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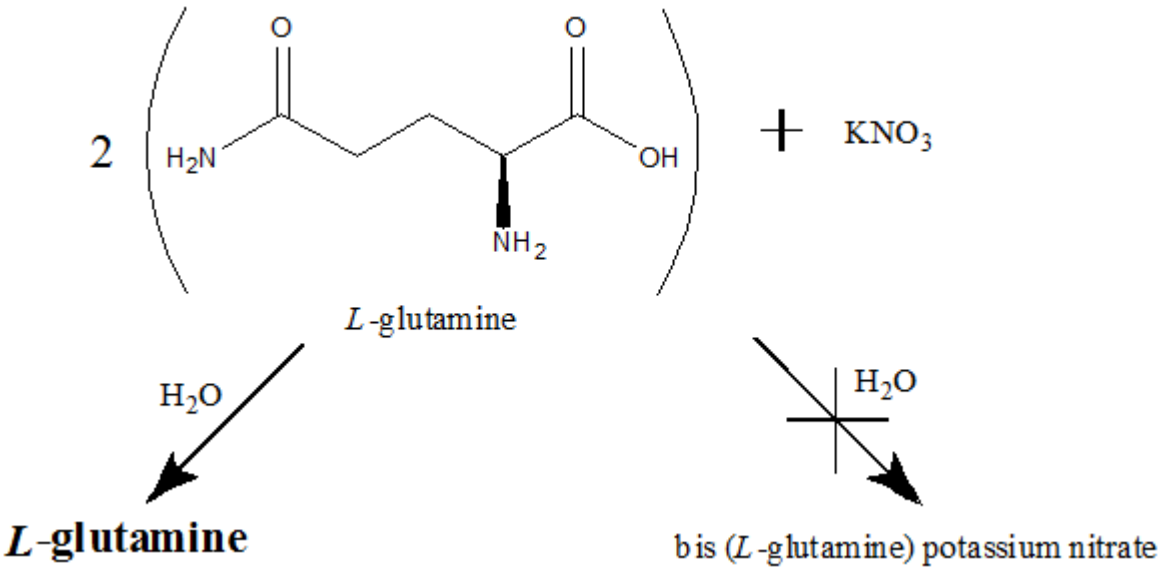
Graphical Abstract



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Highlights

- Reported bis (*L*-glutamine) potassium nitrate crystal is actually *L*-glutamine.
- Reported bis (*L*-glutamine) sodium nitrate crystal is actually *L*-glutamine.
- Use of KNO_3 or $NaNO_3$ does not affect crystal growth of *L*-glutamine.

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Abstract

Slow evaporation of an aqueous solution containing *L*-glutamine and potassium nitrate in 2:1 mole ratio results in the fractional crystallization of *L*-glutamine and not the formation of a so called bis (*L*-glutamine) potassium nitrate as reported recently by Redrothu Hanumantharao and S. Kalainathan, Spectrochim. Acta, A99 (2012) 181–188.

Keywords: *L*-glutamine; potassium nitrate; bis (*L*-glutamine) potassium nitrate; fractional crystallization; infrared spectra; crystal growth.

1. Introduction

The synthesis and characterization of new non-centrosymmetric materials for their nonlinear optical (NLO) properties is a frontier area of research. In order to characterize any new crystalline material, it is essential to unambiguously identify the solid being studied in terms of its correct molecular formula and crystal structure. In a case study of NLO materials Fleck and Petrosyan [1] have shown that formulating new materials based only on unit cell data without any structure refinement is an unscientific procedure of compound characterization. In addition, many reported claims of growth of so called novel NLO crystals based on an assumption that the mixing of a few reagents in aqueous solution will result in the crystallization of a desired new product crystal have also been proved to be incorrect and extensively commented [2-10]. In the literature of amino acid based NLO crystals, it is noted

that some research groups report growth of novel NLO crystals characterized by single crystal method without providing structural details of the asymmetric unit and a proper chemical formula. Many so called novel NLO materials are often represented by an unusual name for example bis (*L*-glutamine) potassium nitrate [11], abbreviated by a strange code. *L*-glutamine is one of the twenty naturally occurring amino acids and is an amide of *L*-glutamic acid. The compound monosodium glutamate, which contains sodium and the monoanion of *L*-glutamic acid, is present in the diet of a majority of the inhabitants of the world [12]. A potassium salt of *L*-glutamic acid is also well documented [13, 14]. To the best of our knowledge, no structurally characterized compound containing both *L*-glutamine and an alkali metal (Na or K) is reported in the Cambridge Structure Database till date. However, the authors of [11] have reported claims of growth of bis (*L*-glutamine) potassium nitrate by mixing potassium nitrate and *L*-glutamine in 1:2 mole ratio in water. The same authors have also claimed growth of another so called bis (*L*-glutamine) sodium nitrate crystal by a very similar method [15]. Interestingly, the unit cell parameters reported for a so called bis (*L*-glutamine) sodium nitrate were in good agreement with those of *L*-glutamine [16] but for an interchange of the *a* and *c* parameters. Since the structure characterization was done based only on unit cell data (Table 1), without any structure refinement, these claims appeared dubious. In view of this, we have reinvestigated the reported crystal growth of bis (*L*-glutamine) potassium nitrate [11] and also bis (*L*-glutamine) sodium nitrate [15] in order to correctly identify the exact nature of the product crystal. The results are described herein.

Table 1. Unit cell data of *L*-glutamine, bis (*L*-glutamine) potassium nitrate and bis (*L*-glutamine) sodium nitrate

Compound	*a* (Å)	*b* (Å)	*c* (Å)	V (Å^3)	Space group	Ref
L-glutamine	16.01(3)	7.76(3)	5.10(3)	633.61	$P2_12_12_1$	[16]
Bis (*L*-glutamine) sodium nitrate	5.09[a]	7.75[a]	16.00[a]	631	$P2_12_12_1$[b]	[15]
Bis (*L*-glutamine) potassium nitrate	6.28[a]	4.98[a]	14.49[a]	---	$P2_12_12_1$[b]	[11]

[a] No esd"s given; [b] Structure determination is not substantiated by CIF data

2. Materials and Methods

All the chemicals used in this study were purchased from commercial sources and were used as received without any further purification. Double distilled water was used as solvent. Infrared (IR) spectra of the samples diluted in KBr were recorded in the region 4000 – 400 cm^{-1} using a Shimadzu (IR Prestige-21) FT-IR Spectrometer, at a resolution of 4 cm^{-1}. ^1H NMR spectra were recorded (in D_2O) using a Bruker 400 MHz (Avance) FT-NMR spectrometer. UV-Vis spectra were recorded using an Agilent-8453 UV-Visible spectroscopy system. Optical rotation of the crystals dissolved in water, were measured in a Rudolph research analytical (Autopol IV) polarimeter.

2.1 Reinvestigation of crystal growth of bis (L-glutamine) potassium nitrate

A mixture of potassium nitrate (0.5056 g, 5 mmol) and *L*-glutamine (1.4615 g, 10 mmol) was taken in water ~15 ml and was stirred well to obtain a clear colorless solution (pH~5.5). H_2O_2 (2 ml) was added to this and the reaction mixture was left undisturbed at ambient temperature. Slow evaporation of the solvent resulted in the separation of crystals in ~7 days, which were isolated by filtration, washed with little cold water and dried in air to yield 1.215 g of crystalline material. This product was labelled as compound **1**. The use of sodium nitrate (0.425 g, 5 mmol) instead of potassium nitrate in the above crystal growth reaction resulted in the formation of transparent crystals (1.147 g) which were isolated by following the same procedure as given above. These crystals were labelled as **2**. In addition, we investigated crystal growth reaction by taking alkali metal nitrate and *L*-glutamine in a 1:1 mole ratio. The products obtained were isolated as before and analysed.

3. Results and discussion

3.1 Synthetic aspects

The reported crystal growth of bis (*L*-glutamine) potassium nitrate [11] and bis (*L*-glutamine) sodium nitrate crystal [15] is reinvestigated to unambiguously characterize the crystalline

product. The reinvestigation was undertaken due to the fact that the single crystal data reported by the authors were not accompanied by CIF file to substantiate the structure refinement. Many claims of the authors of the commented papers for example „*the presence of potassium in the compound has been detected by EDAX analysis'* indicated that the compounds are improperly characterized.

In the present work, the crystal growth reactions were performed using *L*-glutamine and alkali metal nitrate in 2:1 molar ratio. In addition, crystal growth reactions were studied using equimolar quantities of *L*-glutamine and potassium (or sodium) nitrate. H_2O_2 was added to the reaction mixture to inhibit microbial growth. Use of H_2O_2 has earlier been proposed by Yokotani et al. [17] for LAP (L-arginine phosphate monohydrate) and used also by the Petrosyan group for growth of NLO crystals of the LAP family [18]. In the absence of any H_2O_2 microbial growth was observed. In all the cases the product obtained was colourless and the yield of the product was always less than the amount of *L*-glutamine and amounted to ~ 75% of quantity of *L*-glutamine employed in crystal growth. In view of this no efforts were made to isolate the entire amino acid. The product obtained from all the above mentioned crystal growth reactions were studied by spectral methods.

3.2 Usefulness of infrared method for product characterization

In present work, the crystal growth reactions were performed by employing the same conditions as in the earlier reported work and the solid obtained is identified as compound **1** (or **2**). The products of the crystal growth experiments were first investigated by IR spectra. As it is well documented that every compound exhibits a characteristic IR spectrum, a comparison of the IR spectra of the starting reagents and the product material of a crystal growth reaction constitutes an useful procedure for product characterization. A new product crystal is expected to show differences in its IR spectrum in terms of disappearance of

existing IR bands in the spectrum of the starting reagents or appearance of new signals. The spectral changes will be pronounced if the molecular formula of a product differs considerably for example from *L*-glutamine to bis (*L*-glutamine) potassium nitrate. The above reasoning has been made use of for accurate product characterization as shown below.

3.3 Correct formulation of bis (L-glutamine) potassium nitrate and bis (L-glutamine) potassium nitrate

The IR spectra of all the products obtained in each of the crystal growth experiments (irrespective of the amounts of *L*-glutamine and alkali metal nitrate used for crystal growth) were identical indicating that the product formed is one and the same. More interestingly all the IR spectra were coincident with the spectrum of pure *L*-glutamine (Fig. 1) clearly proving that the product formed in all cases is pure *L*-glutamine. A comparison of the IR spectrum of pure *L*-glutamine with the spectrum of an artificial mixture of alkali metal nitrate and *L*-glutamine unambiguously confirms that compounds **1** and **2** are pure *L*-glutamine and not a so called bis (*L*-glutamine) potassium nitrate or bis (*L*-glutamine) sodium nitrate (Fig. S1 - S3). Since IR spectrum is used as a characterization tool for product formation, no band assignments are given.

The formation of pure *L*-glutamine as the only product can be further evidenced by the melting point as crystals of **1** or **2** melt at 186-188 ^{o}C which is same as that of *L*-glutamine. A positive ninhydrin test for **1** or **2** provides additional proof for the formation of an amino acid as the product while standard qualitative spot tests [19] show the absence of the respective alkali metal ions thus unambiguously confirming the crystals of **1** and **2** are nothing but pure *L*-glutamine.

3.4 UV-Visible & NMR spectra, Polarimetry

The optical spectrum of **1** (or **2**) not only matches perfectly with that of *L*-glutamine but also shows the transparent nature of **1** (or **2**) in the 200 – 1100 nm region (Fig S4). The observed

chemical shifts in the ^{1}H NMR spectrum of compound **1** (or **2**) are in good agreement with that for pure *L*-glutamine (Fig S5-S6). In addition, the NMR spectra reveal the purity of **1** (or **2**). The grown crystals exhibit a positive optical rotation confirming them to be same as those of the starting material namely *L(+)*-glutamine. The optical activity data indicate that use of alkali metal nitrate does not result in any isomerisation / racemisation of the amino acid. The present study shows that use of nitrate salts of potassium (or sodium) in the medium does not inhibit the crystal growth of *L*-glutamine. Thus all these results add more credence to the above mentioned IR, melting point data and qualitative spot tests, for the formation of *L*-glutamine as the only product.

3.5 Chemistry of the crystal growth reaction from an aqueous solution containing alkali metal nitrate and L-glutamine

Without taking into consideration the chemistry of the reagents, the authors of the commented papers [11, 15] have assumed that the mixing of a few reagents in aqueous solution in a preferred ratio will result in the crystallization of their desired product crystal namely bis (*L*-glutamine) alkali metal nitrate. Contrary to their assumption, the slow evaporation solution growth from an aqueous solution containing *L*-glutamine and alkali metal salt in 2:1 mole ratio, did not result in the formation of any so called bis (*L*-glutamine) potassium nitrate or bis (*L*-glutamine) sodium nitrate. The present reinvestigation correctly identifies the product of the crystal growth study in the commented papers as pure *L*-glutamine. This can be explained due to no chemical reaction between KNO_3 (or $NaNO_3$) and *L*-glutamine in water at room temperature, leading to the fractional crystallization of the less soluble *L*-glutamine (1 g in 20.8 ml water) [20] with the more soluble KNO_3 (or $NaNO_3$) remaining in solution. Hence, the chemistry of the crystal growth reaction for **1** can be represented as in Scheme 1. A similar scheme (Scheme S1) can be used to describe the crystal growth of **2**. As potassium (or sodium) forms a potassium hydrogen *L*-glutamate

monohydrate [13] by neutralization of *L*-glutamic acid with KOH (or NaOH), the use of two moles of *L*-glutamine with one mole of potassium nitrate (or sodium nitrate) for the formation of the so called bis (L-glutamine) metal nitrate appears to be arbitrary and without proper scientific reasoning.

3.6 Inappropriate use of EDAX for product characterization

It is unfortunate to mention that several claims in both the commented papers not only appear to be unscientific but also many results are questionable. Although the authors claim that bis (*L*-glutamine) potassium nitrate crystals were grown by slow evaporation technique, the statement of the authors under the section titled Materials Synthesis, „... *Then the solution was evaporated at a temperature of 35 ^{o}C in hot air oven and crystalline salt of BGPN was gathered.'* makes one wonder as to how actually the crystal growth was performed. The dubious nature of the so called bis (*L*-glutamine) potassium nitrate can be evidenced from the IR spectrum reported by the authors of [11] which shows no signals till about 2200 cm $^{-1}$ and the band assignments many of which are erroneous. Although the unit cell reported by the authors of [15] for a so called bis (*L*-glutamine) sodium nitrate confirms the fractional crystallization of *L*-glutamine, the assignment of a Sohncke space group ($P22_12_1$) without structure determination is unacceptable. The values of unit cell reported for bis (*L*-glutamine) potassium nitrate differ from that of *L*-glutamine. This claim of the authors of [11] appears to be unreliable in view of the fractional crystallization of *L*-glutamine. The reason for the discrepancy can be due to an incorrect cell. The possibility of listing of some arbitrary values without any cell measurement cannot be ruled out. Likewise the claims of the authors of [11, 15] of confirming the presence of K or Na and doing elemental analysis for C, N with the aid of EDAX study can be dismissed. The % composition of lighter elements like C, H and N is better determined by a microanalysis and not EDAX. Since any potassium (or sodium) containing compound will show presence of K (or Na), mere observation of K (or Na) in an

EDAX study does not confirm the formula of a so called bis (*L*-glutamine) potassium (or sodium) nitrate. The presence of K (or Na) can be attributed to use of a metal (K or Na) contaminated sample for the study. Examples of compounds wrongly formulated due to inappropriate use of EDAX are discussed in recent literature [21-23]. The present work once again points out the risk in identification of new compounds using EDAX data.

4. Conclusions

In summary, it is shown that the slow evaporation of an aqueous solution containing *L*-glutamine and potassium nitrate (or sodium nitrate) in 2:1 or equimolar molar ratio at room temperature, results in the fractional crystallization of *L*-glutamine and not any so called bis (*L*-glutamine) potassium nitrate (or bis (*L*-glutamine) sodium nitrate) crystal. The presence of equimolar or less amounts of metal nitrate in the crystal growth medium does neither inhibit the growth of *L*-glutamine nor affects the optical activity of *L*-glutamine crystals.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/

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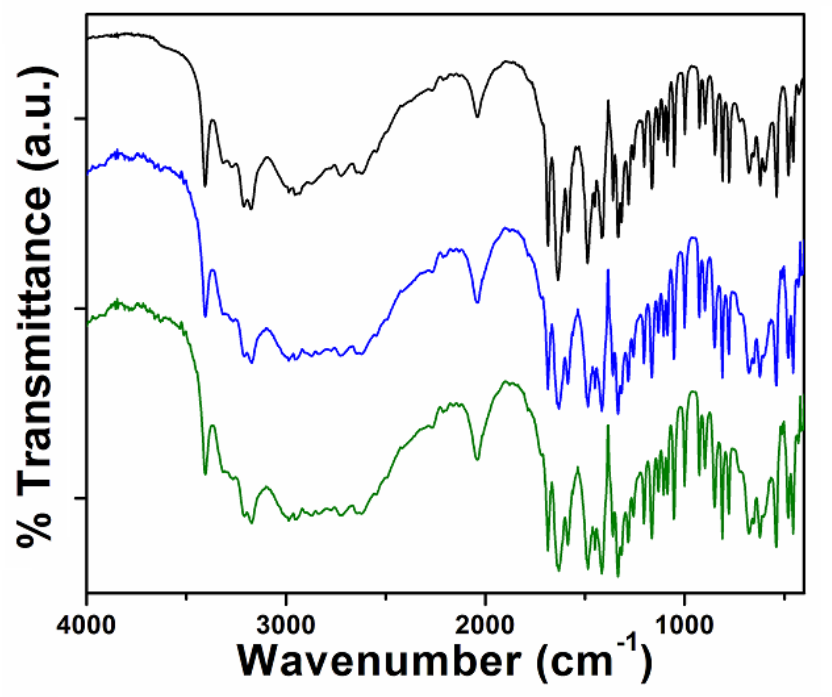
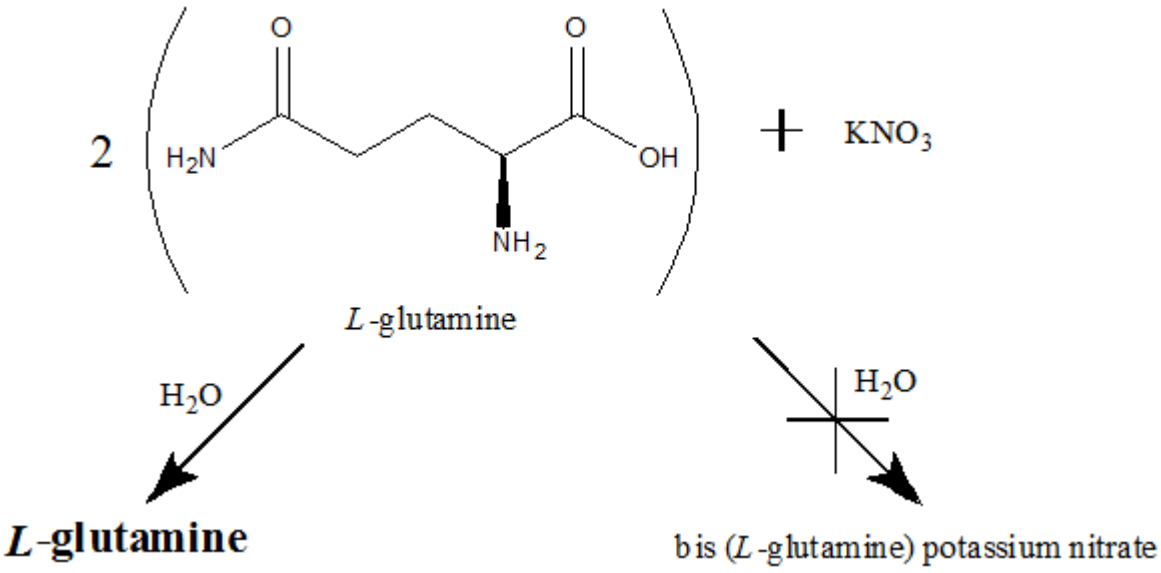


Fig. 1 Infrared spectra of pure *L*-glutamine (top), compound **1** (middle) and compound **2** (bottom)



Scheme 1