

Low pressure phases

Thermodynamic properties of the γ -aminobutyric acid

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Special Chapter dedicated to the memory of dr. Michel Ollivon
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Abstract A metastable form of γ -aminobutyric acid has been prepared by sublimation under vacuum. A theoretical unary phase diagram has been deduced from experimental thermal analysis. Aqueous solution of γ -aminobutyric acid (Gaba) prepared from commercial product (monoclinic) does not cross blood–brain barrier after intra peritoneal administration to mice, hence it displays no anticonvulsion activity. Therefore, we developed a polymorphic form, the solution of which presents such activity. In the present paper the preparation and the physico-chemical properties are reported.

Keywords Gaba · Polymorphism · Low pressure phase

Introduction

Two forms of γ -aminobutyric acid (Gaba) have been described, a monoclinic and a tetragonal. The monoclinic structure was determined by single-crystal X-ray diffraction by Tomita et al. [1]. The parameters are: $a = 719.3$ pm, $b = 1,012.0$ pm, $c = 826.0$ pm, $\beta = 111.05^\circ$, $Z = 4$, $\rho = 1.226$ Mg m⁻³ (calculated), the powder diffraction

data are given by JCPDS (Joint Committee on Powder Diffraction Standards 38-1739) for the commercial form. The molecular geometry is a *gauche* configuration with respect to the $C\alpha$ – $C\beta$ bond. In the tetragonal form, the molecules exhibit a *trans* configuration.

The tetragonal form has been described by Dobson and Gerkin [2], which parameters are: $a = 1,196.3$ pm, $c = 1,528.2$ pm, $Z = 16$, $\rho = 1.253$ Mg m⁻³ (calculated). This form was obtained by dissolution of the monoclinic form in water, followed by slow evaporation of the aqueous solution. For the authors this tetragonal form is strongly suggestive of an intramolecular bridging hydrogen bond involving the N atom, and an O atom and a methylene H atom. In the tetragonal phase of Gaba, the zwitterionic form of the molecule is observed as in the monoclinic form. The hydrogen bonding involves the H atoms bonded to the N atom, and the two O atoms. The authors (2) found that one H atom on the C2 (α) “is the methylene H atom best situated geometrically to form an intramolecular bridge bond (N...H2A...O1) in the *trans* conformation”. The authors are particularly careful for they wrote that “in the absence of information about the charge of H(2A), the present data do not establish that bridge bonding occurs in the *trans* conformer, but they suggest that it is plausible”. In the monoclinic structure, one of the methylene H atoms is more positively charged than the others (H), and this H atom is closer to the negatively charged N and O1 atoms.

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Material and methods

Preparation

We prepared the tetragonal form by sublimation of the monoclinic form (Sigma Ultra) under vacuum (10^{-3} Pa) by

using an Edwards evaporation equipment (Auto 306). The solid is introduced in a molybdenum pan, which is heated under vacuum, and the resulting vapour settles on a Pyrex glass, which is animated with a rotation movement at room temperature.

Analysis

Various methods have been used in view to understand the behaviour of the solid phases and the aqueous solution.

X-ray

The deposited solid is then studied by X-ray analysis, by using the Philips 1050 diffractometer and a 1729 Philips X-ray generator. A computer, which controls the program of measurement and analysis, completes this equipment. We use the program “gonio” and “rayon” [3]. The anode used is Cu $K_{\alpha 1}$ ($\lambda = 1.54051 \text{ \AA}$). Measurements are performed at room temperature.

The data obtained for the monoclinic and the tetragonal forms are presented in Table 1, and the respective spectrums are presented in Fig. 1.

Table 1 X-ray patterns

Gaba (Sigma) <i>m</i>		Gaba (deposited) <i>t</i>	
<i>d</i> /Å	<i>I</i> / <i>I</i> ⁰	<i>d</i> /Å	<i>I</i> / <i>I</i> ⁰
6.6613	4	5.3744	8
6.1457	2	5.0576	2
5.3486	10	4.7765	2
4.2347	100	4.2507	100
3.9938	15	4.0368	5
3.8569	7	3.8048	8
3.7416	5	3.3933	4
3.3731	9	3.3140	5
3.3043	7	3.1486	5
3.1486	13	3.0153	8
3.0074	12	2.8429	4
2.7945	2	2.7877	4
2.6711	2	2.5362	5
2.5307	5	2.3422	3
2.4505	4	2.2651	3
2.3052	2		
2.2564	4		
2.1811	5		
2.1338	3		
2.1148	3		
2.0491	2		
2.0111	2		
1.9270	5		

The Gaba Sigma (*m*) corresponds to the monoclinic form described by Tomita et al. [1] and given in the file of JCPDS (38-1739), commercial Gaba was obtained from Sigma-Aldrich. The deposited form (*t*) corresponds to the tetragonal structure described by Dobson and Gerkin [2].

The two crystal structures demonstrate that both forms have the zwitterion configuration.

Mass density

Measurements have been performed with a Micrometrics AccuPyc 1330 pycnometer under He atmosphere at $24.7 \pm 0.2 \text{ }^\circ\text{C}$.

Data are expressed as the mean value of three series of ten measurements, and of nine series of ten measurements for, Gaba Sigma (*m*) and Gaba deposited (*t*), respectively.

Gaba Sigma <i>m</i>	$\rho = 1.2243 \text{ Mg m}^{-3}$ $\sigma = 0.0005$	calculated value: $\rho = 1.226 \text{ Mg m}^{-3}$
Gaba deposited <i>t</i>	$\rho = 1.2437 \text{ Mg m}^{-3}$ $\sigma = .006$	calculated value: $\rho = 1.253 \text{ Mg m}^{-3}$

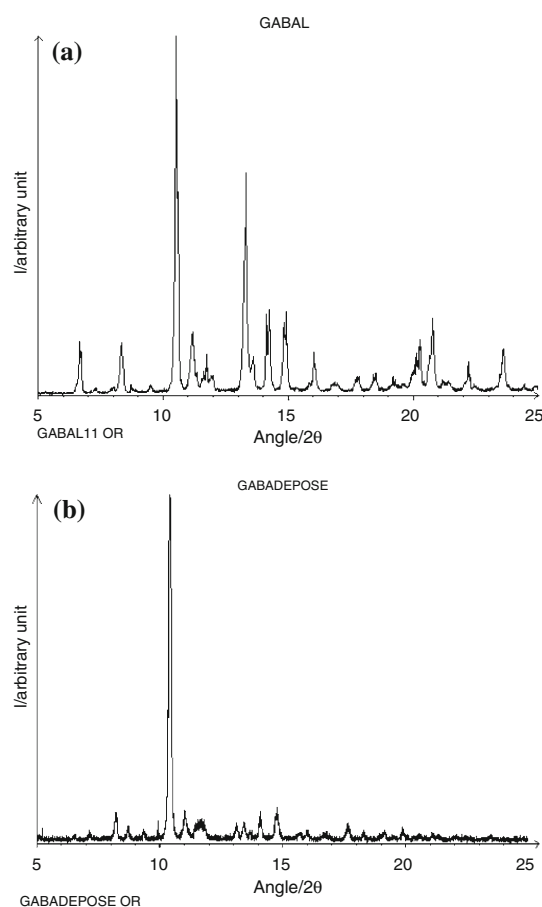


Fig. 1 X-ray patterns for Gaba *m* (a) and *t* (b)

The results of the monoclinic form are not different from the theoretical value. By contrast the tetragonal form exhibits measures different from the theoretical value and more scattered.

FTIR measurements

They are performed in a spectrophotometer (Perkin Elmer Spectrum 2000 ATR diamond) on Gaba powders. Bands are expressed in cm^{-1} with a resolution of 4 cm^{-1} . The results are:

Gaba Sigma: 1660.21, 1638.70, 1574.89, 1505.56, 1448.52, 1425.77, 1398.14, 1384.85, 1338.37, 1306.88, 1281.64, 1170.33, 1124.02, 1060.43, 1005.82, 994.42, 887.00, 868.45, 787.84, 778.02, 645.79.

Gaba deposited: 1646.53, 1568.70, 1554.20, 1531.74, 1473.55, 1447.26, 1436.87, 1427.65, 1395.84, 1381.97, 1360.00, 1338.72, 1304.94, 1282.55, 1266.59, 1240.39, 1162.95, 1123.59, 1063.05, 1027.88, 1006.79, 994.89, 948.52, 902.29, 866.66, 779.04, 753.84, 655.79.

The two spectrums show some similarity, but also a moving of some bands. Moreover, some bands are not present on both spectrums.

Differential scanning calorimetry

Thermal analysis was performed in a Perkin Elmer DSC 7 apparatus, which was calibrated in temperature and energy with the melting points of 156.634 and 231.9681 °C, for indium 5N (NIST—National Institute of Standard and Technology) and tin 5N (Koch-Light), respectively. The enthalpy of fusion is 28.44 and 59.22 J g^{-1} for indium and tin, respectively. These are the recommended values by the American Society for Materials [ASM] [4], for the enthalpy of fusion of indium and tin, and for the melting point temperature by Editor Bull. [Ed] [5]. Calibration was performed for each heating rate.

Pans are in aluminium-based alloys and covered with caps, in which holes ensure a constant pressure.

All experiments are performed under dry nitrogen gas atmosphere, at a flow of $2 \times 10^{-2} \text{ L min}^{-1}$.

Only endothermic phenomenon has been observed between 205 and 245 °C. The results are presented in Fig. 2. Two close peaks are always present, the first one corresponding to the melting of the Gaba and the second one to its vaporisation. It is worthy to notice that for the Gaba Sigma (*m*) the first peak is less intense than the second one, whereas it is the contrary for the Gaba deposited (*t*).

Results are expressed as the average value of five measurements. The onset of the first peak occurs at $212.6 \pm .5$ and 216 ± 1 °C for the Gaba Sigma (*m*) and the Gaba deposited (*t*), respectively. The variation of enthalpy between 200 and 250 °C is $1,188 \pm 8 \text{ J g}^{-1}$

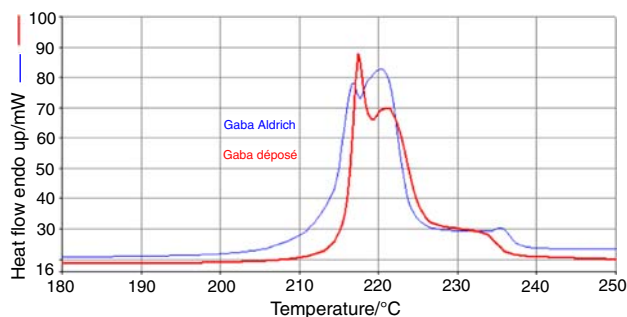


Fig. 2 DSC curves for Gaba *m* (thin curve) and *t* (thick curve)

($122.5 \pm 0.8 \text{ kJ mol}^{-1}$) and $1,147 \pm 28 \text{ J g}^{-1}$ ($118 \pm 3 \text{ kJ mol}^{-1}$) for the Gaba Sigma (*m*) and the Gaba deposited (*t*), respectively. The difference between these two values is mainly due to the difference between the enthalpies of melting of the two solid forms.

When neglecting the difference between the c_p of the liquid phase and the c_p of the monoclinic phase between 212 and 216 °C, it is possible to deduce that the difference between the two enthalpies of fusion is around 4.2 kJ mol^{-1} .

Unexpectedly, the melting temperature of the metastable phase was higher than the stable phase, which is not impossible on a thermodynamic point of view. This point will be discussed later in the present paper.

Other DSC experiments have been performed with various pressures, from 0.1 to 350 MPa , in which no phase transition was observed. Moreover, a decomposition of the molecule occurred.

Thermogravimetry

Enthalpies of sublimation and vaporisation of Gaba *m* have been measured by thermogravimetry using the method described by Price [6]. Experiments have been performed with a TGA 7 (Perkin-Elmer). The results are the average of three runs, enthalpy of sublimation is deduced from measures between 182 and 202 °C and the enthalpy of vaporisation between 220 and 227 °C:

$$\Delta_{\text{sub}}H = 139 \text{ kJ/mol} \pm 4 \text{ or } (1,350 \text{ J/g} \pm 40)$$

$$\Delta_{\text{vap}}H = 87 \text{ kJ/mol} \pm 2 \text{ or } (844 \text{ J/g} \pm 20)$$

Unfortunately for Gaba *t* the dispersion on the measurements are too important and the results cannot be retained.

Calorimetry

c_p measurement c_p measurements have been performed with the C80 calorimeter on Gaba Sigma (*m*) and Gaba deposited (*t*) between 303 and 363 K , according to the method described by Legendre et al. [7].

The results are given on this form: $c_p = A_0 + A_1T$.

Gaba Sigma	$A_0 = 27.423$	$A_1 = 0.3936$	c_p (J K ⁻¹ mol ⁻¹)
Gaba deposited	$A_0 = 72.834$	$A_1 = 0.2575$	c_p (J K ⁻¹ mol ⁻¹)

The measured values for the Gaba Sigma are in the same range than those measured by Skoulika and Sabbah [8].

A study devoted to the thermodynamic of ω amino-acids by Skoulika and Sabbah [8] is interesting in view of checking the validity of our measurements.

The enthalpy of sublimation at T is:

$$\Delta_{\text{sub}}H(T) = \Delta_{\text{sub}}H(298 \text{ K}) + \int_{298}^T (c_p^g - -c_p^s) dT$$

$$\Delta_{\text{sub}}H(298 \text{ K}) = 140 \text{ kJ mol}^{-1}$$

The c_p values given by Skoulika and Sabbah [8] are 135.8 J K⁻¹ mol⁻¹ between 298 and 350 K and 158.1 J K⁻¹ mol⁻¹ between 350 and 485 K for the gas phase. For the monoclinic phase, the c_p are 133.6 and 182 J K⁻¹ mol⁻¹ for the same range of temperature.

From these values, the calculated enthalpy of sublimation around the melting temperature is 137 kJ mol⁻¹, this is in a perfect agreement with the value that we obtained by TGA. The enthalpy of sublimation at the same temperature is equal to the sum of the enthalpies of fusion and vaporisation. However when using the value we measured for the c_p of the solid phase extrapolated up to the melting point the value of the enthalpy of sublimation is equal to 128.5 kJ mol⁻¹, which is similar to our measured value (by DSC) of 122.5 kJ mol⁻¹. Therefore, we can assume that our results are in the same range.

The c_p of the Gaba deposited was measured just after preparation, then 2 weeks later. In these conditions, the values of the two c_p were very close. From these experiments, we deduced that the evolution in the solid state is important.

Heat of dissolution The heat of dissolution in an aqueous solution of NaCl (9 g/1,000 g H₂O) was measured in a C 80 (Setaram) at 36.34 °C.

The results are the mean values of two independent experiments:

Gaba Sigma	$\Delta_s H = -34.1 \pm 0.1 \text{ J g}^{-1}$ (-3,516.4 J mol ⁻¹)
Gaba deposited	$\Delta_s H = -49 \pm 1 \text{ J g}^{-1}$ (-5,090.0 J mol ⁻¹)

Results are always less reproducible with the deposited product than with the Sigma product.

These results suggest that the enthalpy necessary to transform the monoclinic phase into a quadratic phase at 37 °C is 1,574 J mol⁻¹. Nevertheless, this is an approximation as it will be explained later.

Solution

Gaba Sigma and Gaba deposited were dissolved in NaOH 0.1 N aqueous solution. Then UV spectroscopy was performed at 20 °C.

The results are the mean value of 10 measurements.

Gaba Sigma (*m*)

Wavelength	$\lambda = 215.5 \text{ nm}$	$\sigma = 0.24$ (standard deviation)
Absorbance	$A = 0.0800$	$\sigma = 0.0017$
	$\varepsilon = 0.7956 \text{ g}^{-1} \text{ L}$	$\sigma = 0.0556$

Between the 10 successive measurements, a variation with time, from 218.5 to 217.0 nm, was observed in the wavelength for which the absorbance is maximum, and a variation of the absorbance from 0.0455 to 0.062.

Gaba deposited (*t*)

Wavelength	$\lambda = 217.0 \text{ nm}$	$\sigma = 0.71$
Absorbance	$A = 0.058$	$\sigma = 0.0017$
	$\varepsilon = 0.5644 \text{ g}^{-1} \text{ L}$	$\sigma = 0.0556$

Forty hours later the results are:

Gaba Sigma (*m*)

Wavelength	$\lambda = 215.0 \text{ nm}$	$\sigma = 0$
Absorbance	$A = 0.115$	$\sigma = 0.0162$
	$\varepsilon = 1.083 \text{ g}^{-1} \text{ L}$	$\sigma = 0.016$

Gaba deposited (*t*)

Wavelength	$\lambda = 215.0 \text{ nm}$	$\sigma = 0$
Absorbance	$A = 0.109$	$\sigma = 0.009$
	$\varepsilon = 1.061 \text{ g}^{-1} \text{ L}$	$\sigma = 0.089$

These experiments are of major importance, since the physical properties of the two solutions are different. However, the properties of the solution of Gaba deposited (*t*) are time-dependent. This may be explained by a relaxation of the molecule of Gaba deposited (*t*) in solution, the evolution of the solution tending towards the thermodynamic equilibrium. Therefore, we should be careful in the measurement of the heat of dissolution.

In fact, the biologic properties of a solution of Gaba deposited (*t*) are exhibited only for fresh preparations.

Results and discussion

As for the monoclinic form no transition is observed between room temperature and the melting temperature for a pressure of 0.1 MPa the monoclinic—tetragonal transition is not an enantiotropic case. Since the melting temperature of the metastable phase (tetragonal) was higher

that the one of the stable one, it seems that this product presents an unusual behaviour. In view to understand this case, it is necessary to redraw the way of preparation of the tetragonal phase, and to deduce what could be the unary $P = f(T)$ phase diagram.

It is possible to propose a theoretical unary phase diagram $P = f(T)$ for Gaba (Fig. 3), for a pressure of 0.1 MPa the monoclinic phase melt at 212 °C and then one or two degree after crystallise in the tetragonal phase, and melt at 216 °C, the temperature of vaporisation is very close to this melting point. This case is not usual and the question is to know if this is possible on a thermodynamic point of view, we have drawn the curve $G = f(T)$ (Fig. 4) for atmospheric pressure, as we can see the free enthalpy curve of the tetragonal phase (G_t) cross two times the free enthalpy curve of the liquid phase (G_l), and (G_t) cross the free enthalpy curve of the monoclinic phase (G_m) at a higher temperature that the melting temperature of the monoclinic phase. This phase arrangement is not very usual but not impossible and is well known in the solid state for iron for which G_α cross two times G_γ . This hypothesis is supported by the way of preparation of the tetragonal phase and the calorimetric analysis. At room temperature and atmospheric pressure the stable phase is the monoclinic phase, whereas at low pressure it is the tetragonal phase. The pathway leading to the metastable tetragonal phase may be divided into several steps. First, in the evaporator when the vacuum is obtained (A to B) the “m” phase is brought at low pressure but not transformed in “t” phase for kinetic reasons; second, this phase is vaporised (B to C) by heating; third when deposited on the cold glass (at room temperature), the vapour is solidified in “t” phase (C to B). When the vacuum is broken, the “t” phase may be collected. The kinetics of transformation from “t” to “m” is too slow to appear during this operation.

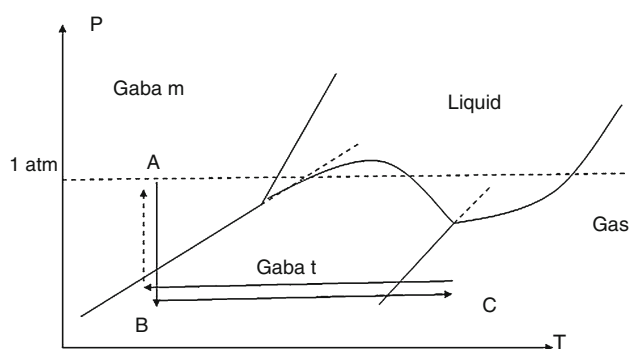


Fig. 3 Theoretical unary Gaba phase diagram $P = f(T)$

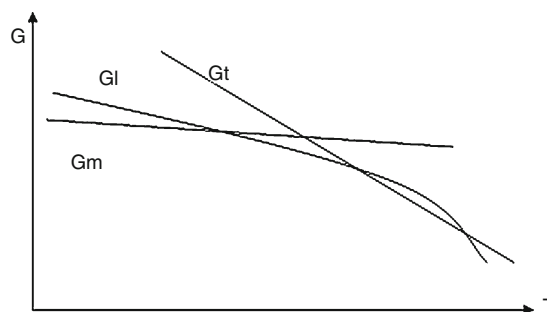


Fig. 4 Theoretical Gibbs energy of Gaba $G = f(T)$

The other interesting point for this compound is the behaviour of the tetragonal phase in solution. Indeed, the heat of dissolution of the two solid phases is different. Moreover, the solution obtained from the tetragonal phase is not immediately in equilibrium, the time of relaxation is rather long (24 h). The kinetics of relaxation will be studied latter on. More importantly, only the fresh recently prepared solution of the tetragonal phase presents anti-convulsant properties compared to a solution obtained from the monoclinic phase [9].

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