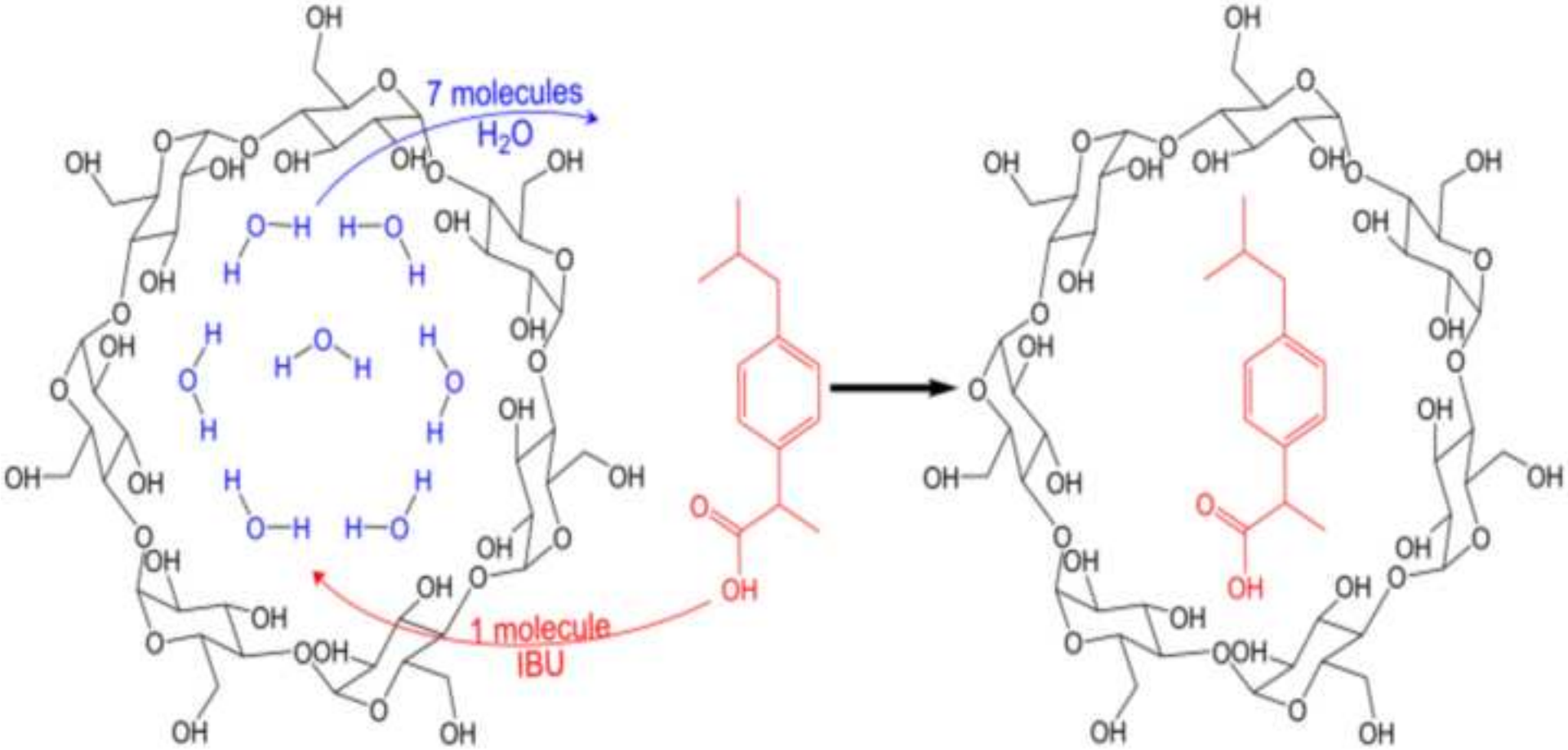
*Graphical Abstract (for review)



Methods:
Ball Milling
Kneading
Solid Dispersion

Ball Milling - easy, cheap, environment friendly, can be applied in industry

Modified Ball Milling with solvent - efficient method for ibuprofen/β-CD complexation

Efficiency of "Cyclodextrin-Ibuprofen" inclusion complex formation

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Abstract

A modified method for ibuprofen / β-cyclodextrin (IBU/β-CD) complex formation, based on ball milling (BM) under controlled conditions was developed and its efficiency with respect to the drug encapsulation yield was compared with the well-known kneading and solid dispersion synthetic approaches. Quantitative evaluation of the efficiency of drug-cyclodextrin interaction applying various methods and experimental conditions as well as characterization of the inclusion complexes were carried out by x-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetry (TG), scanning electron microscopy (SEM), infrared (IR) and nuclear-magnetic resonance (^{1}HNMR) spectroscopy. It was found that the yield of the formed IBU / β-CD complex varies in a large range, depending on the techniques applied. The degree of complexation between IBU and β-CD using the proposed optimized BM method is very high and close to the complete inclusion complex formation achieved by a modified solid dispersion method. Furthermore, using DSC, TG and ^{1}HNMR we proved that the ibuprofen molecules enter the β-CD hydrophobic cavities replacing completely the water molecules present naturally inside, which we determined to be 7.

Keywords: cyclodextrin, ibuprofen, inclusion complex, ball milling, efficiency of drug encapsulation

Introduction

The research and market interest in natural cyclodextrins (CDs) and their derivatives continues to grow. The natural α-, β- and γ-cyclodextrins are cyclic oligosaccharides produced after enzymatic degradation of starch in presence of Bacillus macerans. They consist of 6, 7 or 8 alpha-1,4- linked alpha-D-glucopyranose units which are in "chair conformation" so that the molecules are shaped like a truncated cone. The primary and secondary hydroxyl groups are located on the outer edges of the cone making the external molecular surface hydrophilic. The unique feature of the cyclodexrin molecular structure is the presence of a central apolar cavity with small number of water molecules located in it. This structure determines cyclodextrin's ability to form inclusion compounds with wide range of solid, liquid and gaseous hydrophobic compounds [1,2].

There are many new applications of cyclodextrins in pharmaceutics [2-17], food [17,18], cosmetics [19-25]; in environmental protection, biotechnology [2], cell biology [2], biosensing [2] etc. Among all three CDs β-CD has the lowest aqueous solubility (1.85 g/100 ml H_2O) and is the most used cyclodextrin in the pharmacy, because of its structure, cavity size and price [26, 27].

Ibuprofen ((RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid) is a nonsteroidal anti-inflammatory drug (NSAID). There are many reports on Ibuprofen (IBU) complexes with β-cyclodextrin and its derivatives. These inclusion compounds are widely used and made to increase Ibuprofen water solubility [28, 29, 30]. Variety of methods had been applied for the synthesis of the inclusion compounds such as kneading [31,32,33], solid dispersion [34], spray/freeze drying [28, 32-37], supercritical carbon dioxide [37, 39, 40, 41], microwave treatment [38, 42-45], sealed-heating [31,37,38], co-evaporation [31,32,33,37], co-grinding [31]. Mechanochemical synthesis or co-grinding has been reported as well, however there are no studies comparing wet and dry ball milling synthesis of inclusion compounds between β-CD and ibuprofen. In general the ball milling synthetic approach has a lot of benefits – controlling the particles size, improving ibuprofen solubility and stability and even amorphization of the product (XRD, DSC, SEM) [46,47,48]. Also the method is easy, cheap, environmental friendly and can be apply in an industrial scale.

Phase-solubility technique for determination of the stoichiometric ratio of the inclusion compounds according Higuchi & Conners [49] is presented as well. Most of the authors report of a ratio drug:β-CD = 1:1 [28, 29], but there are also papers reporting ratios such as 1:3 and 2:3 at room temperature (23 °C) and at 37 °C respectively.[30] It is worth

noting that the available results show some discrepancy with respect to the productivity of the synthetic methods. Even sometimes reliable proofs for the complete complex formation are missing. Therefore, the present work aims at comparing the efficiency of several synthetic methods for ibuprofen encapsulation into β-CD, realized by a number of appropriate microstructural, thermal and spectroscopic techniques. For this purpose a modified mechanical micronization of IBU via complexation with beta-cyclodextrin in a ball mill was developed and its complexation efficiency in comparison to kneading and co-evaporation methods was analyzed. Ibuprofen has been chosen as a model drug because of its hydrophobicity and low water solubility. Additionally, some valuable quantitative information concerning the mechanism of the complex formation was presented.

Experimental part. Methods and materials

Materials

2-(4-isobutylphenyl)-propionic acid Ibuprofen 50 (catalytic process) was purchased from BASF AG, Germany and β-cyclodextrin - from Wacker Chemie AG, Germany. All other reagents used were of analytical grade.

Phase solubility studies. The phase solubility studies were conducted according to the method of Higuchi and Connors [49]. Excess amounts of the drug (Ibuprofen) were added to nine flasks containing 50 ml of aqueous solutions having different β-CD concentrations (0, 1, 2, 3, 4, 5, 6, 8 and $12*10^{-3}$ M). The samples were shaken at 25 °C in thermostatically controlled mechanical shaker (Orbital Shake-Incubator, ES-20, Biosan) for 22 hours. Following equilibrium, an aliquot was filtered using a 0.45 μm membrane filter. All samples were analyzed by using UV/Vis (Specord) technique.

Preparation of Ibuprofen - β-CD solid complex

Kneading. Kneading was carried out in a mortar with a pestle by adding ethanol - water solution 1:1(v/v) to powder mixture of IBU/β-CD =1:1 (M/M). The mass was triturated for 45-50 minutes and after that dried in an oven at 42°C for a day.

Solid dispersion technique (SD). IBU/β-CD solid dispersions were prepared in a ratio 1:1 (M/M) by common solvent method.

SD_1. β-CD dissolved in water were gradually added to solution of IBU in ethanol by stirring (electromagnetic stirrer) until a homogenous solution was formed. The solvent was removed in open-air and the solid residue slowly dried in an oven at 42°C for about 22 hours.

SD_2. Modification of Method 1 - The final IBU/β-CD (1:1 M/M) solution was alkalized with ammonia and the solvent removed by rotary vacuum evaporator. The solid residue was dried in an oven at 42 °C for 8 hours.

Physical mixture (PhM). The PhM was prepared in ratio IBU /βCD/ 1:1 (M/M) in a mortar with a pestle by grinding of the powder mixture for 10 min.

Ball milling. Three procedures of milling was used to prepare IBU/β-CD 1:1 (M/M) solid complex in planetary mill Fritch 6, at ball to powder ratio 4:1, varying the milling intensity and duration.

BM_1. 0.306g of Ibuprofen and 1.7g β-CD (IBU/β-CD 1:1 M/M) were mixtured and ball-milled under dry ball-milling conditions at 100rpm for 5 min, 25 min and 1hour and for 2 hours with 300rpm.

BM_2. Powder mixture of Ibuprofen (0.15g) and β-CD (0.85g) (IBU/β-CD 1:1(M/M)) was ball-milled in presence of 0.5 mL aqueous ethanol solution (H_2O/C_2H_5OH (1/1 v/v)). The sample was milled at 300rpm for 50 min.

BM_3. Powder mixture of Ibuprofen (0.15g) and β-CD (0.85g) (IBU/β-CD 1:1 M/M) was ball-milled at 300 rpm for 50 min and every 10 min 0.1 mL (2 drops) of aqueous ethanol (H_2O/EtOH 1:1 v/v) were added.

Characterization of the complex

Differential scanning calorimetry (DSC) and thermogravimetry (TG) – The thermal behavior and stability of the pure substances and inclusion complexes were characterized by Perkin-Elmer DSC - 2C and TG - 2. Samples were heated from room temperature (25°C) to 200°C with a scanning rate of 10°C/min.

Scanning electron microscopy (SEM)

The morphology and particle size of the inclusion complexes were observed by SEM, JEOL JSM-5510.

X-ray diffraction (XRD) - Bruker D8 Advance diffractometer with Cu-Kα radiation was used for the samples microstructure determination.

Infra-red absorption spectroscopy (*IR*) –Nicolet™ FT-IR Spectrometer, Thermo-Electron Corporation, according to the KBr disk method.

Nuclear magnetic resonance (^{1}*HNMR)* – Bruker Avance III 500Mhz, 54mm ASCEND magnet with 11.7 T strength of the magnetic field.

Results and discussion

The phase solubility diagram for ibuprofen as a function of β-CD concentration at 25°C, shown in **Fig. 1,** can be classified as Bs type, according to Higuchi and Connors [49], because of precipitation of the insoluble complex at high concentrations of the carrier [50]. The maximum amount of ibuprofen dissolved reached a constant concentration at $4*10^{-3}$M β-CD. The slope of the linear part of the phase solubility plot is lesser than unity. This fact assumes drug/ligand 1:1(M/M) or 1:3 (M/M) stoichiometry.

Further ^{1}HNMR study in D_2O was carried out to prove the stoichiometry of the IBU/β-CD complex in solution. The results were in accordance with those reported by M. di Cagno et al. [51] and verify substrate/ligand ratio 1:1(M/M) in solution. Thus, all IBU/β-CD complexes object of the present study were prepared in 1:1 M/M ratio.

It is well known that the ball-milling is well approved method not only for production of nano-powders, but also a method for solid state synthesis, especially when non-covalent bonds are created between the reacting components of the powder mixture.

In the present study a modified ball milling method for preparation of IBU/β-CD inclusion compound was developed. The ball milling was carried out under dry and wet (with solvent) conditions. The influence of the process related factors (duration of the mechanical treatment and its intensity) was evaluated to estimate their impact on the yield of the complex. The interaction between IBU and β-CD was followed by the thermal analysis techniques, DSC and TG. The methods chosen for comparison were kneading (mechanical grinding in presence of small amount of solvent) and a solvent dispersion technique using a common solvent.

First, mixtures of IBU and β-CD were milled for different time and intensity. The mixtures were then annealed in DSC from room temperature to above the T^m of IBU. Thus, by measuring the enthalpy of melting of the residual ibuprofen (when it exists), ΔH^m_{IBU}, and knowing its total amount in the sample the quantity of the IBU included in the complex

(practically the complex formation yield) could be determined, **Fig. 2a**. For ensuring the precision of the DSC experiments the endothermic heat effect of pure IBU melting was measured (127.8 J/g), which was found to be exactly its melting enthalpy. The heat effect of IBU melting in the physical mixture was just its melting enthalpy.

Figs. 2b shows the DSC plots of the complexes prepared by different methods and at different conditions. Quantitative information about the degree of complex formation is presented in **Table 1**. As it could be seen from the thermograms in Fig.2a milling both compounds together for very short time (5 min at 100 rpm) at dry conditions results in a negligible reduction of the heat of IBU melting (endothermic effect), while continuous milling (up to 2 h) causes its visible decrease. This effect can only be associated with partial IBU/β-CD complex formation during milling. It is worth to outline that even at relatively long-time (2-3 h) of intensive milling (300 rpm) the process of inclusion complex formation is not taking place to a large extent (< 15%). It has to be also pointed out that after the annealing in the DSC up to temperature above the melting peak of IBU (~ 80^{o}C at 10K/min) and then cooling down to room temperature the amount of the inclusion complex formed is also not very large (~25% only), **Table 1.**

Proving that even at relatively high energy of milling (300 rpm, 10:1 ball to powder mass ratio) the degree of complex formation is low (<25 %) the next step was to add different amounts of solvent (C_2H_5OH/H_2O) to the powder mixture and then to mill it at the already established optimal milling parameters. Two regimes of solvent adding were proven, described as BM_2 and BM_3 in the Experimental part. For the BM_2 experiment the amount of the solvent was the same as at the kneading method [32]. Thus, the ball milling technique simulated the mechanical treatment of the CD/IBU mixture at kneading, because the last method can also be considered as successful with respect to the complexation yield (83%), **Fig.2a and Table 1**. From **Table 1** it can also be seen that the suggested "wet milling" method is equally effective in IBU/β-CD complex formation as the solid dispersion technique with ammonia (SD_2). This result was also confirmed by ^{1}HNMR and IR spectroscopy. One very important advantage of the ball milling method is the possibility to estimate the energy transformed to the powder particles during milling, making in this way the synthetic method better defined and enabling optimization and control of the complex preparation conditions.

Information about the effect of the ball milling treatment on the microstructure of the powder mixture and the formation of the inclusion complexes was obtained by x-ray diffraction analysis. **Fig. 3** shows the XRD patterns of the ibuprofen, β-CD, their physical

mixture and the inclusion complexes formed by the different methods. Clear broadening of the diffraction peaks can be observed, as a result of the milling (milling a mixture of ibuprofen and CD at dry conditions), indicating reduction of the crystal size of both compounds. New diffraction peaks however could not be detected. Qualitatively different are the x-ray diffraction patterns of all inclusion complexes showing the presence of new crystalline peaks and disappearance of these of the initial compounds. The new diffraction peaks are broader and with reduced intensity, revealing a nanocrystalline nature of the complexes formed.

In the temperature range of the DSC experiments thermogravimetric (TG) measurements (**fig. 4**) have also been performed for all above studied mixtures checking for any volatiles, which may release under these heating conditions. The TG curve of ibuprofen also reveals that its decomposition starts at about 130°C. The weight decrease step in the TG curve, which corresponds to the broad endothermic DSC peak of β-CD with a maximum at 115°C (**fig.2**) is due to the release of water from the β-CD. Thus, from the TG analysis of the β-CD the number of water molecules related to 1 molecule β-CD was determined to be about 10. It is known that β-CD contains water molecules in its cavity as well as outside the cavities in the CD crystal lattice, as the total number of water molecules was determined by other authors to be about 9-11 to one β-CD unit [52, 53]. Our analysis confirmed this result, i.e. during heating about 10 water molecules (entrapped into the β-CD cavity plus the intermolecular water) release with broad TG step and DSC peak. Taking into account the number of released water molecules obtained by TG (**fig.4**) and measuring the corresponding heat effect by DSC (**fig.2**) the enthalpy of water release of 44 kJ/mol was determined. This value is only slightly lower than that obtained by Bilal, 49 kJ/mol H_2O [53] and confirms the reliability of both thermal methods (DSC and TG) used for this quantitative analysis.

The thermogravimetric analysis of the IBU/β-CD complexes (**figs.4**) showed substantial decrease of the amount of included water compared to the water content of the initial β-CD and can be used to determine the amount of the water unsubstituted by the ibuprofen molecules. In agreement with the TG analysis the magnitude of the broad endothermic peak (**fig.2**) associated with the release of water from β-CD also varies depending on the complex preparation technique. Looking carefully at the change of the magnitude of the endothermic heat effects (DSC peaks), corresponding to the melting of ibuprofen and release of the included into the β-CD water for all IBU/β-CD complexes (partially or fully formed) it could be realized that parallel with the IBU melting peak area decrease (unreacted IBU quantity decrease) the area of the broad “water release” endo- peak

is also reducing. Measuring both, the enthalpy effect for melting the uncomplexed IBU and that for the water release we could calculate and compare the number of IBU and water molecules existing into the β-CD for all complexes formed. Moreover, from the TG curves of the IBU/β-CD complexes their water content could also be obtained. In the cases of almost full complexation (SD_2 and BM_3), evidenced by the absence of DSC peak of IBU melting (**Fig.2**), about 3 molecules water were found to remain in the β-CD (**fig.4**). This means that 7 water molecules are replaced by the ibuprofen. Both above mentioned numbers of water molecules are referred to a molecule β-CD. Based on the NMR study, revealing that the IBU molecule is located inside the β-CD cavity, it can reliably be assumed that IBU replaces all 7 water molecules from the β-CD cavity. This ratio "included IBU : replaced water molecules" (1:7), referred to one β-CD molecule, is confirmed also for the complexes formed by kneading and BM methods, where the yield of complex formation is above 80% (see **fig.5**). This result is an important proof for the distribution of the water molecules into the β-CD (7 inside and 3 outside the cavity) and confirms the hypothesis that during the IBU/β-CD complex formation 1 molecule IBU replaces all 7 water molecules from the hydrophobic β-CD cavity.

Conclusion:

A modified method for ibuprofen/β-CD inclusion complex formation based on ball-milling was developed and compared to the kneading and solid dispersion techniques for cyclodextrin based inclusion complexes. The efficiency of IBU/β-CD complex formation was quantitatively estimated by means of DSC and TG. The highest complex yield was achieved by the modified solid dispersion technique using an alkalizing agent ammonia and by ball-milling in the presence of appropriate amount of solvent (H_2O/C_2H_5OH = 1:1).

It is also worth to note the important finding of this study that each ibuprofen molecule replaces practically all 7 water molecules, initially occupying the β-CD cavity.

Table 1: Melting enthalpies of the residual (not included into β-CD) IBU and yield of complexation

Methods			**ΔH (J/g IBU)**	**Yield of complexation (%)**
SD_2				100
SD_1			31.8	75.2
Kneading			21.8	82.9
BM_1	Intensity of milling (RPM)	time (min)	122	4.6
	100	5 min		
		25 min	122	4.6
		120 min	117	7.9
	300	120 min	114	10.2
BM_2			16.8	86.8
BM_3			4.1	96.8

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Figure captions:

Figure 1: Phase solubility diagram of Ibuprofen in presence of β-CD at 25 °C

Figure 2a: DSC curves of physical mixture and of inclusion compounds: a) Physical Mixture, b) Ball milling, 5 min, 100 rpm, dry, c) Ball milling, 2h, 100 rpm, dry, d) Ball milling, 2h, 300 rpm (BM_1), e) Ball milling, 0.5 mL solvent (BM_2), f) Ball milling, 0.7 mL solvent (BM_3)

Figure 2b: DSC curves of the inclusion compounds: a) Kneading b) Solid Dispersion (SD_1), c) Solid Dispersion (SD_2), d) Ball milling, 0.7 mL solvent (BM_3)

Figure 3: XRD of a) Ibuprofen, b) β-CD and of Inclusion compounds obtained by c) Ball milling, 2h, 300 rpm (BM_1), d) Ball milling, 0.5 mL solvent BM_2, e) Ball milling, 0.7 mL solvent (BM_3), f) Solid dispersion (SD_1), g) Kneading and h) Solid dispersion (SD_2)

Figure 4: TG curves of Inclusion compounds obtained by a) Ball milling, 0.7 mL solution (BM_3), b) Solid dispersion (SD_2), c) Ball milling, 0.5 mL solution (BM_2), d) Kneading, e) Ball milling, 2h, 300 rpm, f) Ball milling, 2 h, 100 rpm and of g) Physical Mixture

Figure 5: Moles water replaced by ibuprofen (referred to a molecule β-CD)

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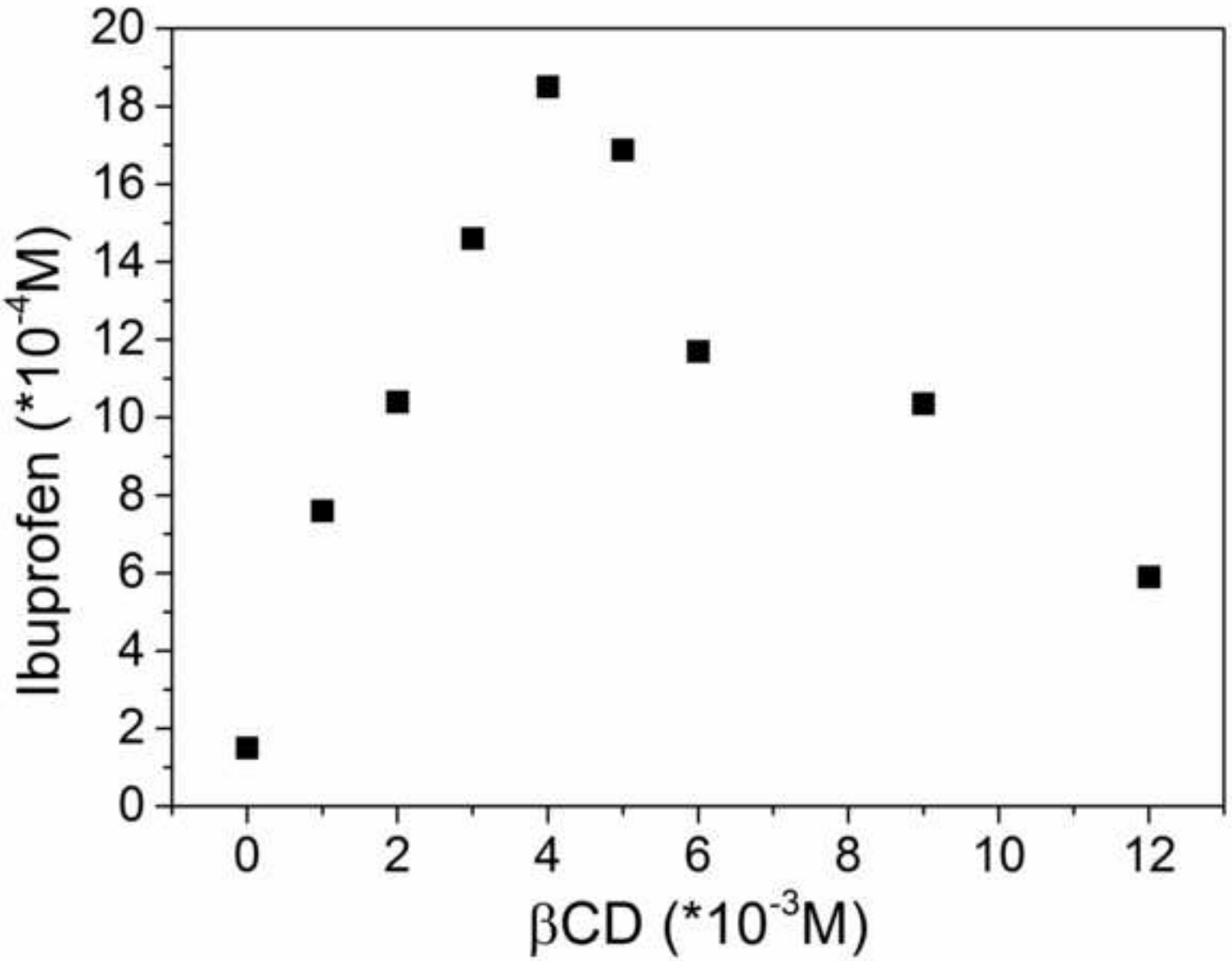

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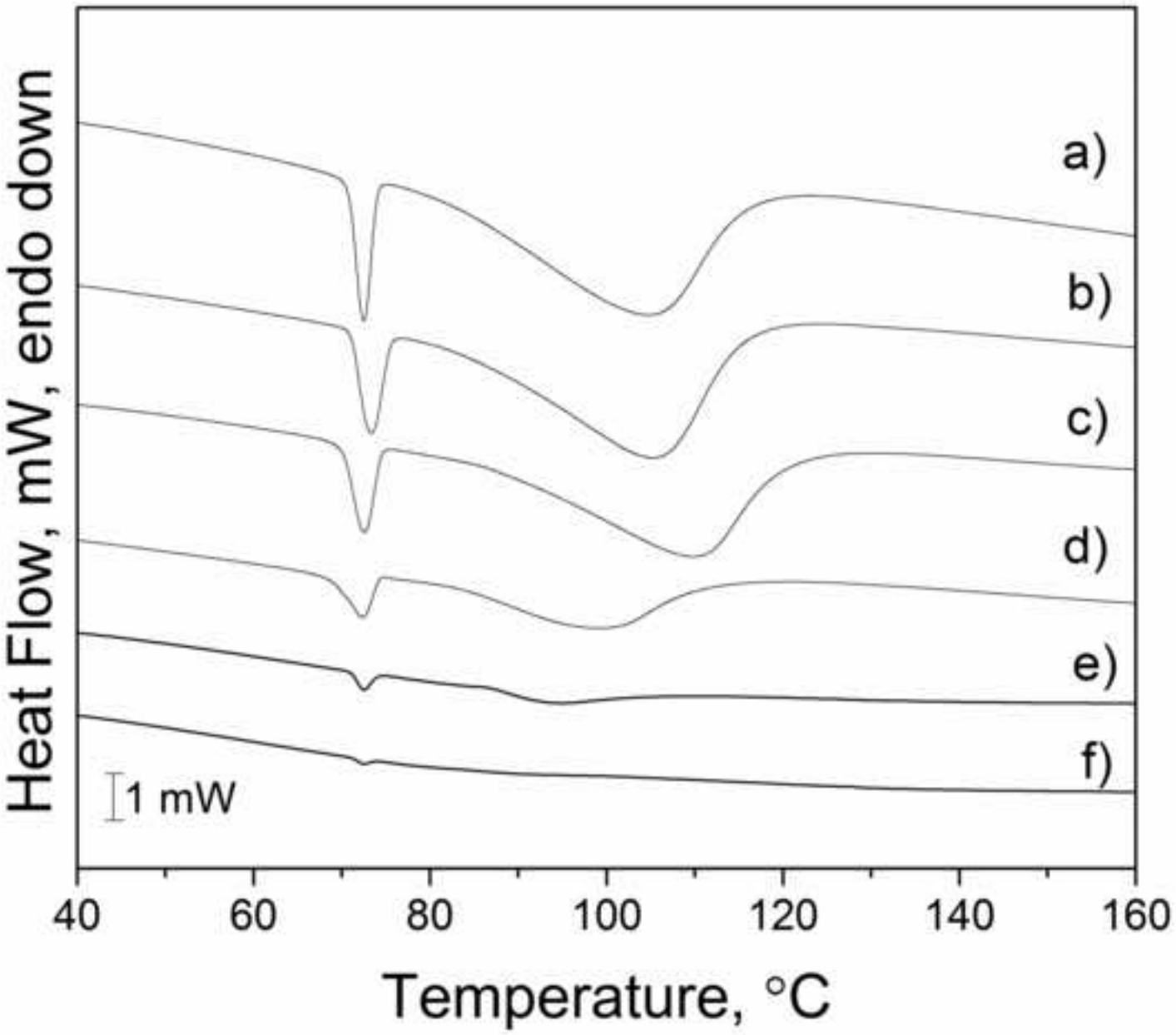

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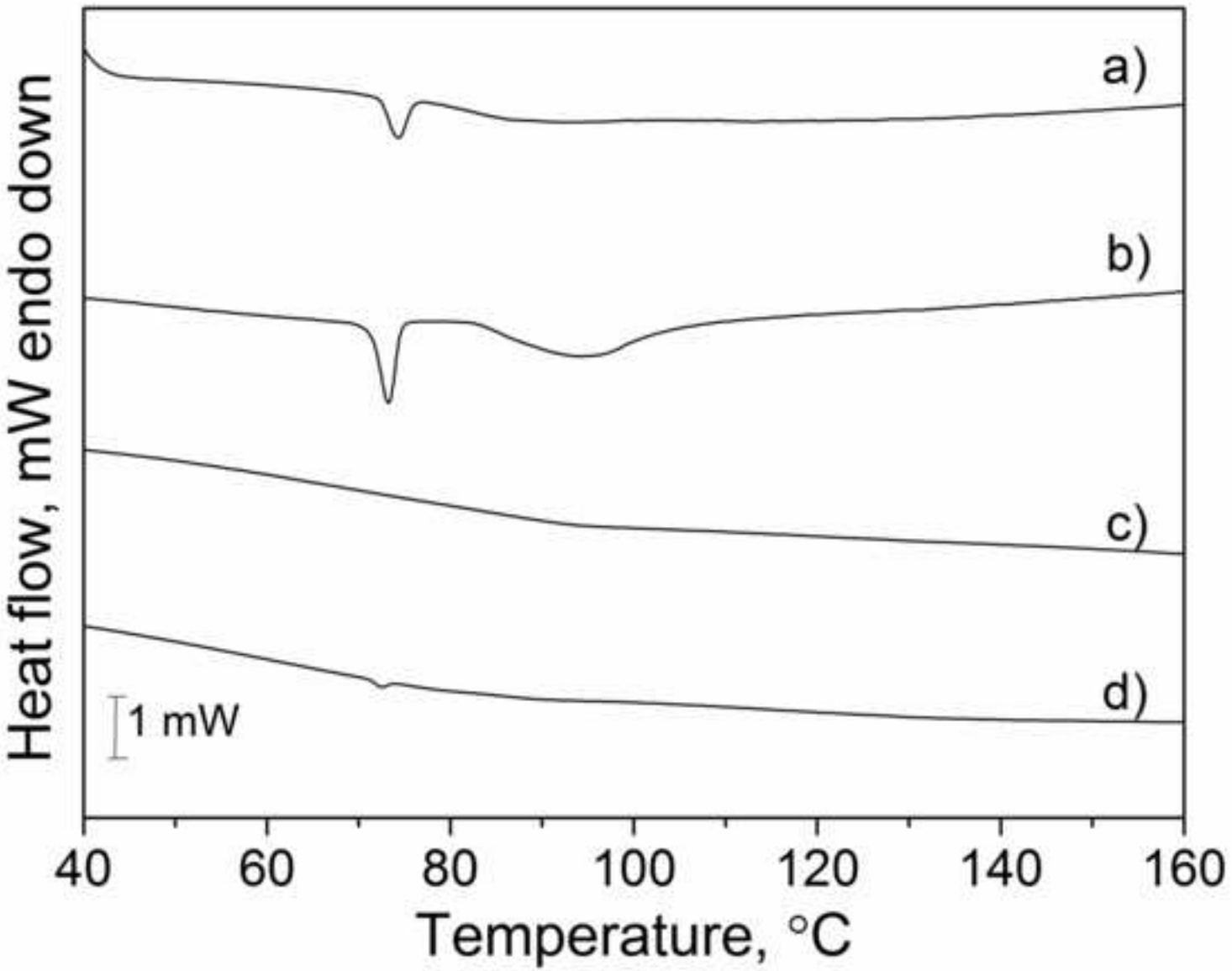

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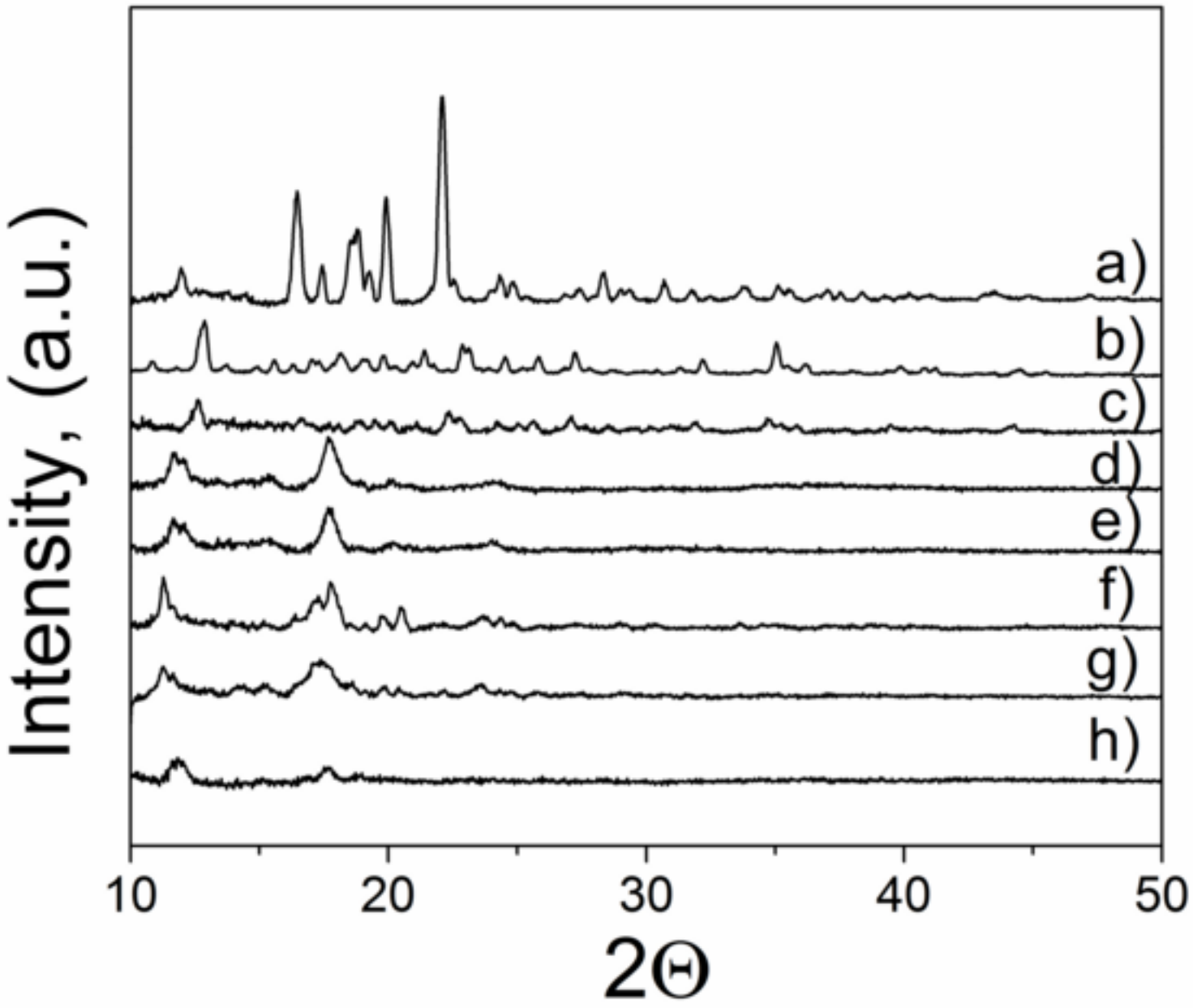

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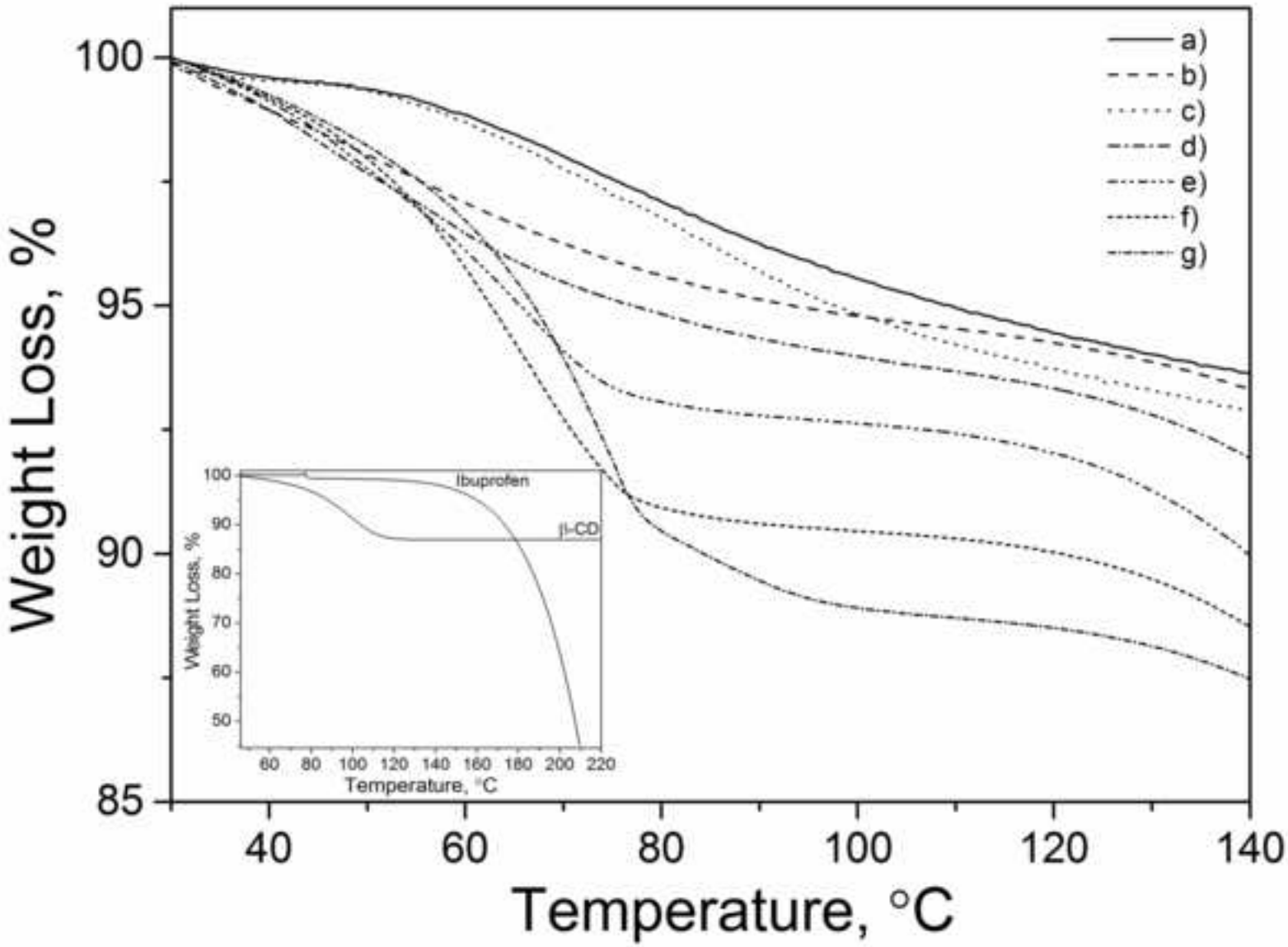

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