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Effects of processing on the solid-state methyl transfer of tetraglycine methyl ester

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Abstract

The thermally induced solid-state methyl transfer of tetraglycine methyl ester (TGME) was followed by HPLC analysis for samples subjected to freeze-drying and milling and compared to that observed for a highly crystalline unprocessed sample. Corresponding measurements by powder X-ray diffraction and differential scanning calorimetry were used to follow physical changes in these samples during the course of the reaction. It was shown that freeze-drying and milling of TGME produced samples with significantly reduced crystallinity, i.e. amorphous structure, as well as an apparent polymorphic transformation. HPLC analysis revealed greatly enhanced chemical reactivity for the processed samples relative to the crystalline sample, but no overall change in the chemical intermediates and products produced. The kinetic curves for the freeze-dried samples were monotonic and could be treated by a first-order model, whereas the curves for the milled samples consisted of two parts, a rapid initial reaction followed by a slow phase. Kinetic data in both cases were treated with the assumption that the reaction proceeds independently and simultaneously in amorphous and crystalline phases. It was shown that reactivity in the amorphous phase for freeze-dried and milled samples is very similar, whereas reactivity taking place in the crystalline regions occurs at a rate almost two orders-of-magnitude greater in freeze-dried samples than in the milled preparations. Thus it is concluded that in addition to the formation of amorphous states processing of TGME such as freeze-drying or milling can introduce disorder into the remaining crystal lattice sufficient to also influence the kinetics of chemical degradation. © 1997 Elsevier Science B.V.

Keywords: Solid-state methyl transfer; Tetraglycine methyl ester; Amorphous phase

1. Introduction

It is well recognized that many drugs undergo chemical change in the solid state when subjected

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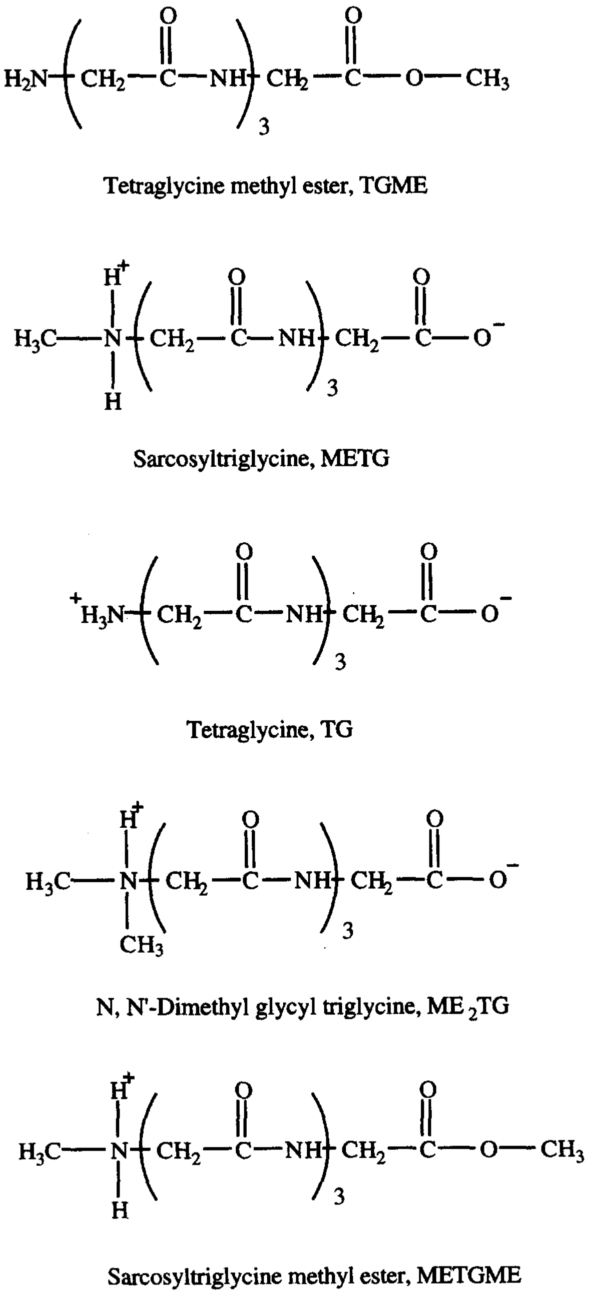
to high temperatures or relative humidities, as well as to other environmental factors such as light and oxygen (Carstensen, 1980; Byrn et al., 1996). Some reactions occur most readily in the crystalline state when compared to reactivity in the melt or solution state because of certain positional requirements within the crystal lattice for reactivity to occur (Sukenik et al., 1975, 1977). More usually in pharmaceutical systems, the introduction of more disordered amorphous character to a crystalline solid, or forming it as a melt or solution, greatly accelerates many reaction rates presumably because of the greater molecular mobility produced in these systems (Pikal et al., 1977; Oberholtzer and Brenner, 1979; Carstensen and Morris, 1993). Such disorder, in the form of an amorphous phase and/or defects in the crystal lattice, can be fully or partially introduced by a number of manufacturing processes, including freeze-drying, spray drying, and milling (Takahashi et al., 1984; Huttenrauch et al., 1985; Saleki-Gerhardt et al., 1994; Tsukushi et al., 1994; Duddu and Grant, 1995). Pharmaceutical processes such as wet granulation or aqueous film coating could also produce such effects. It would be expected, therefore, that such processing could play a role in the chemical instability of drugs when any amount of disorder is introduced into crystalline solids (Pikal et al., 1978; Otsuka and Kaneniwa, 1984, 1990; Kitamura et al., 1989).

Previous studies with the peptide, tetraglycine methyl ester (TGME), have demonstrated significant thermal instability at 100°C in the highly crystalline state via methyl transfer, following the general process shown in Scheme 1 (Sluyterma and Veenendall, 1952; Sluyterma and Kooistra, 1952; Huang et al., 1996). In a recent study, we followed this reaction over the range of 83–115°C, measuring the composition of reactant, intermediates and products by HPLC analysis, along with corresponding measurements by powder X-ray diffraction (Shalaev et al., 1997). It was shown that the methyl transfer of TGME in the crystalline state occurred in what appeared to be an autocatalytic manner, i.e. the reaction accelerates with the reaction extent. It was concluded that the first phase of the reaction, i.e. the fractional extent of reaction, $\alpha < 0.25$, occurred in a single crystalline solid-solution phase, where increasing amounts of product produced sufficient disorder into the crystal lattice to accelerate the reaction. Such acceleration was further enhanced


Tetraglycine methyl ester, TGME

Sarcosyltriglycine, METG

Tetraglycine, TG

N, N'-Dimethyl glycyl triglycine, ME $_2$TG

Sarcosyltriglycine methyl ester, METGME

Scheme 1.

at later extents of reaction by the appearance of a second crystalline phase (Shalaev et al., 1997). It was possible to treat both portions of the kinetic data with different models (single phase and heterophase) and to obtain rate constants for further analysis.

Considering the significant acceleratory effect of introducing disorder into the crystal lattice we would expect that processes such as freeze-drying and milling, that are known to reduce crystallinity, would have significant effects on the rates of methyl transfer in TGME. Indeed, preliminary observations have shown greater rates of TGME loss, relative to that with highly crystalline samples, when exposed to 100°C, for freeze-dried (Huang et al., 1996) and slightly milled (Sluyterma and Kooistra, 1952) samples. In this study we have subjected crystalline TGME to freeze-drying and have followed the loss of TGME and the formation of intermediates and products (see Scheme 1) over the temperature range of 50–115°C. Similar studies at 100°C have been carried out for a sample of TGME milled under one set of conditions. Correspondingly, the physical characteristics of these systems, before and during the reaction were monitored by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). Thus with these systems we were in a position to directly observe how physical changes in the crystal, brought about by processing, might affect the mechanism and kinetics of a model chemical reaction in the solid state.

2. Experimental

2.1. Materials

Tetraglycine methyl ester (TGME) was synthesized in a manner previously described and recrystallized from methanol solution (Huang et al., 1996). Water used in the freeze-drying procedure was passed through a Barnsted water purification system and was twice distilled from potassium permanganate and dilute sulfuric acid solutions. Acetonitrile and trifluoroacetic acid, used in the HPLC mobile phase, were HPLC grade solvents. LiF, used as an internal standard in the X-ray diffraction measurements, was obtained from Aldrich at reagent grade.

2.2. Methods

2.2.1. Sample preparation

Freeze-dried TGME was prepared using a Dura-Stop, FTS Systems (Stone Ridge, NY) Freeze-drier. Aqueous solutions, 2% w/w in concentration, were placed into 2-ml ampuls (0.8 ml per ampul), cooled with liquid nitrogen at cooling rates of 30–40°C/min, then evacuated at −40°C for 3–5 days (residual pressure at 60–75 mT) and held at increasing temperature (10–20°C/day) up to 25°C for 3–5 more days. HPLC analysis of such freeze-dried material revealed that no more than 5% TGME was hydrolyzed to tetraglycine (TG) during the entire process.

Milled samples of TGME were prepared with a Wig-L-Bug Heavy Duty Laboratory mill (Spectratech) using a stainless steel vial and ball. In preliminary experiments various grinding times, from 5 s to 15 min, were used. Optimal reproducibility in all respects was obtained at and above 5 min grinding and, thus, this time period was chosen for preparation of the milled sample used in this study. Ground samples used in the kinetic study weighed between 0.30 and 0.35 g. HPLC analysis of milled samples did not show any degradation associated with milling.

2.2.2. Specific surface area

Specific surface area measurements were carried out with a model 1M0325 Quantasorb Surface Area Analyzer (Quantachrome, Syosset, NY) and a Quantasorb Flow Control Accessory, using nitrogen gas for the freeze-dried and milled samples and krypton gas for the crystalline samples in the BET analysis (Brunauer et al., 1938). The molecular cross-sectional areas of nitrogen and krypton were taken to be 16.2×10^{-20} and 19.5×10^{-20} m^2/molecule, respectively.

2.2.3. Kinetic measurements

The freeze-dried and milled samples of TGME were heated in 2-ml ampuls sealed under vacuum. The ampuls before sealing were subjected to vacuum over P_2O_5 for a period of at least 2 days for the milled samples and 5 days for the freeze-dried samples. One of the freeze-dried preparations was additionally dried in vacuum at 56°C for 15 h. The reaction kinetics for this preparation was identical with those dried only at room temperature. Hence, we conclude that the drying at room temperature is enough to remove residual solvents. Details for treatment of crystalline samples were described in an earlier publication (Shalaev et al., 1997). Kinetic studies were carried out with the freeze-dried sample at 49.8 ± 0.6, 82.3 ± 0.2, 100.0 ± 0.25 and 114.3 ± 1.0°C in laboratory ovens. Studies with the milled samples were carried out at 100.0 ± 0.25°C. The ampuls were immersed in oil to avoid thermal inhomogeniety and were removed periodically at set time points for analysis. Chemical analysis was carried out by an HPLC method described in detail earlier (Huang et al., 1996; Shalaev et al., 1997). The procedures used for DSC and X-ray analyses are described below.

2.2.4. *Differential scanning calorimetry (DSC)*

DSC measurements were carried out on a Seiko SSC/5200 differential scanning calorimeter at a heating rate of 10°C/min. The instrument was calibrated using the melting points of indium, gallium, tin, water, and mercury. Samples from about 3 to 13 mg were placed into sealed aluminum pans with pin holes. Before analysis, all materials were stored in a refrigerator in tight containers with a desiccant.

2.2.5. *X-ray diffraction*

Powder X-ray diffraction measurements were obtained on a Scintag PADV instrument under the following conditions: CuK_α radiation, 30 mA, 45 kV, scan rate, 5°C/min. Samples were placed on a quartz sample holder with zero background and measurements were carried out at room temperature. The procedure for quantitative X-ray diffraction analysis with an internal standard has been described earlier (Shalaev et al., 1997).

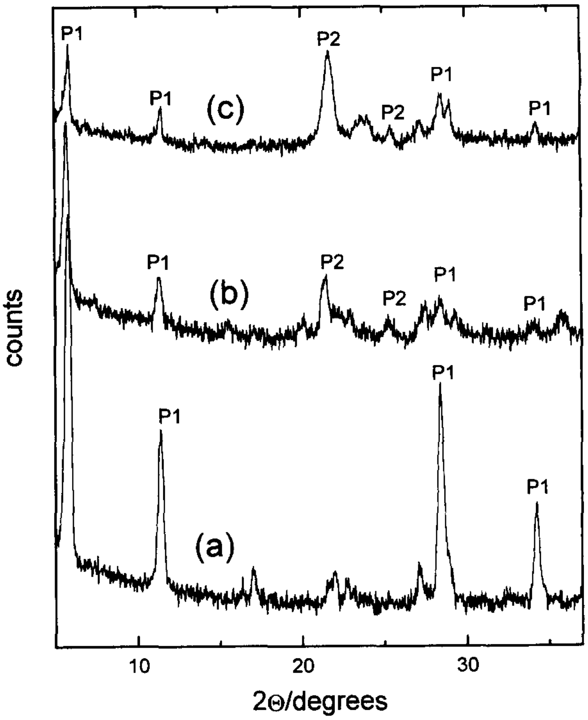


Fig. 1. Representative X-ray powder diffraction patterns for TGME. (a) Initial crystalline preparation; (b) freeze-dried preparation; (c) milled preparation. P1 marks four main peaks of the initial TGME crystalline phase and P2 marks peaks of the second crystalline phase created as a result of processing.

3. Results

3.1. *Characterization of processed samples*

3.1.1. *Specific surface area*

The surface areas for the three samples used in this study, estimated by BET analysis, are as follows: initial crystalline sample, 0.32 m^2/g; freeze-dried, 8.4 m^2/g; and milled, 2.5 m^2/g. Clearly, freeze-drying and milling in this system have introduced a significant amount of energy into the solid to be able to produce such a large specific surface area change, particularly freeze-drying.

3.1.2. *X-ray diffraction*

Fig. 1 contains the X-ray diffractograms for the three samples (crystalline, freeze-dried, and milled) before being subjected to thermal degradation. The four main peaks of crystalline TGME are still present in the freeze-dried and milled samples, however, their intensities are considerably reduced relative to the highly crystalline sample. Moreover, there appear to be a few peaks on the diffractograms of the freeze-dried and milled samples that were either absent or almost negligible with the initial crystalline sample. These peaks occur at $2\theta = 21.4$, 25.2, 29.3 and 35.8° for the freeze-dried sample and 21.4, 23.6, 25.3 and 29.0° for the milled sample. Thus freeze-drying and milling of TGME, in the manner used here, do not produce a fully amorphous structure and may cause some polymorphic transition to occur. All attempts to produce fully amorphous TGME by these techniques failed. It appears most likely that at least two polymorphic forms may exist, one the original crystal form characterized by the main peaks at $2\theta = 5.7$, 11.3, 28.4 and 34.2° and the second form at $2\theta = 21.4$, 25.3 and 29.0–29.3°. A very small amount of this second form may be present in the original sample since in Fig. 1(a) there is some indication of a very small peak at $2\theta = 21.4°$. Using purely crystalline samples of TGME mixed with LiF, we were able to establish and approximate percent of the original crystal form by X-ray analysis, as described previously (Shalaev et al., 1997), to be 37% w/w and 14% w/w for the freeze-dried and milled samples, respectively. We were unable to determine the weight fraction of the second crystalline form produced by processing since we had no standards available for calibration. From the relative peak areas under the new major X-ray peak at $2\theta = 21.4°$, however, we estimate that the amount of new crystalline phase in the milled sample was about 1.5 times higher than in the freeze-dried sample.

3.1.3. Differential scanning calorimetry

Representative DSC curves for crystalline, freeze-dried and milled samples are given in Fig. 2. As depicted in Fig. 2, whereas the crystalline sample shows no thermal events over the temperature range reported, the freeze-dried and milled samples show an apparent glass transition with accompanying enthalpic relaxation at 35 ± 3 and 30 ± 6°C, respectively, on a first scan. If a thermal cycle of heating up to 100°C followed by cooling, then heating again, was used, as expected, the enthalpic relaxation was greatly reduced. Thus it appears that the process of freeze-drying and milling TGME has introduced some amorphous character. It should be mentioned that it is remotely possible that the 'glass transition' observed here is actually related to the presence of a new 'plastic crystalline phase', where the transition observed is associated with greater molecular rotation in the crystal lattice rather than translational motion in an amorphous phase (Meyer and Duparc, 1993). Although we cannot at this point clearly establish the presence of one or the other new forms in the processed material, it is reasonable to conclude that such a new solid phase exists in state of greater disorder relative to the initial crystalline form of TGME and that it is most likely an amorphous form.

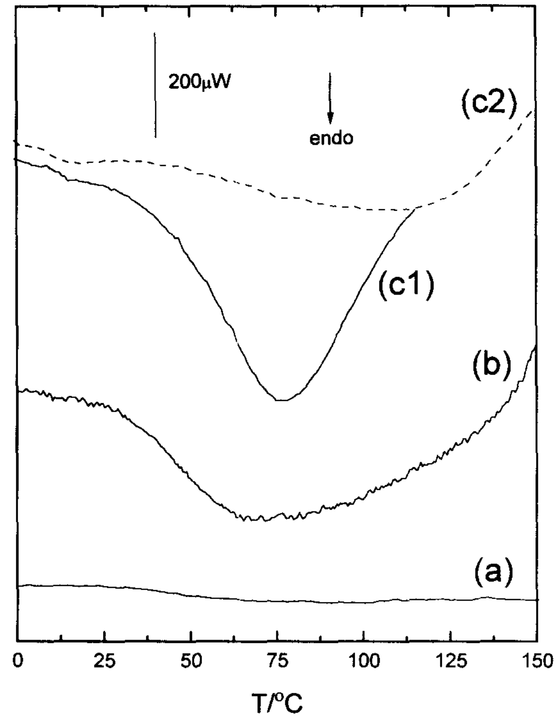


Fig. 2. Representative DSC curves for (a) initial crystalline TGME; (b) freeze-dried TGME; and (c) milled TGME. Curves c_1 and c_2 represent the thermal cycling experiment (heating up to 100°C (scan c_1), cooling (scan is not shown), heating (scan c_2)). Heating and cooling rates are 10°C/min.

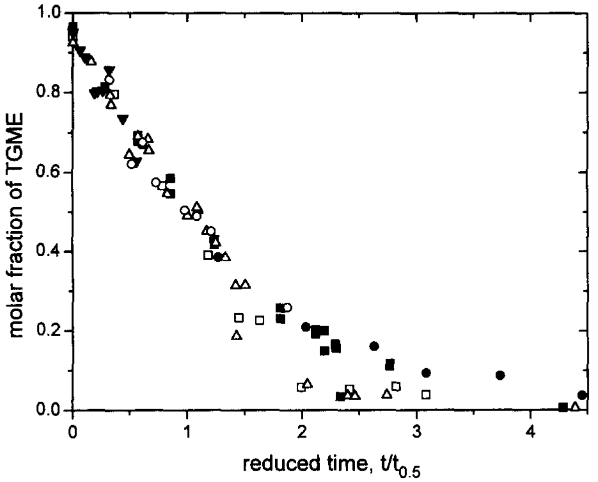


Fig. 3. Kinetics curves for degradation of freeze-dried TGME as a function of reduced time, $t/t_{0.5}$. ■, □: 100°C; ●, ○: 83°C; △: 115°C; ▼: 50°C. Open symbols, preparation which exhibits crystallization of product phase beyond $\alpha = 0.78$; closed symbols, preparations without crystallization.

3.2. Kinetics of methyl transfer

Fig. 3 shows the loss of TGME and Fig. 4 shows the appearance of TG, METG and METGME for the freeze-dried sample at four temperatures, plotted in terms of reduced time, $t/t_{0.5}$ where $t_{0.5}$ is the time required for 50% of TGME to react. Table 1 provides values for $t_{0.5}$ at the various temperatures. The superimposition of all data would indicate that the underlying mechanisms involved in this reaction are the same over the temperature range studied. However, beyond the point at $t/t_{0.5} = 1.5$ the curves split into two parts that can be understood on the basis of X-ray diffraction results to be discussed below. Fig. 5 indicates the loss of TGME at 100°C for the milled samples. Included in this figure are the plots of TGME loss at 100°C for the crystalline sample, as previously reported (Shalaev et al., 1997) and for the freeze-dried sample, described above. Note for the milled sample that there is an initial rapid loss of TGME followed by a slower phase, with behavior that appears intermediate to the freeze-dried and crystalline samples.

In Fig. 6 are given X-ray diffraction patterns for the freeze-dried samples taken after different extents of reactivity. Three independently freeze-dried materials were studied in this manner, and it is interesting to note that two of them revealed no new peaks during the entire reaction while one freeze-dried preparation produced a new weak and broad peak at $\alpha = 0.77$ at $2\theta = 30.6°$. At the end of the reaction in this latter case 2 more peaks appeared at $2\theta = 6.0$ and 12.1°. The same peaks at $2\theta = 6.0$, 12.1 and 30.6° were observed earlier for the latter stages of reaction involving the crystalline sample (Shalaev et al., 1997). These are assumed to represent a new crystalline phase involving the final products of the reaction. It should be noted that samples which show crystal-

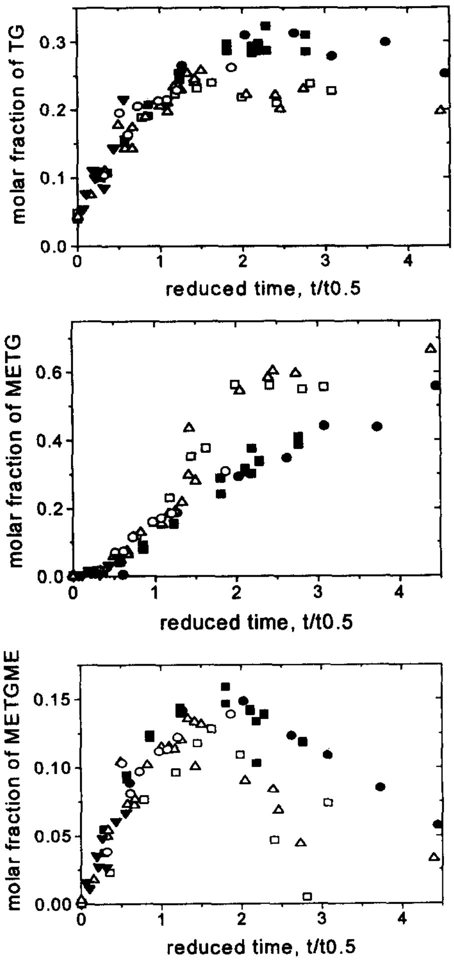


Fig. 4. Molar fraction of the products and intermediate as a function of reduced time, $t/t_{0.5}$. (a) TG; (b) METG; (c) METGME. Symbols are the same as in Fig. 3.

Table 1

Time required for 50% TGME to react, $t_{0.5}$ and first-order rate constants, k, for reactivity of freeze-dried TGME

T (°C)	$t_{0.5}$ (h)	k (1/h)
49.8	(1314)[a]	0.00049
82.3	36.2	0.0191
	50.2	0.0125
100.0	8.3	0.0801
	10.5	0.0685
114.3	3.0	0.2096

[a] This number has been obtained by extrapolating experimental data to $\alpha = 0.5$ using a first-order model (Eq. (3)).

lization of a product phase during the reaction also exhibit acceleration for reactant consumption and the accumulation of products and intermediates compared to materials not exhibiting such crystallization (Figs. 3 and 4). A phenomenological description of how crystallization of a product phase may influence chemical degradation is given by Shalaev and Zografi, 1997. For all three samples the reaction is accompanied by a reduction in the intensity of the X-ray peaks associated with the initially crystalline sample, while the peak at $2\theta = 21.4°$, believed to be associated with a new crystalline form of TGME, does not change during the entire course of the reaction.

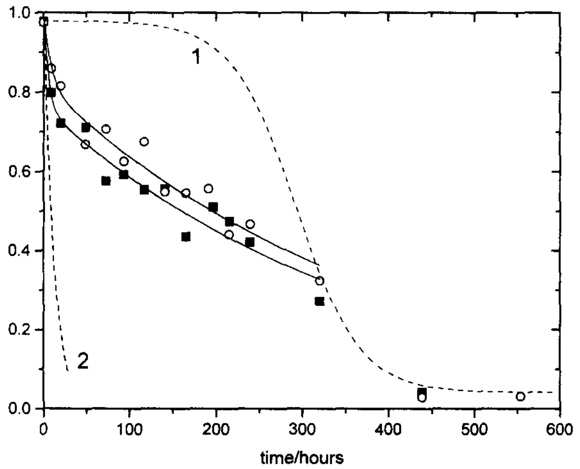


Fig. 5. Kinetics curves for degradation of two milled TGME preparations at 100°C. Dotted curves represent the summary of data for reactivity of crystalline TGME (Shalaev et al., 1997) (curve 1) and freeze-dried TGME (curve 2, this paper). Solid curves represent fitting of experimental data to Eq. (2). Symbols represent experimental data points for reactivity in two milled preparations.

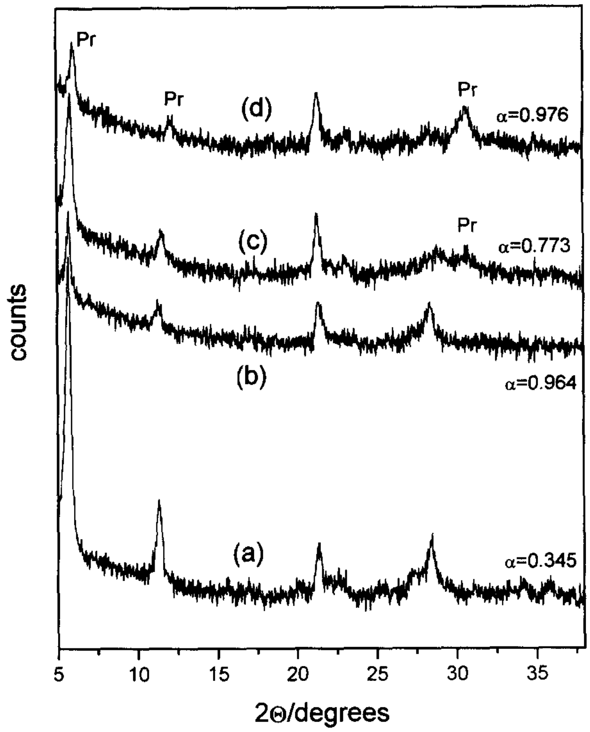


Fig. 6. Representative X-ray powder diffraction patterns for freeze-dried TGME with different extents of conversion. The numbers indicate the fraction of conversion, α. Pr marks designate peaks of a new product phase appearing at the end of the reaction. Curves (c) and (d) represent one of three preparations which exhibit crystallization of the product phase, and curve (b) represents two other preparations without any crystallization. Curve (a) for an intermediate reaction extent is typical for all three preparations.

X-ray patterns for two milled preparations, measured over the time course of reactivity at 100°C, are given in Fig. 7. Here, we see no changes in these patterns and no losses in intensity up to $\alpha = 0.77$ (see also Table 3). At $\alpha = 0.85$ the peaks presumed to be associated with products, as described above, appear and any peaks associated with the initial crystalline form of TGME have essentially disappeared. The peak at $2\theta = 21.4°$ does not change during the entire reaction, similar to freeze-dried preparations.

To observe the extent to which the chemical degradative pathway might have been influenced by processing, as opposed to effects due to changes in the physical state of the solid, we present in Fig. 8 plots of molar fraction for the products and intermediates, TG, METG and METGME, as a function of α, the extent of reaction, for the crystalline, freeze-dried and milled samples. Clearly in all respects the initially crystalline and freeze-dried samples do not show any differences in overall chemical changes. The results for the milled sample also are quite similar to those for the crystalline and freeze-dried samples, however, there appears to be some difference in the pattern for the extent of accumulation of the intermediate, METGME. We would conclude at this point, therefore, that the major effect of processing on the rates of methyl transfer in TGME arise from changes in the physical state of the solid and most likely from the degree of local solid-state disorder introduced.

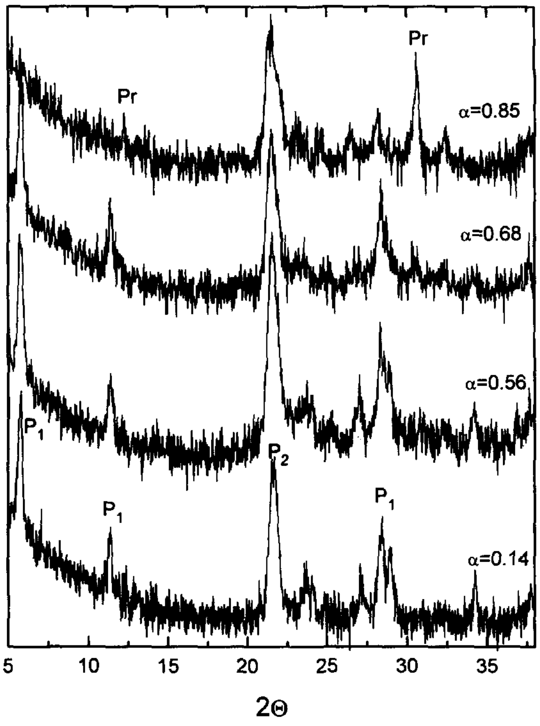


Fig. 7. X-ray powder diffraction patterns for milled TGME with different extent of conversion. See Figure 1 and 6 for explanation of marks.

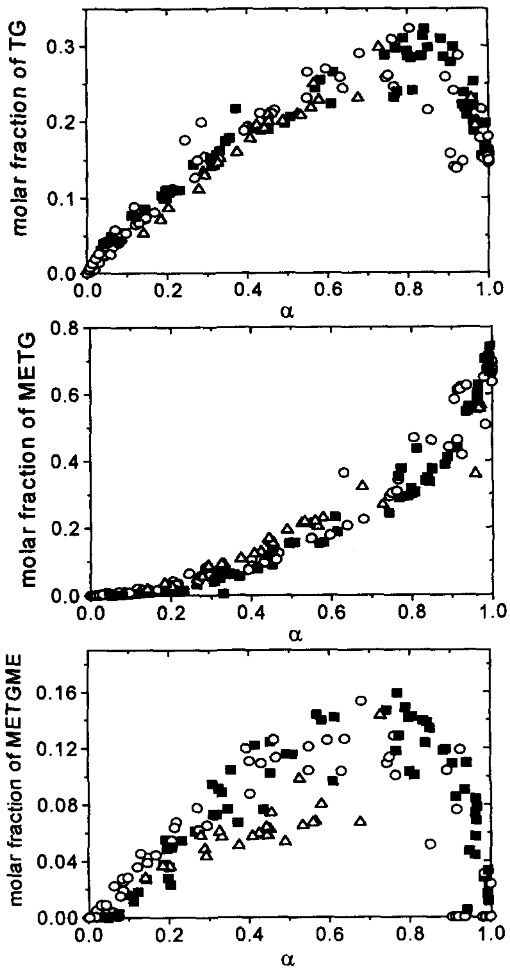


Fig. 8. Molar fraction of the products and intermediate as a function of the extent of conversion. ■: freeze-dried; ○: crystalline; △: milled.

4. Discussion

The results of this study show that the rate of solid-state thermally induced methyl transfer is significantly affected by processing without changing the chemical mechanisms involved. For the freeze-dried sample before degradation, at least two crystalline phases of TGME and an amorphous phase appear to exist. A similar pattern of physical change occurs upon milling the initially crystalline sample. The processing of TGME also produced significant changes in specific surface area of each sample, i.e. from 0.32 m^2/g for the crystalline sample to 8.4 and 2.5 m^2/g for the freeze-dried and milled samples, respectively. If the reaction would have involved more than one component in the solid, liquid, or gaseous state, available surface area would be expected to be a critical factor. In this reaction it appears, however, that two TGME molecules in the solid-state are involved in the initial step (see Scheme 1), suggesting a less important role being played by the surface area. It is possible that molecules in the surface may be more disordered than in the bulk phase or that the reactions start at the surface and move into the bulk phase although we have no microscopic evidence to suggest a moving boundary into the bulk. From a comparison of the patterns of loss of TGME as a function of time for the three samples shown in Fig. 5, it also does not appear that the kinetics of the reaction are solely determined by surface area changes since the initial rates up to $\alpha = 0.20$ of the freeze-dried and milled samples are very similar (8.4 vs. 2.5 m^2/g, respectively). Thus we would conclude that the effect of processing in this reaction arises to the greatest extent from changes in the state of disorder in the bulk phase.

The concurrent use of X-ray diffraction during the course of monitoring the rate of reaction has allowed us to observe changes in the initial crystalline states of TGME and whether or not any new crystalline forms appear. In our earlier study with a highly crystalline sample of TGME (Shalaev et al., 1997), it was shown that the first 25% of the reaction occurred in a single crystalline phase, most likely consisting of a solid solution of intermediates and products in the original TGME lattice. Beyond this point a second crystalline phase, believed to be that of a solid solution (mixed crystalline phase) of products, occurred with a greater accelerated rate of reactivity. It is interesting to note that up to about $\alpha = 0.70$ of the reaction for the freeze-dried and milled samples no new crystalline phase appeared. However, for all milled samples and for one of three independently freeze-dried samples nucleation and growth of a new crystalline phase did eventually occur. This new phase was identical to the phase that appeared at $\alpha > 0.25$ for the crystalline sample (Shalaev et al., 1997). Apparently, the point at which this new crystalline phase appears, i.e., the point at which we can no longer maintain a supersaturated metastable state of products, will vary depending on the exact history of the processed material.

Although simply fitting kinetic data to various models does not confirm the existence of a particular reaction mechanism, it appears appropriate to consider the most likely mechanisms and their associated kinetic models and to estimate rate constants for further kinetic analysis. In previous studies it was concluded that methyl transfer in crystalline TGME, up to $\alpha = 0.25$, occurred as an acceleratory, single-phase reaction followed by a further acceleration as the reaction mechanism switched from a single-phase to a heterophase process. Thus this reaction was treated kinetically in two parts, one a homogeneous reaction and the other a heterogeneous reaction (Shalaev et al., 1997). In the case of the freeze-dried sample, we see in Fig. 5 that the kinetics appear to be much faster than that of the crystalline sample and to be monotonic up to $\alpha = 0.70$, despite the presence of two crystalline phases of TGME and an amorphous phase. It appears, therefore, very likely for the freeze-dried sample that this reaction, which goes to completion in terms of remaining TGME, must take place concurrently in all three phases with no domination of the kinetics by any one phase. On the other hand, even though X-ray diffraction patterns and quantitative X-ray diffraction analysis indicate similar phase compositions for the freeze-dried and milled samples up to $\alpha = 0.70$, and DSC analysis reveals similar values of Tg, the rates of chemical change and the patterns of change are quite different. Whereas the freeze-dried sample produces a monotonic loss in TGME, the milled sample shows what appears to be at least two rate-limiting regions up to $\alpha = 0.70$. Some differences in the initial amounts of the second crystal form of TGME occur (about 1.5 times), but this does not appear to be enough of a difference to govern the different kinetic patterns observed.

To analyze this situation in more quantitative terms, rate constants for the degradation in the crystalline and amorphous phases need to be determined. The principal complication in kinetic analysis of such heterophase systems is that only the average concentration of a reactant in a system can be determined experimentally whereas mechanistic kinetic analysis must deal with reactant concentration in every phase (Shalaev et al., 1997), as given in the following equation,

$$A(t) = A_1(t)(1 - X(t)) + A_2(t)(X(t)) \tag{1}$$

where $A(t)$ is the average reactant concentration at time, t, $A_1(t)$ and $A_2(t)$ are the current reactant concentration in phases 1 and 2, respectively and $X(t)$ is the weight fraction of phase 2. However, even in this situation it is possible to estimate rate constants in crystalline and amorphous phases in freeze-dried and milled materials, as will be shown below.

To do this we first can carry out a simulation of a situation in more quantitative terms. Let us consider solid-state reactivity in a material consisting of two solid phases under the following conditions: first-order reaction takes place simultaneously in both phases and the amount of each phase does not change as the reaction proceeds. The question we ask is, how different must the relative rates be to shift the kinetic pattern that is simply monotonic to one that is more complex? To do this, let us consider two reactions that occur via first-order kinetics, so that the integral kinetic equation which describes change in average reactant concentration can be written as follows:

$$A = A_{01}(1 - X)\exp(-k_1 t) + A_{02}(X)\exp(-k_2 t) \tag{2}$$

here A is the average reactant concentration at time, t, A_{01} and A_{02} are the initial concentrations in phase 1 (e.g. more reactive, amorphous phase) and in phase 2 (e.g. less reactive, crystalline phase), respectively, and k_1 and k_2 are the first-order rate constants for the reaction in phase 1 and 2, respectively, and X is weight fraction of phase 2.

First, we can generate kinetic curves using Eq. (2) and various parameters listed in Table 2. Representative curves are given in Fig. 9 as symbols. We then attempt to fit these kinetic curves to a simple single first-order expression

Table 2
Parameters used in simulations of model kinetic curves with Eq. (1) (X_{cr}, k_{am}, k_{cr}) and results of fitting of the model curves to single-phase first-order model (Eq. (3))

X_{cr}	k_{am}	k_{cr}	k fitting	χ^2
0.2	0.9	0.4	0.767	6.46×10^{-5}
0.2	0.9	0.1	—[a]	0.00196
0.2	0.9	0.05	—[a]	0.00458
0.2	0.9	0.01	—[a]	0.01267
0.8	0.9	0.4	0.47	5.84×10^{-5}
0.8	0.9	0.1	—[a]	0.00207
0.8	0.9	0.05	—[a]	0.00495

[a] Single-phase model (Eq. (3)) does not allow a reasonable description of the data.

$$A = A_0 \exp(-kt) \tag{3}$$

checking the quality of fit by using χ^2 analysis and by visual observation of the plots (solid curves in Fig. 9). From such an analysis, as seen in Table 2, we can conclude that at a ratio of k_{am}/k_{cr} (amorphous/crystalline) of 2.25 (curve 1) the one-phase model gives a very good description

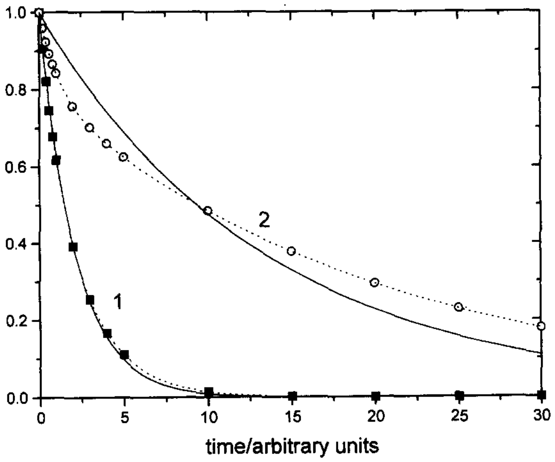


Fig. 9. Representative model kinetic curves obtained from Eq. (1), and the result of fitting to the single-phase first-order model (Eq. (3)). Points were obtained from Eq. (2) using fixed values of parameters (X_{cr}, k_{am}, k_{cr}) at different times; values of parameters are given in Table 2, row 1 for curve 1 and row 3 for curve 2. Solid lines were obtained by fitting curves 1 and 2 to Eq. (3) using k as a fitting parameter and dotted lines by fitting curves 1 and 2 to Eq. (2) using X, k_1, k_2 as fitting parameters. The fitting procedure with Eq. (2) gave exactly the same X, k_{am} and k_{cr} values as were used to generate the curves.

of kinetic data and it gives rate constants that are close to the true rate constants in the major phase (in this case this is an amorphous phase). At a ratio of k_{am}/k_{cr} equal to nine or more (curve 2), there are two distinct regions and the single-phase model does not allow one to obtain a reasonable description of the simulated kinetic data. Rather, it requires fit to Eq. (2) (dotted lines). Hence, we would conclude that if a two-phase reacting system has a smooth monotonic kinetic curve the ratio of rate constants for the individual phases is likely to be less than an order-of-magnitude different and most likely about two to three times. If the kinetic curve exhibits a distinct discontinuity, there is most likely at least one-order of magnitude difference within the two phases and individual rate constants can be obtained using Eq. (2) (assuming first-order kinetics). Thus the conclusion from this analysis is that kinetic curves for the freeze-dried preparations can be treated with a single rate equation, $A = f(t)$, whereas for milled preparations, two kinetic equations for reactivity in two phases must be used, $A_1 = f_1(t)$ and $A_2 = f_2(t)$. To choose the most appropriate equations, we considered the fact that the freeze-dried and milled preparations have differently shaped kinetic curves, but they also have one important common feature; both preparations exhibit deceleratory kinetic curves during the entire reaction. There are three types of kinetic models that can be used to treat deceleratory curves (Brown et al., 1980): models for contracting geometry (surface reactivity); models for diffusion-controlled reactions; and models based on the law of mass action. Since surface reactivity in this particular solid-state reaction is very unlikely (Shalaev et al., 1997), we have eliminated the use of a contracting geometry model. Diffusion normally can play an important role in solid-state reactions involving two substances. However, this particular reaction starts with 100% reactant and only involves the interaction of two molecules in close proximity to one another. Thus we conclude that it is most appropriate to choose a kinetic model based on the law of mass action. To choose the specific model, kinetic data for the freeze-dried preparation were fit to first- and second-order expressions and it appeared that the first-order fits were much better than the second-order fits.

For the case of TGME reactivity in the freeze-dried preparation, therefore, we have fit the kinetic data to Eq. (3) and have obtained rate constants k for the various temperatures (see Table 1). Fit of these constants to the Arrhenius expression gives values of E_a, the activation energy and A, the pre-exponential factor, of 98.4 kJ/mole and 29.11, respectively. The estimated activation energy is very close to the value of 100–107 kJ/mole obtained for the highly crystalline, unprocessed material (Shalaev et al., 1997). This provides further evidence for the earlier conclusion that processing does not change the overall chemical mechanism of this reaction.

For the milled sample, up to $\alpha = 0.70$, the kinetic data have been fit to Eq. (2), using $A_0 = 1$ and assuming that X is constant. That this assumption is reasonable can be seen in Table 3 which indicates a nearly constant amount of the original crystalline phases over the extent of reaction covered by this analysis. Table 4 provides the pertinent data for two milled samples, indicating an approximate ratio of k_{am}/k_{cryst} of about 50–70.

To further evaluate the impact of processing on the TGME system, we can attempt to interrelate the kinetic analysis of the crystalline, freeze-dried and milled samples at 100°C where we have data for all three systems. From Table 1 we can see that the rate constant from Eq. (3) for the freeze-dried sample is 0.07–0.08 h^{-1}. Applying our conclusion that the ratio of k_{am}/k_{cryst} should be no greater than about 3, we would expect the rate constant for the more reactive phase (amorphous) to be about 0.1–0.2 h^{-1} and for the less reactive phase (crystalline) about 0.05–0.07 h^{-1}. The rate constant for the faster portion of the reaction involving the milled sample (presumed to be the amorphous phase) is estimated from Eq. (1) to be about 0.15 h^{-1} (see Table 3) which is reasonably consistent with the values of 0.1–0.2 h^{-1} obtained for the freeze-dried sample. However, estimates of the rate constant for the crystal portion of the freeze-dried and milled samples are quite different, i.e. 0.0025 h^{-1} for the milled sample and 0.05–0.07 h^{-1} for the freeze-dried sample. Certainly these are highly oversimplified analyses of a complex kinetic situation, but the significant difference in apparent rate constants for the crys-

Table 3
Change in the amount of first and second crystalline phases for milled TGME during the reaction

Reaction extent, α	TGME crystalline phase 1		TGME crystalline phase 2
	Weight fraction	Relative amount[a]	Relative amount[a]
0.0	0.14	1.0	1.0
0.14	0.14	0.98	0.86
0.31	0.12	0.84	0.84
0.45	0.13	0.91	0.91
0.56	0.13	0.92	0.95
0.68	0.11	0.76	0.75
0.85	0.00[b]	0.00	0.81

[a] Relative amount has been calculated as S_i/S_0 where S_0 and S_i represent peak areas for nonreacted sample and reacted sample, respectively; peaks at $2\theta = 5.7$ and 21.4° were used for the crystalline phase 1 and 2, respectively; peak areas were normalized using a LiF internal standard.

[b] Appearance of new crystalline phase.

talline portions of the milled and freeze-dried state would suggest that processing not only affected chemical degradation of TGME through the formation of an amorphous state. Rather, it also could have resulted in residual crystalline states that have been altered with regard to their degree of disorder in different ways. Hence, freeze-drying in addition to producing an amorphous state could have produced a more reactive crystalline state of TGME than does milling of the type used in this study.

5. Conclusions

The thermally induced methyl transfer in tetraglycine methyl ester readily occurs in the highly crystalline state with an apparent autocatalytic kinetic profile. Such acceleration of the reaction with reaction extent in a single-phase region is due to increasing disorder in the crystal lattice caused by the formation of a crystalline solid solution of products in the TGME lattice. Processing, such as freeze-drying and milling, known to introduce disorder (most likely an amorphous state) into the solid, produce samples that show markedly enhanced reaction rates while producing the same intermediates and products and the same overall activation energy as seen with the crystalline sample.

The kinetic patterns, however, are different for the two processes, wherein the freeze-dried sample changes in a fairly monotonic manner and the milled sample shows an initial rapid loss of TGME followed by a slower phase. Model simulations, based on parallel reactions in the crystalline and amorphous phase, and analyses of experimental kinetic data lead to the conclusion that reactivity in the amorphous phase in both processed preparations is fairly similar. However, reactivity in the crystalline phase of the freeze-dried sample is more than one order-of-magnitude faster than that occurring in the crystalline phase of the milled sample. This strongly suggests that in addition to creating an amorphous phase with greatly enhanced disorder, processing increases the extent of disorder in the remaining crystal

Table 4
Rate constants for reactivity of two milled TGME preparations obtained by fitting experimental data to Eq. (2)

Parameter of Eq. (2)	First preparation	Second preparation
k_{am}	0.186	0.123
k_{cr}	0.0026	0.0025
X	0.763	0.822

k_{am} is the rate constant for the more reactive (amorphous) phase and k_{cr} is the rate constant for the less reactive (crystalline) phase; X is the weight fraction of the crystalline phase.

lattice. Furthermore, it appears that such processes produce different extents and types of disorder, e.g. different concentrations of point, linear (dislocations) and planar defects. This study indicates, therefore, that it is generally important to try to completely characterize the physical state of a solid material after it undergoes processing to better understand and predict the rates at which solid-state chemical reactions can occur.

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