Physico-Chemical Characterization of γ-Amino *n*-Butyric Acid Nanoparticles

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This work deals with the preparation and characterization of high-purity nanoparticles of γ-amino *n*-butyric acid (GABA) in order to enhance the efficiency of this drug. A sublimation procedure at low pressure was applied to GABA after improving the experimental parameters of this physical transformation. The elaboration of the molecule is solvent-free. The process does not change the chemical formula of the compound but modifies its physico-chemical characteristics. In this work, we present the experimental parameters for preparing monoclinic GABA nanoparticles. Their identification and physico-chemical properties were determined with a large number of investigations: Powder X-ray diffraction (PXRD), density and purity measurements, differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA), calorimetric measurements ($\Delta H_{dissolution}$ and C_p), thermally stimulated current (TSC), and electrochemical impedance.

Key words γ-amino *n*-butyric acid; nanoparticle; sublimation; polymorph; X-ray diffraction

For some decades, extensive research has contributed to the development of new pharmaceutical molecules or to the discovery of new therapeutic uses for already existing molecules. The elaboration process constitutes the essential operation in the shaping of the solid. Controlling this process is therefore a crucial parameter since it enables not only the desired phase or polymorph, but also the desired size, to be obtained. Polymorphism is the ability of a crystalline material to exist in more than one crystal structure. A significant number of pharmaceuticals exhibit this phenomenon. Investigations dealing with the polymorphism of drug substances usually focus on the characterization of the different forms and on the determination of their different physical properties. Polymorphs can be produced by a variety of strategies, including conventional crystallization from solution using different solvents, crystallization in the presence of additives that promote nucleation of a specific polymorph or by physical transformation. Understanding and controlling such crystallization processes are clearly of paramount importance in many aspects of polymorphism research.

For several years, we have been carrying out a study on amino acid polymorphs synthesized by sublimation such as glycine,[1] γ-amino butyric acid (GABA)[2] and leucine. The polymorphism exists in the crystal structures of amino acids with different packing arrangements and hydrogen bonding patterns of the zwitterions.

This paper presents new results on γ-amino butyric acid (GABA). Its formula is $H_2N–(CH_2)_3–COOH$. However, in solution and in the solid state, the chemical species can be in the form of zwitterions $^+H_3N–(CH_2)_3–COO^-$ while in the gas phase only the neutral form exists. This molecule has particular physico-chemical properties, due to the presence of the proton donor carboxyl acid (–COO) group and the proton acceptor amino ($–NH_2$). GABA exists in two polymorphic forms: either the monoclinic structure with the space group ($P2_1/a$)[3—6] or the tetragonal structure with the space group ($I4_1cd$).[7] The network of the intermolecular hydrogen bonds plays an essential role in the zwitterionic organisation of these crystal structures. The monoclinic and tetragonal crystals can be obtained respectively by crystallization in ethanol[3,4] or aqueous solution.[7]

GABA is a major inhibitory neurotransmitter in the central nervous system and its biological characteristics were investigated in a previous paper.[2] As the GABA molecule cannot cross the hemato–encephalic barrier (HEB), its neurotransmitter activity is only observed from GABA present in the body and released by neurones, and not by food intake.

Biological analyses[2] have demonstrated the therapeutic activity of sublimated GABA. When administered intraperitoneally, a dose of 25 mg/kg proved effective against audiogenic seizures, whereas for non-sublimated GABA, doses up to 100 mg/kg were unable to prevent audiogenic seizures. A considerable biological improvement was observed by modification of the experimental parameters of the process.

The work reported here has focused on a sublimation mechanism that produces a polymorph. The sublimation of GABA (organic compound) was carried out with an apparatus generally used for metals. However, it was first necessary to determine the best experimental parameters (pressure, sublimation temperature, weight of the sample, speed of rotary substrate, speed of deposition rate, and distance of the collecting surface from the material).

The present work proposes new experimental parameters to prepare a novel form of GABA so as to optimize its therapeutic potential. Identification of the compound generated by sublimation of GABA was prompted by its biological behaviour, which is distinct from that of non-sublimated GABA and sublimated GABA prepared as in reference.[2]

Experimental

Material and Preparation of the Nanoparticles The GABA (Sigma Co., Germany) used is 97% pure. The molecule will be referred to as 'commercial GABA' hereafter. When it has been submitted to a physical procedure, it will be referred to as 'sublimated GABA.'

The apparatus used was an Edwards AUTO 306 Joule effect evaporator. Its description and the sublimation process have been detailed in a previous paper.[1] In this work, the process parameters of GABA sublimation were improved. The deposition rate was fixed at 0.1 nm/s. This is a relatively low speed, given that it can reach 999.9 nm/s. The sublimation temperature was reduced from 250[2] to 190 °C by changing the transformer and the evapora-

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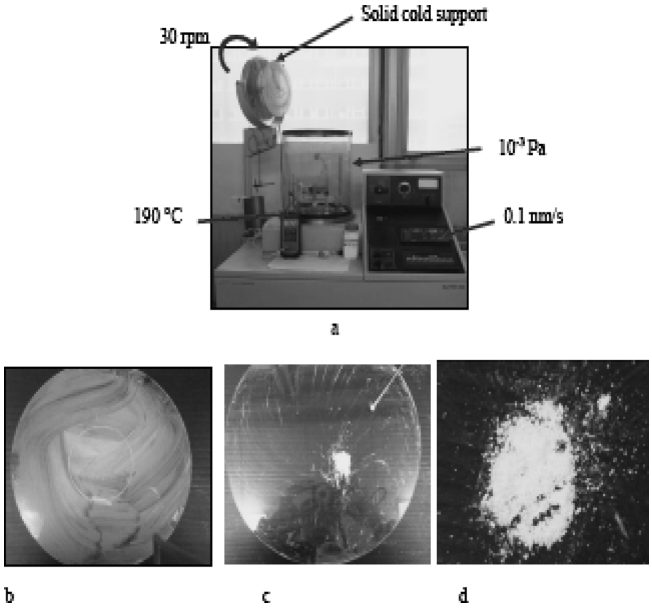

Fig. 1. Sublimated GABA onto a Solid Cold Support (a), Sublimated GABA Collected (b—d)

Sublimated GABA is prepared according to the procedure presented in the text.

tor meter. The apparatus had been previously designed and used for the deposition of metals and its maximum temperature was 800±10 °C. Currently the temperature is limited to 300±1 °C.

A weight (40 mg) of commercial GABA was placed in a molybdenum crucible connected to the adjustable power supply. GABA was introduced at room temperature and at 10^5 Pa pressure in a bell jar. The pressure was then lowered down to 10^{-3} Pa. An increase in temperature (190 °C) allowed the sublimation of the commercial GABA at a pressure lower than the triple point. Crystallization occurred from the gas phase, without formation of liquid phase. Cooling was achieved by heat transfer of the gas onto a disc of Pyrex® glass (Fig. 1a). This rotary substrate carrier is situated at a distance from d=17 cm, vertically above the molybdenum crucible. Its temperature is comprised between 20 °C and 25 °C and its rotation speed was controlled by a solid state speed controller. The rotation speed selected was r=30 rpm (from a possible range of 20 to 60 rpm). The sublimated GABA was then collected (Figs. 1b—d) and placed in a container into which nitrogen was introduced; a vacuum was then applied. No solvent was used. This process yields layers that are between a few Angstrom and some micrometers thick. The thinness of the layers can induce different properties from those of the same material prepared in larger amounts.

Characterization. Powder X-Ray Diffraction (PXRD) Diffraction measurements were made on a high resolution prototype diffractometer based on a Rigaku UltraX 18 generator with a copper rotating anode used under 300 mA and 50 kV equipped with a graphite back monochromator. Recordings were made at ambient temperature between 10 and 50° 2θ using steps of 0.04° 2θ counting 30 s per point. Calibration of the apparatus was verified with Si.

Particle Size Analysis In order to investigate the size of the particles (mean diameter), they were observed by transmission electron microscopy (TEM EN208 Philips). For analysis, the samples were placed on copper grids with Formwar® films then diluted with demineralised water. Water was filtered with a paper filter. The sample was dried under vacuum. Particle size was calculated for several preparations.

Density Density was determined with an AccuPyc 1330 Pycnometer by measuring the pressure change of helium in a calibrated volume.

Thermal Analysis Thermal analysis was performed with a TA Instrument Q1000 under nitrogen flow. The software used is 2002 TA Instrument Advantage version 2.8.0. Samples were introduced in aluminum pans and covered with holed caps in order to avoid uncontrolled variations in pressure. Calibration of temperature was performed with indium (156.634 °C) and tin (231.9681 °C) of quality 5N for heating rates of 5 °C/min, the values recommended by the American Society of Materials (ASM).[8] For the calibration of purity analysis, benzoic acid was used at a heating rate of 1 °C/min. The parameters obtained from this analysis were 99.97 mol% for purity and 1.899% for correction.

Thermo Gravimetric Analysis (TGA) TGA was performed with a TA Instrument Q500. The software used was 2002 TA Instrument Advantage version 2.8.0. Calibration was performed at different temperatures using the Curie magnetic transition for the recommended alloys, Alumel (163 °C) and Nickel (354 °C). Calibration (weight) was performed using a standard mass of 100 mg and calibration of the furnace was done between 100 and 900 °C to verify the thermocouple operation. All the experiments were performed under dry nitrogen with a flow of 6.10^{-2} l/min. Analysis of compounds under nitrogen purging was performed at 20 °C/min in order to determine the precise percentage of weight loss.

Calorimetric Measurements C_p Heat capacity was determined with a C80 calorimeter (Setaram) by varying the temperature by 0.1 °C/min between 50 and 100 °C. Calibration and energy were performed with the melting points of indium and tin. A weight of about 200 mg was put in a glass cell which was then introduced into a stainless steel vessel. The C_p was measured using the continuous method:

$$C_p = \frac{1}{m}\left(\frac{\delta Q}{\delta T}\right)_p = \frac{1}{m}\left(\frac{\delta Q}{\delta t}\right)\left(\frac{\delta t}{\delta T}\right)_p$$

in which m is the weight of the sample, Q is the heat (at constant pressure), T the temperature and ($\delta T/\delta t$) is the scanning rate used at this temperature.

Calorimetric Measurements $\Delta H_{dissolution}$ The C80 calorimeter (Setaram) was used to determine the heat of dissolution ($\Delta H_{dissolution}$) in water with an isotherm mode (298 K). A reversal mixing vessel was used. The GABA weight and water volume were known. GABA and water were placed in two chambers of the reversal mixing vessels into the calorimeter. Thermal equilibrium was reached after 7 h, ensuring that the two separate components were at the same temperature. Mixing was performed by reversing the calorimeter. Ten rotations of the calorimeter were necessary in order to obtain a good mix. The duration of one experiment was 10 h. The calorimeter calibration was performed with the dissolution of potassium chloride in water.

Thermally Stimulated Current (TSC) The process consists in the accumulation and discharge of electrically active species occurring at specific locations within the sample. Experiments were carried out with a TSC Setaram. The temperature range studied was between −170 °C and 300 °C. The system comprised three electrodes: a calibration electrode composed of a stainless steel electrode and a receptacle in which a sample of indium was placed; an electrode for films and pellets composed of a point electrode and a circular electrode; an electrode for liquids composed of a point electrode and a capsule with an upper removable part. A pellet of about 25 mg and 1 mm thick was under a vacuum of approximately 0.05 mbar applied in the chamber which was filled with dry helium (overpressure of 1100 mbar). The sample was then heated to polarization temperature T_p=160 °C and held there for a polarization time t_p=1 min. An electric field of 200 V/mm was applied to allow the different mobile entities of the material to orient themselves according to the field. This configuration was then fixed by quenching to freezing temperature T_0=−170 °C for a time t_0=1 min with liquid nitrogen, which freezes the dipole orientation. At T_0, the field was cut off. The sample was then heated to final temperature T_f=200 °C at the rate 7 °C/min. The depolarization current was recorded from −170 to 200 °C with a Ketley electrometer.

Electrochemical Impedance Measurements The impedance measurements in aqueous solution were carried out using VoltaLab 80 (Radiometer Analytical) with a 2-pole cell based on two plates of platinum. The frequency range studied was 100 kHz—100 mHz. The signal amplitude was 100 mV. The solution preserved under nitrogen was placed in a double-walled glass cell ensuring thermal stabilization as described in ref. 1. Measurements were performed at 20 °C. The electrochemical impedance $Z(\omega)$ of the sample between the two plates of the conductivity cell is a complex number which can be represented in polar coordinates by its module $|Z|$ and its phase, and in cartesian coordinates by its real part Z' and its imaginary part Z'' with $Z(\omega)=Z'+iZ''$. At the time of frequency scanning, the experimental points were distributed on a curve having the form of an arc of a circle. The curve is called a Nyquist diagram ($-Z''$ *versus* Z'). Z' (at low frequency) is the impedance when 'Z' crosses the OZ axis (real axis). Demineralised water is prepared with an ion exchange resin type E500, N°2204, R=3.7 MΩ.

Results

In this work, the product was obtained by sublimation at low pressure using an Edwards Auto 306 evaporator. During

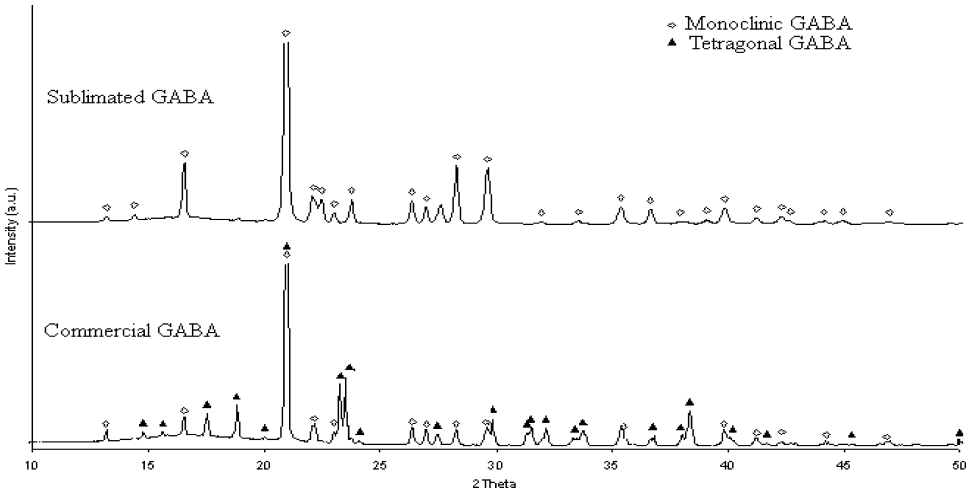

Fig. 2. X-Ray Powder Patterns of Commercial GABA and Sublimated GABA Obtained at Ambient Temperature between 10 and 50° 2θ

On the *Y*-axis: intensity of the diffraction peaks. On the *X*-axis: diffraction angle (2θ). Their indexing was obtained from monoclinic GABA monocrystals[4] and tetragonal GABA monocrystals.[7]

this step, it is important to determine the best experimental parameters responsible for the synthesised product. It is evident that the reproducibility of the product is essential to preserve its therapeutic virtues. This procedure does not modify the molecular formula *i.e.* the starting organic compound and the end product both have the same chemical formula, that is the same qualitative and quantitative atomic composition. This process presents a considerable advantage in toxicity terms because no solvent is used during the physical transformation of the product. The potential of the therapeutic function of the synthesised product was studied from its physico-chemical analysis.

Several experimental methods (structural study by X-ray diffraction, density measurements, differential scanning calorimetric analysis (DSC), thermo gravimetric analysis (TGA), calorimetric measurements (C_p and $\Delta H_{dissolution}$), thermally stimulated current (TSC), and electrochemical impedance) were used to highlight the specific physico-chemical properties.

In order to identify the polymorphs before and after the change, an X-ray crystallographic study on powder was carried out. Figure 2 clearly shows different XRD patterns. To interpret these patterns, two hypotheses can be considered: one is the JCPDS (Joint Committee on Powder Diffraction Standards); the other draws on work on monoclinic GABA monocrystals[4] and tetragonal GABA monocrystals.[7] Under the first hypothesis, several diffraction peaks of the two patterns were not indexed. The second hypothesis was therefore adopted and Tables 1 and 2 confirm a good indexation of the diffraction peaks. The indexing was carried out from literature data obtained at ambient temperature on monoclinic GABA monocrystals[4] and tetragonal GABA monocrystals.[7] The reticular distances of the diffraction peaks and their attribution are presented in Tables 1 and 2 for respectively the commercial GABA and the sublimated GABA. Indexing demonstrates that the commercial GABA is a mixture of monoclinic and tetragonal forms, whereas the sublimated GABA is composed only of the monoclinic form.

Moreover, an increase in the width of the X-Ray diffraction peaks of sublimated GABA is observed. This is often in-

Table 1. Diffraction Peaks Indexing Commercial GABA X-Ray Powder Patterns

2θ	*dhkl* (Å)	Intensity (%)	Polymorph	*h*	*k*	*l*
13.160	6.7222	2.8	monoclinic	1	0	0
14.760	5.9967	2.6	tetragonal	0	2	0
15.619	5.6690	2.5	tetragonal	1	1	2
16.538	5.3559	4.8	monoclinic	−1	1	1
17.533	5.0541	5.4	tetragonal	1	2	1
18.811	4.7135	6.6	tetragonal	0	2	2
20.004	4.4350	1.8				
20.947	4.2375	100.0	monoclinic	0	2	1
			tetragonal	2	2	0
22.158	4.0085	4.0	monoclinic	1	1	1
22.560	3.9380	1.4	monoclinic	−1	2	1
22.999	3.8637	2.6	monoclinic	0	0	2
23.285	3.8170	9.8	tetragonal	0	0	4
23.481	3.7856	10.9	tetragonal	1	3	0
23.761	3.7416	2.1	monoclinic	−1	1	2
24.120	3.6867	1.3	tetragonal	1	2	3
26.382	3.3755	3.3	monoclinic	−2	1	1
26.985	3.3014	2.9	monoclinic	1	2	1
27.450	3.2466	2.2	tetragonal	2	3	1
28.268	3.1544	3.0	monoclinic	−2	0	2
29.623	3.0131	3.1	monoclinic	−1	3	0
29.840	2.9917	4.6	tetragonal	0	4	0
31.321	2.8535	2.4	tetragonal	4	1	1
31.484	2.8392	3.2	tetragonal	2	2	4
32.124	2.7840	3.2	tetragonal	3	2	3
33.380	2.6821	1.6	tetragonal	3	1	4
33.737	2.6545	2.7	tetragonal	1	2	5
35.441	2.5307	2.9	monoclinic	0	4	0
36.741	2.4441	2.0	tetragonal	1	1	6
37.960	2.3684	1.9	tetragonal	3	4	1
38.346	2.3454	5.8	tetragonal	2	0	6
39.872	2.2591	2.6	monoclinic	−2	2	3
40.200	2.2414	1.7	tetragonal	3	2	5
40.762	2.2118	0.8	monoclinic	1	3	2
41.227	2.1879	1.6	monoclinic	1	4	1
41.680	2.1652	0.9	tetragonal	4	3	3
42.300	2.1349	1.2	monoclinic	−1	3	3
44.235	2.0459	1.3	monoclinic	0	3	3
45.315	1.9996	0.8	tetragonal	5	1	4
46.907	1.9353	1.2	monoclinic	3	1	1
49.983	1.8232	1.1	tetragonal	2	3	7

Table 2. Diffraction Peaks Indexing Sublimated GABA X-Ray Powder Patterns

2θ	*dhkl* (Å)	Intensity (%)	Polymorph	*h*	*k*	*l*
13.198	6.70269	2.1	monoclinic	1	0	0
14.395	6.14784	2.6	monoclinic	0	1	1
16.557	5.34983	13.4	monoclinic	−1	1	1
18.863	4.7005	1.7				
20.925	4.24175	100	monoclinic	0	2	1
22.113	4.0165	6.3	monoclinic	1	1	1
22.458	3.95557	5.7	monoclinic	−1	2	1
23.025	3.85939	2.9	monoclinic	0	0	2
23.752	3.74295	5.7	monoclinic	−1	1	2
26.361	3.3781	5.3	monoclinic	−2	1	1
26.985	3.3014	4	monoclinic	1	2	1
27.601	3.2291	4.5				
28.295	3.15145	12.7	monoclinic	−2	0	2
29.615	3.01391	12.2	monoclinic	−2	1	2
30.744	2.90581	0.9				
31.927	2.80076	1	monoclinic	−2	2	0
33.539	2.66977	1.2	monoclinic	1	3	1
35.372	2.53549	4.1	monoclinic	0	4	0
36.645	2.45026	3.6	monoclinic	−2	1	3
37.927	2.37035	1.1	monoclinic	−1	4	0
39.062	2.30406	1.5	monoclinic	−2	3	2
39.852	2.26017	3.9	monoclinic	−2	2	3
41.222	2.18816	1.9	monoclinic	1	4	1
42.311	2.13433	2.2	monoclinic	−1	3	3
42.68	2.11673	1.3	monoclinic	−3	2	2
44.117	2.05105	1.2	monoclinic	−3	2	0
44.946	2.01515	1.3	monoclinic	−2	0	4
46.952	1.93362	1.1	monoclinic	3	1	1

dicative of a modification in texture. Size measurements of these particles determined by transmission electron microscopy confirm this, showing a decrease in particle size. The mean diameters deduced from thirty measurements for commercial GABA and sublimated GABA are about 800 nm and 120 nm respectively.

During the sublimation process, GABA (solid phase) was transformed into neutral species (gas phase). From an ordered packing arrangement (solid phase), it changed directly to a disordered structure (gas phase) without liquefying. During the condensation process, it recrystallized in an ordered packing arrangement, *i.e.* a monoclinic structure. This process in gas phase consisted in generating a vapour leading to the formation of a nanoparticle solid. During condensation, similar to a rapid quench, the particles have not time to grow. It should be noted that at the nano scale, ordinary substances can exhibit novel physico-chemical and biological properties which do not exist on a larger scale.

The density measurements confirmed the transformation of the GABA crystallographic structure after sublimation. Thirty density measurements were performed, and gave different values for commercial GABA and sublimated GABA. The mean values for commercial GABA and sublimated GABA were 1.219 ± 0.001 g/cm^3 and 1.246 ± 0.001 g/cm^3 respectively. Sublimation led to a highly dense material with a small particle size.

Two kinds of measurements in thermal analysis were carried out: differential scanning calorimetric analysis (DSC) and thermo gravimetric analysis (TGA). The first shows thermograms (Fig. 3) of the two GABA forms at a heating rate of

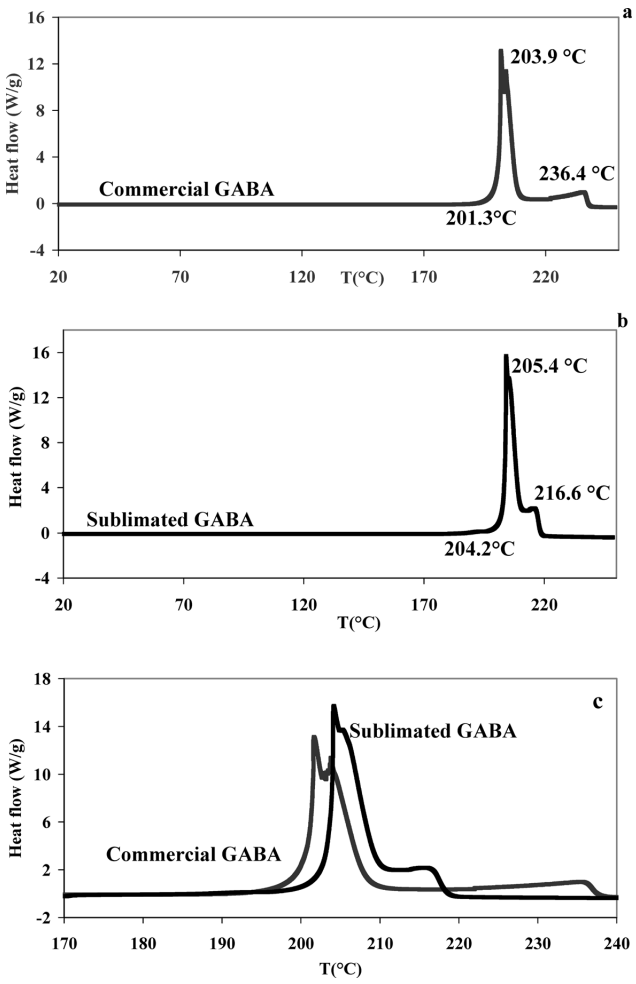

Fig. 3. DSC Curves of Commercial GABA and Sublimated GABA Obtained at a Heating Rate of 5 °C/min between 20 and 250 °C (a, b) and between 170 and 240 °C (c)

For each thermogram, the temperature of the first peak is read at the onset, and the other two at the top. On the *X*-axis, the temperature readings, expressed in degrees Celsius (°C). On the *Y*-axis, the heat flow signal, expressed in watts per gram (w/g).

5 °C/min with a similar general curve. No phase transition was observed (Figs. 3a, b). They presented three endothermic peaks between 200 and 240 °C that correspond to melting and vaporization. Their melting temperatures differed by 3 °C. The mean values of fifteen measurements of each form were at the onset 201.3 °C and 204.2 °C for commercial GABA and sublimated GABA respectively. The values of enthalpy variations were 121.82 kJ/mol for commercial GABA and 104.87 kJ/mol for sublimated GABA. During the cooling run of thermal analysis, no peak was observed. The second measurement shows no significant difference between the two products. The thermo gravimetric analysis was carried out from 30 to 300 °C (Fig. 4). The curves showed a weight loss for commercial GABA and sublimated GABA of 99% and 99.9% respectively. These weight losses start at the same temperature and correspond to the decomposition of the two GABA materials. This analysis also showed that they do not contain water.

The commercial GABA and sublimated GABA have different heat capacity C_p and heat of dissolution properties. The heat capacity C_p data were processed with the Setaram C_p program which proposes a polynomial for the analytical

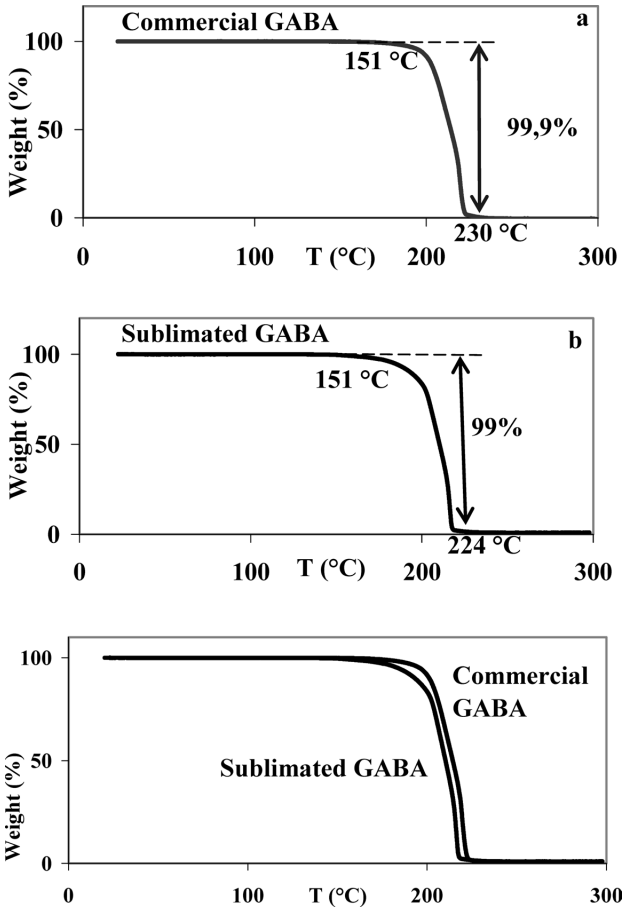

Fig. 4. TGA Curves of Commercial GABA and Sublimated GABA Obtained at a Heating Rate of 20 °C/min between 20 and 300 °C

On the *X*-axis, the temperature readings, expressed in degrees Celsius (°C). On the *Y*-axis, the heat flow signal, expressed in weight %.

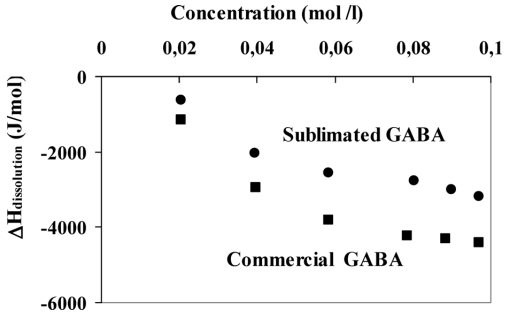

Fig. 5. Dissolution Enthalpy of Commercial GABA and Sublimated GABA at 25 °C

On the *X*-axis, the concentration readings, expressed in mol per liter (mol/l). On the *Y*-axis, the heat of dissolution $\Delta H_{dissolution}$, expressed in joule per mol (J/mol).

description of C_p: $C_p = a + bT + cT^2 + \cdots$, C_p in J/g K and T in K. For commercial GABA and sublimated GABA, it was necessary to obtain the best polynomial fit. The results are distinct for the two GABA materials:

commercial GABA:

$$C_p = 37.51978 - 2.15917E - 002T + 3.20155E - 004T^2$$

sublimated GABA:

$$C_p = 8.90153 - 4.42029E - 002T + 6.79828E - 005T^2$$

For the sublimated GABA (monoclinic form), the C_p value at 373 K is 1.872 J/g K or 193.1 J/mol K which agrees with that measured (182.0 J/mol K) by Skoulika and Sabbah.[9]

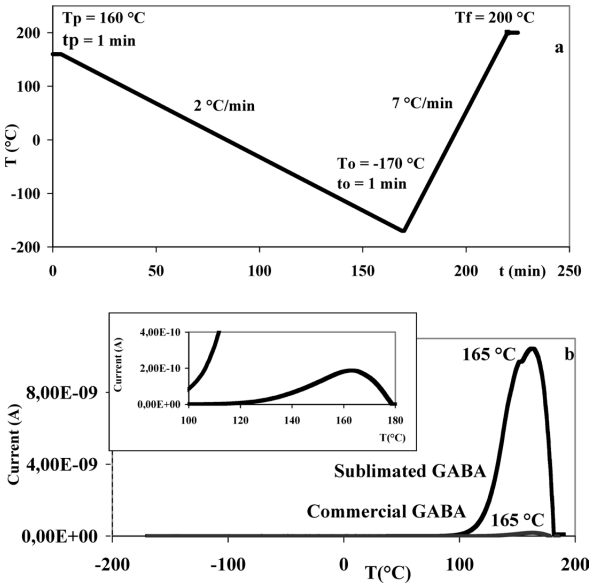

Fig. 6. Schematic Representation of the TSC Procedure (a) and TSC Global Spectrum of Commercial GABA and Sublimated GABA Obtained at a Heating Rate of 7 °C/min between −170 and 150 °C (b)

The experimental conditions were the same for the two GABAs: polarizing electric field, E=200 V/mm; polarization temperature, T_p=160 °C; freezing temperature, T_0=−170 °C; final temperature of the heating ramp, T_f=200 °C; heating rate, r=7 °C/min.

The two GABA have an exothermic heat of dissolution (Fig. 5). Substances that evolve heat as they dissolve must show a decrease in solubility with an increase in temperature.

Thermally stimulated current and electrochemical impedance measurements enabled us to determine the mobility of these products. Thermally stimulated current was applied for the characterization of the molecular mobility of the GABA compound whose molecule is asymmetric and carries a sizable permanent dipole moment. The schematic representation of the TSC procedure and the variation in the depolarization current *versus* temperature corresponding to the TSC spectrum are shown in Fig. 6. One depolarization peak corresponding to relaxation of the oriented dipoles was observed for commercial GABA and sublimated GABA. Analysis of these TS peaks showed that they correspond to a dipolar motion and are indicative of molecular mobility. These peaks have an identical temperature location in both GABAs and have a temperature of maximum current (2×10^{-10} A for commercial GABA and 1.1×10^{-8} A for sublimated GABA) at 165 °C. The GABA molecule contains two functional groups (carboxyl acid and amino groups) which induce molecular mobility due to the presence of labile hydrogen. This phenomenon can be attributed to dielectric relieving which is related to charge movements. However, the current is much higher for sublimated GABA than for commercial GABA. Relaxation at 165 °C in sublimated GABA is much greater than that in commercial GABA. The difference in structure leads to the decrease or the increase in the dielectric strength of relaxation in the material. Two explanations can be proposed for sublimated GABA: the increase in the dipole number of the entities involved, and/or in the amplitude of the dipolar reorientations.

An analysis of aqueous solution GABA was also carried out by electrochemical impedance measurements. The Nyquist curves of the commercial and sublimated GABA of the 5×10^{-3} mol/l concentration are plotted in Fig. 7. The existence of a semi-circle is typical of the presence of a simple charge transfer. At low frequency, the impedances of the commercial GABA (361.2 kohm cm^2) and sublimated GABA (202.6 kohm cm^2) solutions were distinctly different, indicating that the mobility of sublimated GABA was greater than that of commercial GABA. Compared to the Nyquist curve for water, its impedance is 654.5 kohm cm^2. The electronic transfer between the sublimated GABA in water and the platinum cell is easier than for commercial GABA.

During the elaboration process, GABA (solid state) receives an amount of energy by joule heating. This energy contributes to its sublimation. From a mainly zwitterion configuration, it converts to a molecular configuration (neutral form). During the condensation process, a proton is transferred from a COOH group to an NH_2 group and GABA regains its zwitterion configuration. Thermally stimulated current and electrochemical impedance measurements show that sublimated GABA has a higher relaxation and a greater conductivity. This can be explained by the formation of a greater number of zwitterions for sublimated GABA than for commercial GABA.

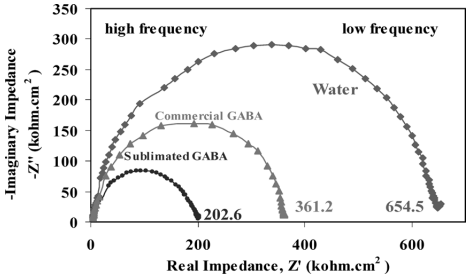

Fig. 7. Impedance Spectra of Commercial GABA and Sublimated GABA Solutions (5×10^{-3} mol/l) and Water at 20 °C

The *X*-axis represents real impedance, the *Y*-axis, imaginary impedance. The given values correspond to the impedance of each solution.

Pharmaceutical substances are generally required in a state of high purity. The sublimation process is mentioned in several papers that report its efficacy for the purification[10—14] of organic or inorganic substances such as salicylic acid, benzoic acid, or magnesium. It is also verified in this work, as a high degree of purity of sublimated GABA was obtained. The determination of the purity of sublimated GABA, based on the Van't Hoff principle, is 99.87% with a correction of 5.5×10^{-3} % (Fig. 8). However, no comparison is possible between the two materials because commercial GABA is a mixture of two polymorphs.

Discussion and Conclusion

During the elaboration process, the control of polymorphism is an essential issue for the pharmaceutical industry. Depending on the polymorphic shape selected, the properties of the future drug can be different. At this stage of generation of the polymorphic shape, elaboration remains the essential process to be mastered because any modification in an experimental parameter may modify the polymorph.

In this work, the sublimation of GABA was carried out with the following experimental parameters: temperature $T=190\pm1$ °C, pressure $P=10^{-3}$ Pa, deposition rate $v=0.1$ nm/s, weight $m=40$ mg, speed of rotation $r=30$ rpm, and distance of the collecting surface from the material $d=17$ cm. The identification and the physico-chemical properties of the sublimated GABA and the non-sublimated GABA were determined with a large number of investigations. Four groups of data were collected: structural, thermal, calorimetric and molecular mobility. X-Ray powder crystallography and thermal analysis are widely used and create very convenient routine methods for the analysis of polymorphic phenomena; heat capacity measurements and dissolution heat give access to the calorimetric data. More sophisticated techniques such as thermally stimulated current and electrochemical impedance measurements are needed to determine molecular mobility of phases. It was demonstrated by X-ray diffraction that the commercial material is a mixture of monoclinic and tetragonal forms while the sublimated material is in mono-

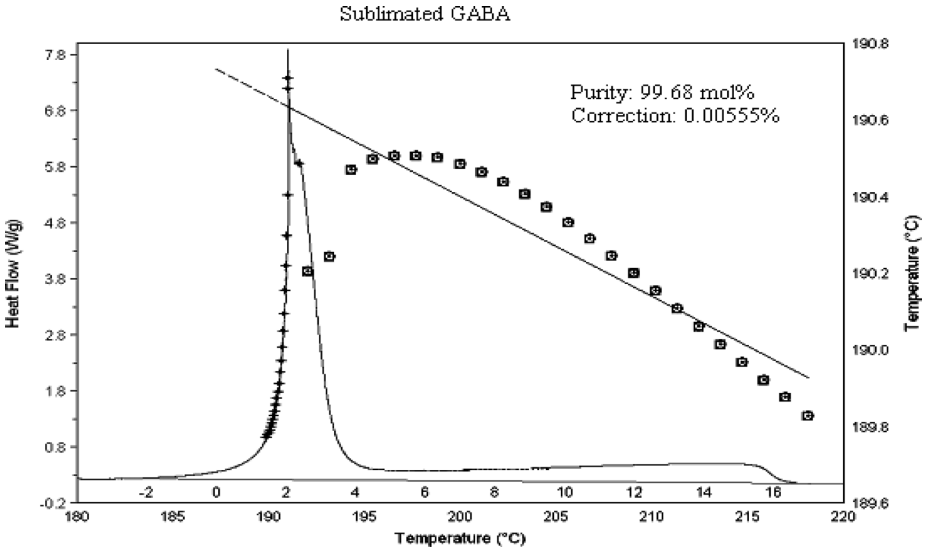

Fig. 8. DSC Curves of Purity Analysis of Sublimated GABA Obtained at a Heating Rate of 1 °C/min

The software (2002 TA Instrument Advantage version 2.8.0) used the Vant'Hoff equation to determine purity.

clinic form. The presence of the monoclinic form only in the sublimated sample implies differences in all the analyses performed: the density measurement, the different thermal behaviour and the calorimetric data. Moreover, sublimation induces a significant reduction in particle size, a higher relaxation and a high purity.

Biological tests have confirmed the therapeutic activity of sublimated GABA prepared as in ref. 2. They show that when administered intraperitoneally non-transformed GABA, even at a strong dose (100 mg/kg), is inactive at the level of the central nervous system. After transformation, the GABA synthesized as in ref. 2 is active with a dose of 25 mg/kg, suggesting passage of the hemato–encephalic barrier (HEB). For the new sublimated GABA, a dose of 7 mg/kg only is sufficient. It is therapeutically more active. Its physico-chemical properties are also more effective than those of the sublimated GABA prepared as in ref. 2 (*i.e.* with the following experimental parameters $T=250\pm10$ °C, $P=10^{-3}$ Pa, $v=0.4$ nm/s). Thermally stimulated current (1.1×10^{-9} A for one depolarization peak to 165 °C) and electrochemical impedance (202.6 kohm cm^2) measurements indicate a molecular mobility lower than that of the new sublimated GABA. Moreover, marked differences in the two sublimated GABAs can be noted in other physico-chemical characteristics, as evidenced by X-ray diffraction, differential scanning calorimetry and calorimetric measurements. It was observed that the diffraction peaks of the new GABA are wider, indicating a reduction in particle size. While the thermograms of the two sublimated GABAs show a similar general curve, the enthalpy variation of new sublimated GABA is lower (104.87 kJ/mol and 118.28 kJ/mol for sublimated GABA[2)]). This results from a decrease in the number of intermolecular hydrogen bonds because of a decrease in the particle size. The polynomial functions describing the heat capacity of the two sublimated GABAs are clearly distinct. All the data presented here indicate that we have synthesized a new drug candidate, monoclinic GABA nanoparticles, with specific physico-chemical and biological properties. The reduction in size can have a great influence on the bioavailability of the molecule. Thus, we may conclude that the therapeutic impact of the use of an organic compound synthesized by this process is dependent on the experimental parameters used, and the literature[15)] confirms the influence of the sublimation parameters on the form and the size of product.

By its ability to generate a new polymorph, this sublimation process at low pressure, free of toxic by-products and solvents, can be applied to polar molecules having at least one hydroxyl, carboxyl or amino such as amino acids (leucine, glycine[1)]), caffeine or theophylline These physico-chemical analyses will be described in forthcoming papers.

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