European Journal of Chemistry 1 (4) (2010) 319-321

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Determination of enantiomeric excess of leucine and valine by X-ray powder diffraction

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ARTICLE INFORMATION

Received: 26 June 2010
Accepted: 06 August 2010
Online: 31 December 2010

KEYWORDS

Leucine
Valine
Enantiomeric excess
X-ray powder diffraction

ABSTRACT

Two amino acids, leucine and valine, were studied by X-ray powder diffraction (XRPD). The linear correlations between intensity of racemate (decrease) or enantiomer (increase) and enantiomeric excesses were observed in each case.

1. Introduction

Leucine [1,2] and valine [3,4] are two of the twenty most common amino acids on Earth. Only their L-enantiomers appear in mammalian proteins. They are essential in metabolism and necessary in the diet, as the human body cannot synthesize them from simpler metabolites. They are branched-chain amino acids (BCAA). BCAA help to maintain muscle tissue and are also needed during times of physical stress and intense exercise. Young adult humans need about 31 mg of leucine and 23 mg of valine per day per kilogram of body weight.

Leucine, $(CH_3)_2CHCH_2CH(NH_2)COOH$, is a hydrophobic amino acid that is found as a structural element in the interior of proteins and enzymes. It was isolated from cheese in an impure form in 1819 and from wool in the crystalline state in 1820. Leucine stimulates muscle protein synthesis and may be the major fuel involved in anabolic (tissue-building) reactions. It helps with the regulation of blood-sugar level, growth hormone production, wound healing and energy regulation. It is found in protein-containing foods, brown rice, beans, nuts and wheat.

Valine, $(CH_3)_2CHCH(NH_2)COOH$, was first isolated in 1888 by Weyl [5]. It has a stimulating effect on muscle metabolism and is needed for muscle metabolism, repair and growth of tissue and maintaining the nitrogen balance in the body. Good sources for this amino acid are dairy produce, meat, grain, mushrooms, soy and peanuts.

Today, many athletes use leucine and valine in the L-enantiomeric form as supplemental nutrients, so easy and fast determination of enantiomeric excess (ee) of these amino acids is very important. It is known that two enantiomers give different pharmacological responses, so pharmaceutical companies tend to produce chiral drugs in single enantiomeric form and any new method able to control the enantiomeric purity is strongly desired. The X-ray powder diffraction method (XRPD) is successfully used for determination of ee of pharmaceuticals such as Ibuprofen (anti-inflammatory, analgesic and antipyretic agent [6,7]) [8] and cyclophosph amide (anticancer agent [9-11]) [12]. Now extension of the XRPD technique to the determination of enantiomeric excess in the mixtures of leucine or valine is presented.

2. Experimental

The samples of racemeric (DL) and enantiomeric (L) leucine were mixed in different proportions to prepare physical mixtures. The ee values were 5%, 10%, 25%, 40%, 50%, 60%, 75%, 90%. Analogous samples were prepared for valine. The physical mixtures and recrystallized physical mixtures ($MeOH:H_2O$, 1:1) of these amino acids were prepared. Recrystallized physical mixtures were prepared in different ways: slow evaporation techniques and fast evaporation in a rotary evaporator (Pressure 100 mm Hg, Temperature: 19 °C; Pressure: 500 mmHg, Temperature: 40 °C). In all cases the same mixtures recrystallized by different methods gave identical diffraction patterns, i.e. the peaks have the same positions and intensities. To avoid errors associated with change of ee during the recrystallization the ee was monitored also by ^{13}C CP/MAS and ODESSA NMR experiments.

2.1. Instrumentation

The substances and mixtures discussed above were milled in the planetary ball corundum mill for 5 minutes and the X-ray diffractograms were then recorded using a D-5000 diffractometer and CuKα (Ni-filtered) radiation with Bragg-

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.1.4.319-321.224

Brentano focusing geometry. The measurements were made in the 2θ = 2-80° range with 2 seconds per 0.01° step. The area of peak was computed by EVA version 3.09 from diffrac at program PACKAGE with ω-2θ scan mode after appropriate background determination [13].

3. Results and discussion

The structures of L-leucine [14-16] / L-valine [17-19] and their racemates (DL-leucine [20,21] / DL-valine [22-25]) are different (for details see supplementary materials), which result in different powder patterns. This allows both phase identification and quantitative analysis. Recorded diffractograms of the pure amino acids, leucine and valine in racemic and enantiomeric forms, are depicted in Figure 1 and 2. The sample X-ray powder diffraction patterns of prepared mixtures are depicted on Figure 3 and 4.

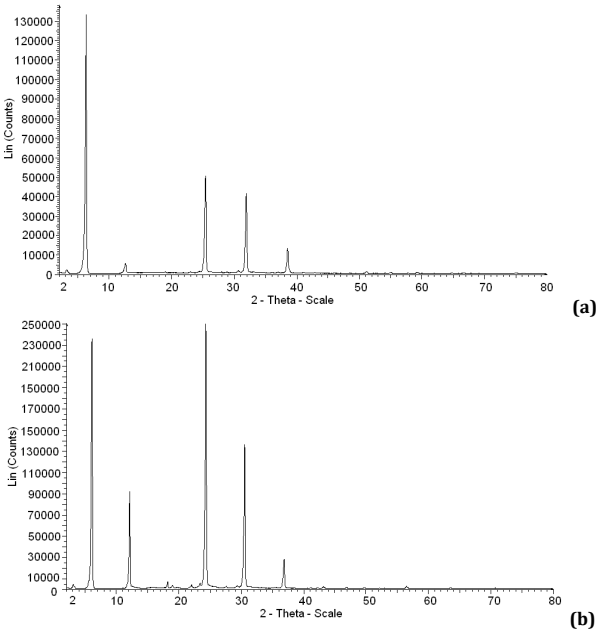

Figure 1. Diffractogram of (a) leucine (racemate), (b) leucine (L-enantiomer).

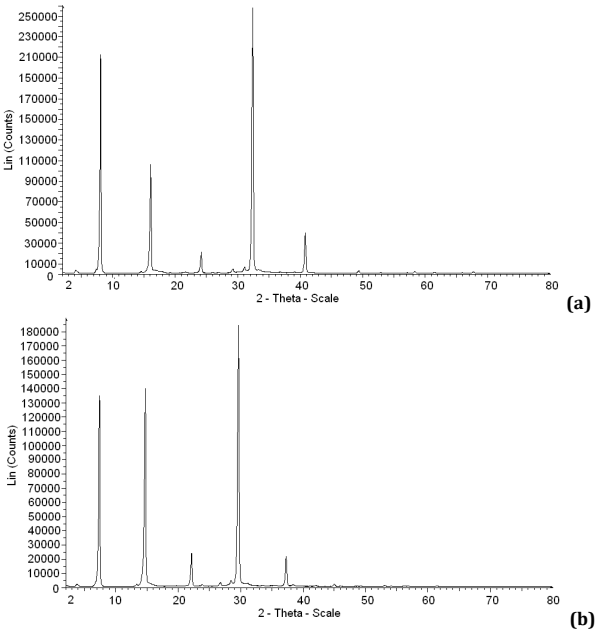

Figure 2. Diffractogram of (a) valine: racemate, (b) valine: L-enantiomer.

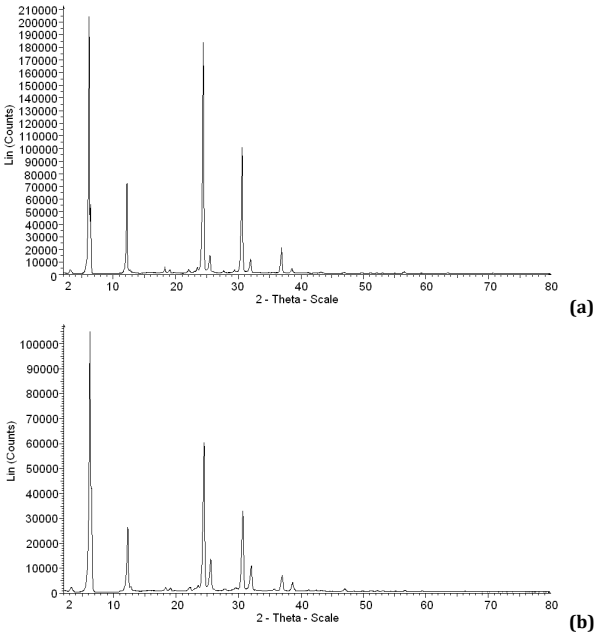

Figure 3. Diffractogram of leucine (*ee* = 75%) (a) physical mixture. (b) recrystallized mixture.

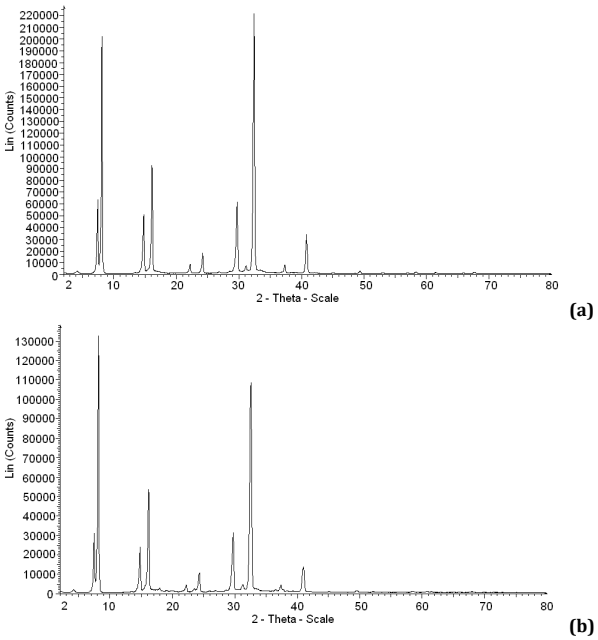

Figure 4. Diffractogram of valine (*ee* = 25%) (a) physical mixture, (b) recrystallized mixture.

For each pattern one non-overlapping independent diffraction peak for the L-amino acid and the racemate can be selected (for the leucine L-enantiomer at 2θ = 24.3° and for the leucine racemate at 2θ = 25.3°; and for valine at 29.5° and 32.4° for the L-enantiomer and racemate, respectively). Thus the classic approach based on the intensity of one peak can be applied instead of the Rieteveld method. Fitting the whole diffraction pattern to the calculated intensities for the various phases that are present can give more precise results, but in the case discussed here the classic approach gives a linear correlation between the intensity (the area of the peak) and the ee in each cases (Figure 5 and Figure 6) with R^2 values greater than 0.99.

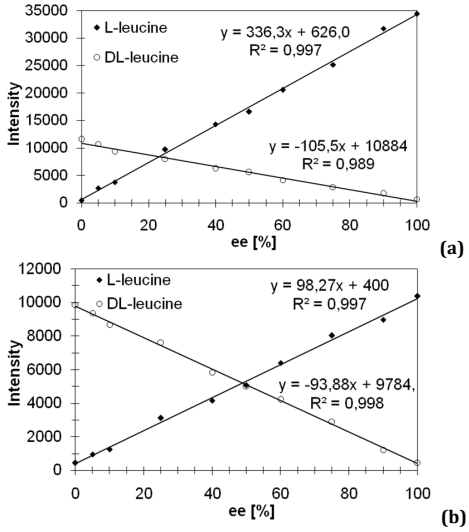

Figure 5. Linear correlation between intensity and *ee* in samples of leucine (physical mixture) – reflection: 2θ = 24.3° (enantiomer); reflection: 2θ = 25.3° (racemate) (a) physical mixture, (b) recrystallized mixture.

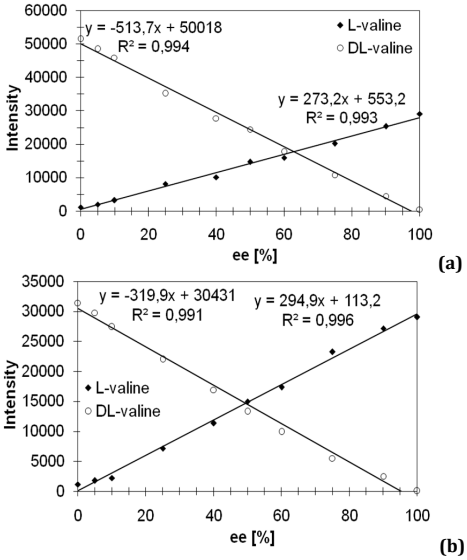

Figure 6. Linear correlation between intensity and *ee* in samples of valine (physical mixture) – reflection: 2θ = 32.4°(enantiomer); reflection: 2θ = 29.5° (racemate) (a) physical mixture (b) recrystallized mixture.

As it is shown in Figure 5 and Figure 6 the regression coefficients differ for physical mixtures and recrystallised samples. It is caused by differences in the degree of crystallinity of determined compounds. Thus all standard samples must be recrystallised to obtain reliable results.

Prepared physical mixtures diffractograms did not show a dependence on method of preparation - slow evaporation or fast evaporation in a rotary evaporator (Pressure: 100 mm Hg, Temperature: 19 °C; Pressure: 500 mmHg, Temperature: 40 °C). In all cases of recrystallization for the same physical mixture recorded diffraction patterns are identical i.e. the peaks have the same positions and intensities. Thus the recrystallization process can be fast and using the same crystallization medium (MeOH:H_2O, 1:1) gives repeatable results.

4. Conclusion

The X-ray powder diffraction is a powerful and fast technique for the identification of polycrystalline samples [26-28]. However, quantitative analysis is not a common practice. It is mostly involved with problems occurring during determination of the amount of phase in a multiphase sample. The quantity of a specific compound or compounds in a multicomponent sample can be determined from the intensities of the peaks in a powder diffraction pattern [13]. The main problem is the accurate determination of peak intensities. The absolute intensity measurement practically almost cannot be made, but measurement of relative intensity is sufficient if the comparative analytical method is used [29]. Thus one of two methods can be considered: the calibration curve and the internal standard method. As shown in the amino acids samples considered here, the relative intensity is linearly correlated with the ee, and, as expected, it changes with the degree of crystallinity. Thus choosing the calibration curve method after fast recrystallization from the same medium is quicker and more reasonable than the internal standard method, because in the second case the problem of the crystallinity of the standard exists. Though this problem can be overridden by a one additional recrystallization, the internal standard method will be still more laborious, as depicted in the work of Phandnis and Suryanarayanan [7].

Acknowledgement

This work was supported by funds allocated by the Ministry of Science and Higher Education to the Institute of General and Ecological Chemistry, Technical University of Lodz.

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