing product is present in the starting compound as an impurity, or a simple transformation of the starting compound to the product occurs simultaneously with the autocatalysis. Both versions are kinetically indistinguishable. For the second version, the following scheme of transformation of the starting compound **A** to product **B** operates:

$\mathbf{A} \rightarrow \mathbf{B} + \mathbf{C}$ (simple reaction),

$\mathbf{A} + \mathbf{C} \rightarrow \mathbf{B} + \mathbf{C}'$ (autocatalytic reaction),

where **A** is the starting aspartic acid, **B** is water, **C** and **C**´ are oligomeric or polymeric molecules, differing in the molar mass.

Then the corresponding differential equation will have the following form:

$$\mathrm{d}b/\mathrm{d}t = k_2(b + k_1/k_2)(1 - b), \tag{1}$$

where k_1 is the rate constant of the simple reaction, k_2 is the rate constant of the autocatalytic reaction, $b = \Delta P_t/\Delta P_{\max}$ is the unitless extent of the Asp transformation, ΔP_t is the weight loss (wt.%), $\Delta P_{\max}$ is the maximum weight loss equal to 27%.

Equation (1) is solved in analytical form, that allows one to obtain the following linear dependence:

$$\ln[(b + k_1/k_2)/(1 - b)] = k_2t + c. \tag{2}$$

In this equation, only the value of parameter k_1/k_2 is initially unknown. However, when the extent of reaction is high enough its value virtually does not affect the value of parameter k_2 to be determined. A dependence of the Asp weight loss from time at 208 °C in the flow of Ar, transformed in the coordinates of Eq. (2) is shown in Fig. 4. Parameter k_1/k_2 was taken equal to 0.01. It is seen that at $t > 50$ min, the shape of the curve is close to linear and this section is described by the equation

$$y_{\mathrm{end}} = 0.0052t - 1.25 \tag{3}$$

with the correlation coefficient $R^2 = 0.9993$.

Thus, using Eqs (2) and (3) it is possible to calculate kinetics of the TGA curve last section. Kinetics of the first section is obtained by the subtraction of the results calculated using Eq. (3) from the corresponding experimental data. Subsequent analogous transformation leads to a curve described by the following formula:

$$y_{\mathrm{st}} = 0.147t - 2.72\ (R^2 = 0.997). \tag{4}$$

Figure 5 allows one to compare two sections of the experimental TGA curve of Asp registered at 208 °C in the flow of Ar with analogous sections calculated using Eqs (2)—(4). It is seen that the sections of the curves at $t > 50$ min virtually coincide. Thus, after the extent of the Asp transformation reaches 30%, the polycondensation kinetics can be satisfactorily described by the equation of the first order, which suggests autocatalysis by the reaction product. Results of the experimental measurements and calculated data for the first section of the curves are close enough only in the region of measurable reaction rates, that follows the induction period.

Before the induction period is completed, various processes apparently take place in the system, for example, transformation of the crystal lattice due to the appearance of the first portions of the amorphous product. The kinetic scheme suggested does not make allowance for this step of the solid-phase polycondensation. Therefore, the calculated function of the weight loss in the induction period became negative, that has no physical meaning. The deviations from the calculated kinetic scheme observed are related to the very low extents of transformation: ~1% of the loss in weight from the starting Asp. Further transformation is quantitatively described by two autocatalytic reactions of the first-order rate law to the starting Asp and to the product, that catalyzes the reaction.

The rate constants of autocatalysis for both steps of the polycondensation are compared in Fig. 6. Despite a wide range in which the constants are changed, the Arrhenius dependencies remain linear, that is not characteristic of the solid-phase reactions. Effective activation energies for the autocatalytic reaction of the chain growth at the first

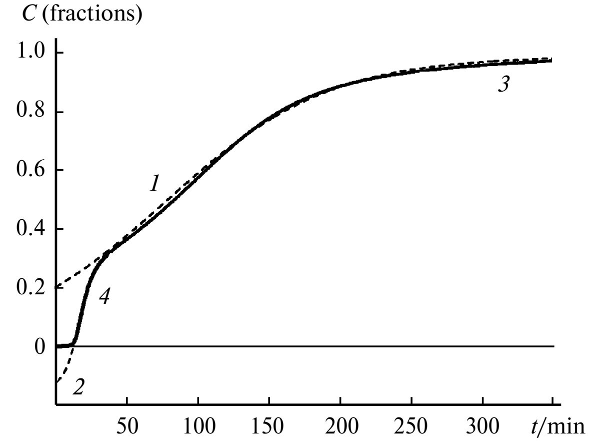

Fig. 5. Comparison of theoretical (*1*, *2*) and experimental (*3*, *4*) TGA curves for Asp (208 °C, Ar); *1*, *3* are the last sections, *2*, *4* are the first sections.

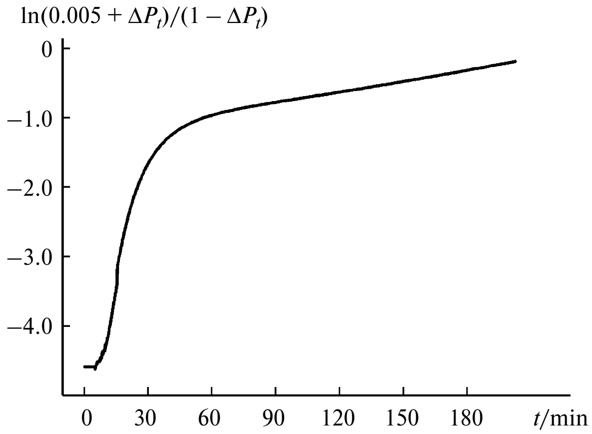

Fig. 4. Transformation of the TGA curve of Asp (208 °C, Ar) in the coordinates of the first order autocatalysis; ΔP_t is the extent of the monomer transformation: $\Delta P_t = (100 - P_t)/27$, where P_t is the current percentage of the sample weight with respect to the starting weight.

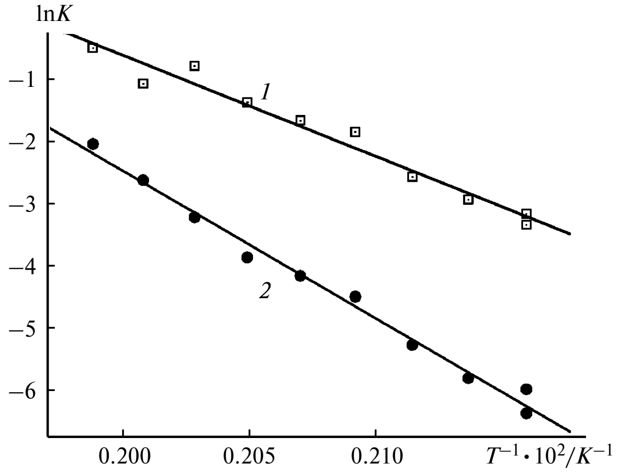

Fig. 6. Arrhenius transformation of the rate constants of autocatalysis on the two sections of the TGA curves of Asp in Ar: *1* is the first section $(k_2)_{\mathrm{st}}$, $y = -15796x + 30.957$ ($R^2 = 0.96$); *2* is the last section $(k_2)_{\mathrm{end}}$, $y = -23320x + 44.149$ ($R^2 = 0.9875$).

and second steps are 31.6 and 46.6 kcal mol^{-1}, respectively. Such high values are not typical at all of the polycondensation of amino acids, however, they were observed earlier[12] in the study of the solid-phase polycondensation reactions.

Determination of the rate constant of the autocatalytic reaction in this case is a result of rather rough simplifications of the differential equations system describing two successive reactions. The contribution of the second reaction, *viz.*, the dehydration of the polyaspartic acid *n*-mer leading to PSI, had to be neglected due to the mathematical difficulties in solving the systems. In order to evaluate contribution of the dehydration, we performed computer calculations of the TGA isotherms using the NETZSCH Thermokinetics program for thermochemical measurements. Optimization of the kinetic scheme parameters was performed for ten measured TGA isotherms at once. Two parallel pathways for the process, consisting of two successive reactions, have been suggested in the scheme. The pathway is accomplished in its own zone of the solid phase. Such a model of the solid-phase reactions was suggested earlier.[13] A more extensive discussion of this model is given below. Each of the pathways consists of autocatalytic reaction of the chain growth, followed by dehydration with the formation of a succinimide ring.

Results of calculations using the NETZSCH program are shown in Fig. 7. The experimental data fit well the calculated curves. Activation energies of the autocatalytic reaction in the first and second zones obtained by computer calculations are 37.5 and 43.6 kcal mol^{-1}, respectively. A comparison with the values obtained using an analytical function (31.6 and 46.6 kcal mol^{-1}, respectively) shows that the deviation is not very high and apparently results from the rather rough simplifications used in the analytical solution.

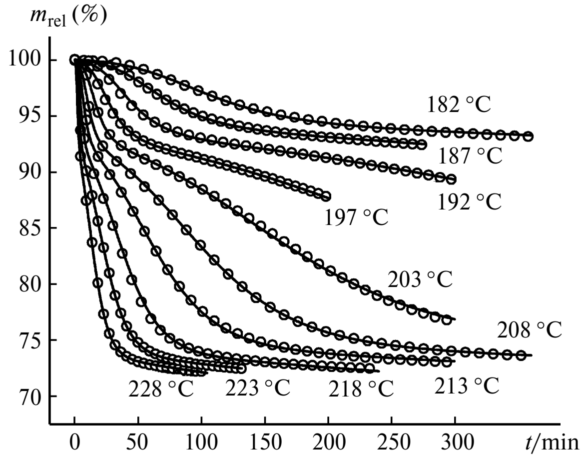

Fig. 7. Results of computer calculations of the Asp polycondensation kinetic scheme for the TGA isotherms using the NETZSCH program: the circles show the experimental data, the solid lines show calculation; temperatures are given near each curve, m_{rel} is the relative weight.

In the temperature range studied, the rate constant of the first autocatalytic reaction $(k_2)_{st}$ is always significantly higher than the constant of the second reaction $(k_2)_{end}$. This difference grows with the temperature reduction. The experimental and calculated data obtained show that the very difference in the rate constants of two parallel reactions results in the complex shape of the kinetic curve with two maxima and a minimum.

One of the possible explanations for the phenomenon observed is given by a hypothesis suggested in the course of studies of other solid-phase processes:[13] a solid phase consists of two spatial regions in which a reaction can take place. The first region can be called a potential reactive space formed yet before the reaction. The second "dynamic" region of the matrix is formed in the course of the chemical process itself. In our case, the first step (the first pathway of the process) can be explained by the reaction in the "potential" space, the first retardation of the reaction, by a decrease in the volume of the reaction zone. The second step (the second pathway of the process) of the solid-phase polycondensation of Asp proceeds in the "dynamic" part of the reaction space. During this period of time, retardation of the reaction is caused already by the pure chemical reason, *viz.*, exhaustion of the monomer. Our data can be explained if independence of these two zones is postulated, whereas in the work[13] it is suggested that the reaction in the first zone initiates its development in the second, *i.e.*, the process in the second zone depends on the reaction in the first zone.

The measured values of activation energies appear to be too high for usual polycondensation reactions. It is likely that these values are associated with the reaction proceeding in the condensed phase. At the same time, the facts that the kinetics observed fits usual kinetic equations and the Arrhenius dependence operates within a wide range of temperatures and rates of the process are in contrast with existing concepts on the solid-phase reactions as the processes taking place in the kinetically heterogeneous medium. Apparently, in this case the reaction in each of the two regions of the solid phase proceeds in homogeneous, but very dense medium, hindering motion of the molecules. All the processes leading from the heterogeneous crystal structure of Asp to this highly dense homogeneous medium, probably, occur during induction period and/or prior to very low extents of transformation.

It was shown by kinetic analysis that the reaction of thermal polycondensation of Asp in the solid phase is considerably more complicated than it has been suggested in all the preceding studies. The polycondensation consists of two parallel processes, each of which proceeds independently from other in its own region of the reaction space. Both begin with an autoaccelerating reaction. The manual and computer calculations of the corresponding kinetic scheme give close Arrhenius parameters. The rate constants significantly differ for each of the regions where chemical transformations proceed. This results in a very complex shape of the TGA and DSC isotherms with three inflection points: two maximum and one minimum rates of polycondensation.

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