

Formation of low melting point binary systems comprising ketoprofen and an amide local anaesthetic

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ABSTRACT

Liquid forms of active pharmaceutical ingredients, ionic liquids (ILs) and deep eutectic mixtures (DEMs), offer several potential benefits in respect to advancing pharmaceutical formulations. The aim of this study was to develop and characterise ILs/DEMs composed of two active molecules: ketoprofen (KET), as the acidic component, and a local anaesthetic (LA), lidocaine (LID), mepivacaine (MEP) or bupivacaine (BUP), which constituted the basic component. A mechanosynthetic approach was successfully applied to obtain LA-KET low melting systems. Composition/temperature phase diagrams were determined by differential scanning calorimetry. The amide LA-KET mixtures showed a eutectic behaviour during heating and formed viscous liquids upon quench cooling. Considering the quench cooled LA-KET mixtures, LA crystallisation was observed only in the LA-rich mixtures. LID, MEP and BUP formed disordered complexes with KET at an approximate 1:2 stoichiometry. Infrared spectroscopy studies revealed that the mixtures were composed mainly of hydrogen bonded acid and base molecules, but small amounts of carboxylate anions were detected. The formation of LA-KET complex not only suppressed the high crystallisation tendency of the LA molecules in the dry state, but also eliminated the crystallisation of KET and LA molecules induced by moisture, as revealed by dynamic vapour sorption studies.

1. Introduction

Deep eutectic mixtures (DEMs) and ionic liquids (ILs) are emerging as promising strategies to obtain favourable forms of active pharmaceutical ingredients (APIs) (Moreira et al., 2015; Griffin et al., 2014; Balk et al., 2015; Berton et al., 2017; Wojnarowska et al., 2018; Umerska et al., 2020a). Such systems have fewer issues associated with the use of highly crystalline APIs, such as polymorphism, limited solubility and bioavailability. DEMs offer many advantages in drug delivery such as increasing drug dissolution and improving drug penetration. They could be potentially incorporated in both, liquid and solid-state formulations. The dual functional ILs and DEMs, obtained by combining biologically active cations and anions via reversible intermolecular interactions, offer the advantage of lowering either the melting point (Mp) or the glass transition temperature (Tg) of the API in addition to providing a second biological function, an approach very attractive for pharmaceutical combination products (Hough et al., 2007; Hough and Rogers, 2007; Wang et al., 2014; Umerska et al., 2020a). They differ from co-amorphous systems as those are defined as single phase amorphous solids. There is a wide range in ionisation degree in these

multicomponent API forms, from the ILs (ionised species), through oligomeric ILs (mixtures of ionised and neutral molecules), to DEMs (hydrogen bonding between the parent compounds) (Griffin et al., 2014; Balk et al., 2015; Berton et al., 2017). The ionisation degree and the molar ratio between the components are important for pharmaceutical applications since they influence the solubility and pharmacokinetics of the API (Berton et al., 2017).

Local anaesthetic agents (LAs) have a wide range of clinical uses and there is an interest in low melting form of these drugs, since they can improve bioavailability via routes such as the transdermal (Berton et al., 2017). Among the LAs, lidocaine (LID) is undoubtedly the most widely investigated component of the ILs/DEMs. LID has been shown to form ILs with counterions such as saccharinate (Moreira et al., 2015), ace-sulfamate (Moreira et al., 2015), docusate (Moreira et al., 2015; Berton et al., 2017; Mann et al., 2020) and dicarboxylic acids (Zotova et al., 2020) as well as DEMs with carboxylic acids such as decanoic (Griffin et al., 2014), stearic and oleic acid (Bica et al., 2011). Considering the LID-based DEMs/ILs, those with non-steroidal anti-inflammatory drugs (NSAIDs) attracted a great deal of attention. Combinations with ibuprofen (Wang et al., 2014; Berton et al., 2017; Mann et al., 2020), but

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Table 1

Physicochemical properties of investigated molecules (determined experimentally or obtained from [Wishart, 2006](#)).

Molecule	Ketoprofen	Lidocaine	Bupivacaine	Mepivacaine
Molecular weight	254.28 g/mol	234.34 g/mol	288.43 g/mol	246.35 g/mol
Melting point	94.7 ± 0.4 °C ¹	68.4 ± 0.1 °C ²	106.1 ± 0.4 °C ²	153.2 ± 0.1 °C ²
ΔH	106.0 ± 1.3 J/g ¹	68.6 ± 0.2 J/g ²	89.8 ± 0.2 J/g ²	85.8 ± 0.9 J/g ²
pKa ³	3.88	7.75	8.09	7.25
Hydrogen donor counts	1	1	1	1
Hydrogen acceptor counts	3	2	2	2
Crystallisation tendency	III ⁴	I ⁴	I ²	I ²

¹ Obtained experimentally by [Umerska et al. \(2020a\)](#).

² Obtained experimentally in this work.

³ Strongest acidic for KET, protonated amine for Las.

⁴ According to [Baird et al. \(2010\)](#).

also with other NSAIDs such as indomethacin ([Shimada et al., 2013](#)), naproxen ([Fiandaca et al., 2020](#)), diclofenac ([Moreira et al., 2015](#), [Maneewattanapinyo et al., 2019](#)), aceclofenac ([Marei et al., 2021](#)), etodolac ([Miwa et al., 2016](#)), flurbiprofen ([Moreira et al., 2015](#), [Marei et al., 2021](#)), ketoprofen ([Marei et al., 2021](#)), flufenamic acid ([Moreira et al., 2015](#)), tenoxicam and meloxicam ([Marei et al., 2021](#)), and salicylate ([Bica et al., 2010](#)) have been reported. Bupivacaine (BUP) has been less investigated than LID. BUP has been shown to form ILs with docusate, acesulfamate and saccharinate counterions ([Moreira et al., 2015](#)), and DEMs with flurbiprofen, diclofenac and flufenamic acid ([Moreira et al., 2015](#)). To the best of authors' knowledge, there has been no report on mepivacaine-based DEMs/ILs thus far. The combinations of LAs with NSAIDs offer the unique possibility of having dual functional analgesics, desirable from the therapeutical point of view ([Bica et al., 2010](#)).

In many cases LID and BUP form DEMs rather than ILs, but most of the researcher groups tested only equimolar LA-NSAID combinations. However, there have been reports on complexes formed between two molecules of acid and one molecule of LID, such as that with decanoic acid ([Griffin et al., 2014](#)), indomethacin ([Umeda et al., 2007](#), [Umeda et al., 2009](#)) and naproxen ([Fiandaca et al., 2020](#)). Moreover, in the case of a eutectic system the whole range of compositions should be investigated, because the eutectic can form at component ratios that can be non-stoichiometric ([Umerska et al., 2020a](#)). To this end, the construction of a thermodynamic phase diagram can be of a significant benefit ([Wang et al., 2014](#), [Fiandaca et al., 2020](#), [Umerska et al., 2020a](#)). Considering dual LID-API combinations, full phase diagrams have been constructed with ibuprofen ([Wang et al., 2014](#)), indomethacin ([Shimada et al., 2013](#)) and naproxen ([Fiandaca et al., 2020](#)), with the latter also describing a solid-state form of naproxen and LID with an atypical 2:1 stoichiometry. No phase diagram has been constructed for BUP thus far and since BUP and MEP have similar structure to LID, they could also potentially form complexes with NSAIDs at a 1:2 M ratio.

Considering NSAIDs, KET-based ILs have not been extensively investigated ([Balk et al., 2015](#), [Ribeiro et al., 2016](#), [Ossowicz et al., 2020](#)) despite the low melting point and low crystallisation tendency of this molecule, thus making it an ideal drug component for DEM formation. Among the KET-local anaesthetic systems, only our previous work that describes the DEMs between KET and ester local anaesthetics has been published thus far ([Umerska et al., 2020a](#)). The ester local anaesthetics described in that work (benzocaine, tetracaine and procaine) differed in their pKas, hence ionisation tendency, and exhibited different crystallisation propensity. Both parameters, i.e. the pKa and crystallisation tendency may be of importance as regards the formation of DEMs, as they may influence the magnitude of interactions within the DEM. The disadvantage of ester local anaesthetics is the incidence of

allergic reactions due to their metabolite (p-aminobenzoic acid) ([Eggleston and Lush, 1996](#)). Amide local anaesthetics are alternative to esters and do not undergo such metabolism. Amides are very stable, whereas esters are relatively unstable in aqueous solution. The physicochemical properties of amide LAs investigated in this study are shown in [Table 1](#). In contrast with the heterogeneous ester LA group, amide LAs show similar pKa values (7.75, 7.25 and 8.09 for LID, MEP and BUP, respectively, [Table 1](#)). LID is well known to have a high crystallisation tendency and low glass forming ability ([Baird et al., 2010](#)), similarly to BEN described in the previous study ([Umerska et al., 2020a](#)), but in terms of ionisation tendency LID resembles more procaine and tetracaine than benzocaine. Therefore, investigation into interactions between KET and LID would provide better understanding of DEMs/ILs. There is no information about the glass forming ability of either MEP or BUP, thus determination of their crystallisation tendency was one of the aims of this study.

The aim of this work was to investigate the thermal behaviour, solid-state properties, and crystallisation tendency of physical and quench cooled binary mixtures comprising ketoprofen (KET) and one of the following LAs: lidocaine (LID), bupivacaine (BUP) or mepivacaine (MEP). A whole range of compositions was investigated to determine the ratios where the mixtures can be regarded as DEMs and the strength of intermolecular interactions. The latter was accomplished by infrared spectroscopy. To develop straightforward synthesis for these new formulations, without the risk of solvent, halide and metal contamination, a mechanochemistry approach was used, whereby KET as a free acid was mixed with the LA as a free base. Also, as it was deemed a critical property from the stability point of view, selected systems were examined in terms of the effect of moisture/humidity on the preservation of their physicochemical character.

2. Materials and methods

2.1. Materials

Ketoprofen (KET), mepivacaine (MEP) and bupivacaine (BUP) were purchased from Fluorochem Ltd., (UK). Lidocaine (LID) was purchased from Sigma-Aldrich (Ireland). All chemicals were used as supplied with the exception of MEP, which was heated to 130 °C for 5 min in order to remove the hydrate from the starting material, manifested as an endotherm at 108.4 °C (by differential scanning calorimetry). Water HPLC grade used for DVS experiments was obtained from Fisher Scientific (UK).

2.2. Methods

2.2.1. Sample preparation

A mechanosynthetic process described previously ([Umerska et al., 2020a](#)) was used to prepare approximately 200 mg of binary KET-LA mixtures containing 5–95 mol% of KET. KET and LA were accurately weighed using an MT5 Mettler Toledo microbalance (Switzerland) and ground in an agate mortar using an agate pestle until a homogenous mixture was obtained.

2.2.2. Preliminary thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) experiments

Preliminary TGA and DSC experiments were performed using Mettler Toledo system as described previously ([Umerska et al., 2015](#), [Mesallati et al., 2017](#), [Umerska et al., 2020b](#)) to determine thermal stability of the samples and the temperature range for further DSC analysis using the Perkin Elmer system as described below. Samples were heated from 25 to 250 °C at a rate of 10 °C/min using nitrogen as the purge gas. Samples were considered as stable at temperatures at which the mass loss determined by TGA was less than 5% of the initial weight ([Umerska et al., 2020b](#)).

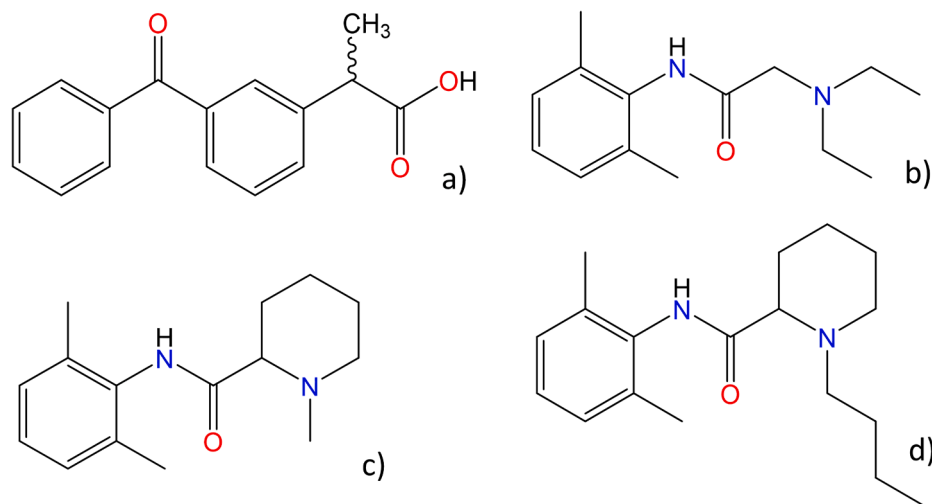


Fig. 1. Chemical structures of the investigated molecules (a) ketoprofen (KET), (b) lidocaine (LID), (c) mepivacaine (MEP) and (d) bupivacaine (BUP).

2.2.3. Differential scanning calorimetry (DSC)

To examine the thermal behaviour of KET-LA powder mixtures and quench cooled KET-LA systems a Perkin Elmer DSC was used as described previously (Umerska et al., 2020b). Approximately 3–5 mg of samples were accurately weighed into 18 μ l aluminium pans with cover, held at either 25 $^{\circ}$ C (BUP-KET and MEP-KET mixtures) or at 0 $^{\circ}$ C (LID-KET mixtures) for 1 min in the DSC unit, then heated at a rate of 10 $^{\circ}$ C/min (=first heating), cooled at a nominal rate of 300 $^{\circ}$ C/min, held at either –50 $^{\circ}$ C (BUP-KET and MEP-KET mixtures) or –60 $^{\circ}$ C (LID-KET mixtures) for 5 min and reheated at a rate of 10 $^{\circ}$ C/min (=second heating).

Phase diagrams were constructed using the experimental thermal data and the average and standard deviations of three independent experiments are presented. For melting events the onset is presented, whereas for the glass transition event the midpoint is shown. The data from the first heating were compared to theoretical phase diagrams of an ideal eutectic mixture calculated using the Schröder van Laar equation (Eq. (1)) (Meltzer and Pincus, 2012; Umerska et al., 2020a):

$$\ln x_i = -\frac{\Delta H_{fi}}{R} \left(\frac{1}{T} - \frac{1}{T_{fi}} \right) \quad (1)$$

where x_i is the mole fraction of the component i at temperature T (in Kelvin degrees), R is the gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), ΔH_{fi} is the molar enthalpy of fusion of component i , and T_{fi} is the melting temperature (onset) of pure component i (in Kelvin degrees). ΔH_{fi} and T_{fi} were determined by DSC.

The percentage of crystallinity of LA in the second heating cycle was calculated using Eq. (2) (Blaine, 2002; Umerska et al., 2020a):

$$\% \text{ crystallinity} = \frac{\Delta H}{\Delta H_f} \cdot 100\% \quad (2)$$

where ΔH is the enthalpy of melting obtained from DSC and corrected for the LA content and ΔH_f is the enthalpy of melting of the reference, which was considered 100% crystalline (i.e. ΔH of LID and MEP from the second heating, 67.6 J/g and 83.0 J/g, respectively).

2.2.4. X-ray diffraction (XRD)

Diffraction patterns in the range 5–40 2theta degrees at a step size of 0.05 $^{\circ}$ per second were obtained by using a Rigaku Miniflex desktop X-ray diffractometer (Japan) equipped with Ni-filtered Cu K α radiation ($\lambda = 1.5408 \text{ \AA}$) (Umerska et al., 2018). Samples were spread on a low background silicon mount and the measurements were carried out at room temperature.

2.2.5. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra at a range of 650–4000 cm^{-1} were recorded on a PerkinElmer Spectrum 100 FTIR spectrometer (USA) as described previously (Umerska et al., 2020a).

2.2.6. Polarized light hot stage microscopy

Microscopy experiments were conducted using an Olympus BX53 polarising optical microscope equipped with a U-POT cross polariser, a Linkam hot stage (model LTS420) and a Q IMAGING Fast 1394 camera (Olympus, Japan) at $\times 80$ magnification. BUP powder was heated from room temperature to 120 $^{\circ}$ C at 10 $^{\circ}$ C/min, cooled down to –60 $^{\circ}$ C at 50 $^{\circ}$ C/min and reheated to 120 $^{\circ}$ C at 10 $^{\circ}$ C/min.

2.2.7. Dynamic vapour sorption (DVS)

DVS studies were performed using an Advantage-1 automated gravimetric vapour sorption analyser (Surface Measurement Systems Ltd., London, U.K.) at $25.0 \pm 0.1 \text{ }^{\circ}\text{C}$ (Mesallati et al., 2017). Nitrogen was used as a dry carrier gas. Approximately 25 mg of the quench cooled LA-KET sample in the sample basket was placed in the instrument and equilibrated at 0% relative humidity (RH) until a constant mass was obtained ($\text{dm}/\text{dt} \leq 0.002 \text{ mg}/\text{min}$). The reference mass was recorded and sorption-desorption analysis was then carried out between 0 and 90% RH in steps of 10% RH. At each stage the sample mass was equilibrated ($\text{dm}/\text{dt} \leq 0.002 \text{ mg}/\text{min}$ for at least 10 min and the maximum equilibration time was set as 480 min) before the RH was changed. An isotherm was calculated from the complete sorption and desorption profile. Following DVS analysis, the samples were analysed by XRD in order to detect any crystallisation.

3. Results and discussion

3.1. Physicochemical properties of investigated molecules

KET is a derivative of propionic acid and it bears a negative charge at physiological pH (7.4) due to the ionisation of carboxylic group ($\text{pK}_a = 3.88$). LID, MEP and BUP are amino-amide anaesthetic agents with the aromatic head and the hydrocarbon chain that are linked by an amide bond (Fig. 1). All the above mentioned LAs are derivatives of 2,6-xyli-dine (2,6-dimethylphenylamine). Unlike LID, the terminal amino portion of BUP and MEP is contained within a piperidine ring; these agents are known as piperidyl xyli-dines. These LAs bear a positive charge at physiological pH due to ionisation of the amino groups (pK_a of 7.25, 7.75 and 8.09 for MEP, LID and BUP, respectively). KET and LAs have 1 hydrogen donor group each. KET has 3 hydrogen acceptors,

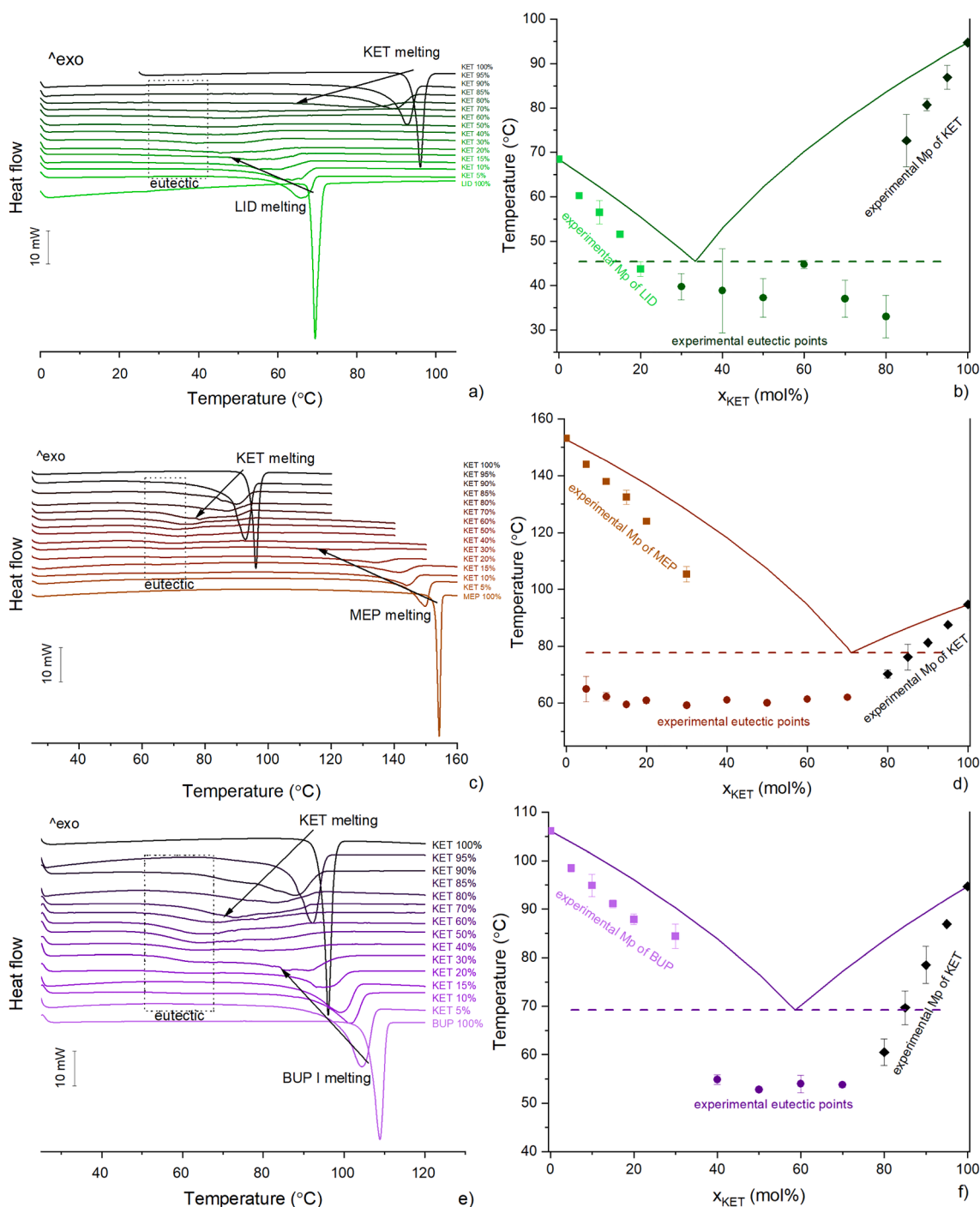


Fig. 2. Thermal analysis of LA-KET systems; DSC thermograms from first heating of (a) LID-KET, (c) MEP-KET (e) and BUP-KET mixtures; phase diagrams based on thermal analysis of DSC first heating of (b) LID-KET, (d) MEP-KET and (f) BUP-KET mixtures. In the thermograms the rectangle indicates the position of the eutectic peak while the arrows show the position of API melting peaks. In the phase diagrams the solid lines show the theoretical liquidus curves, while the broken line indicates the position of the eutectic, all calculated using Eq. (1).

whereas each LA molecule has 2 hydrogen acceptors (Table 1).

3.2. Thermal behaviour of KET-LA powder mixtures

In this work mechanosynthesis was used to obtain LA-KET mixtures. Apart from numerous advantages such as high product yield, purity and no need for organic solvents (Umerska et al., 2020a) the use of mechanosynthesis enables precise control of system stoichiometry, in contrast with metathesis, whereby the composition of the final product is influenced by extraction/isolation process and cannot be precisely

controlled, particularly for compounds that form eutectics.

To analyse the thermal behaviour of KET and LA powder mixtures, binary physical mixtures were subjected to heating in the DSC apparatus. The experimental melting points and ΔH of starting materials are reported in Table 1. All starting materials gave sharp melting endotherms in the first heating, with the onset at 94.7 ± 0.4 , 68.4 ± 0.1 , 106.1 ± 0.4 and 153.2 ± 0.1 °C for KET, LID, BUP and MEP, respectively.

In the binary systems, i.e. LID-KET, BUP-KET and MEP-KET, the melting point of each API decreased and the melting endotherms

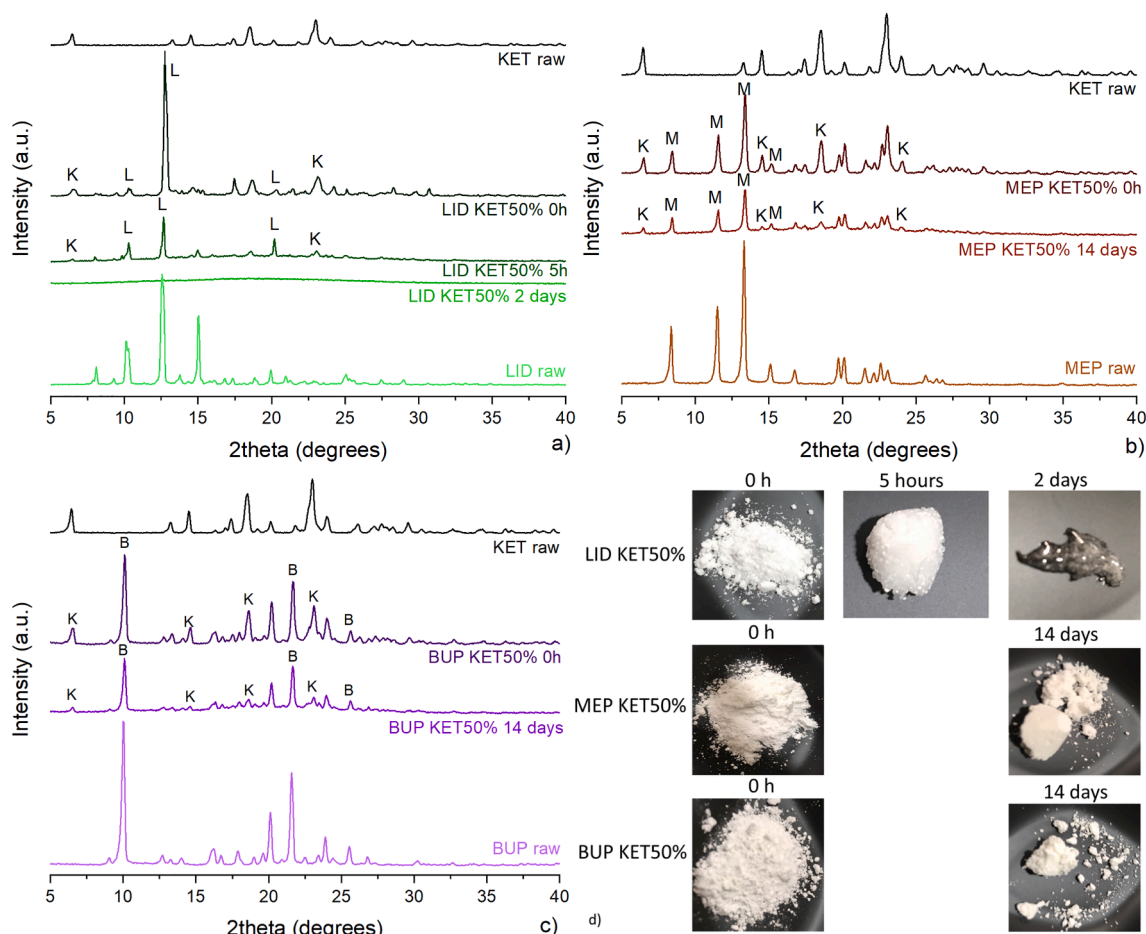


Fig. 3. XRD of starting material (raw) KET, LAs and equimolar LA-KET systems freshly prepared (0 h) and after storage at room temperature: (a) LID-KET, (b) MEP-KET and (c) BUP-KET systems. Morphologies of the equimolar mixtures are presented in (d).

became broader corresponding to its decreasing content in the mixture (Fig. 2). A melting peak of MEP and BUP was detected in mixtures containing at least 70 mol% of these drugs. Melting of LID was observed in mixtures containing at least 80 mol% of LID. KET melting was detected in samples containing at least 80, 80 and 85 mol% of KET for MEP-KET, BUP-KET and LID-KET, respectively. The mixtures with a composition closer to equimolar showed broad, flat endotherms, with onsets lower than that of any of the constituents, a feature characteristic of eutectic systems. The broadness of these peaks could be due to the low tendency to crystalline of the eutectic phases and also affected by the heating rate used (Rycerz, 2013; Umerska et al., 2020a). In all systems tested deviations of experimental melting points of starting components (particularly in KET-rich mixtures) from those calculated using the Schröder van Laar equation (Eq. (1)) were observed. Theoretical eutectic compositions were 33.35, 71.7 and 58.7 mol% of KET for LID-KET, MEP-KET and BUP-KET systems, respectively. This difference in theoretical eutectic compositions is attributed to different melting points and melting enthalpies of LAs (Martins et al., 2019). Unfortunately, it was impossible to determine the experimental eutectic composition using a Tamman plot as previously described for the KET-benzocaine system (Umerska et al., 2020a) due to uncertainty in determination of enthalpy of the eutectic peak. The calculated eutectic temperature for the LID-KET system (45.4°C) showed a reasonable agreement with the experimental data, however some points departed from the theoretical eutectic line. However, the calculated eutectic temperature of the MEP-KET system (78.3°C) was notably higher than the experimental melting point of eutectic (between 59.2 ± 0.1 and $64.9 \pm 4.5^{\circ}\text{C}$). Similarly, the calculated melting temperature of the BUP-KET eutectic (69.2°C) was

higher than the experimental eutectic melting point (between 52.8 ± 0.2 and $54.9 \pm 1^{\circ}\text{C}$). The negative deviations from the behaviour of an ideal eutectic system for all tested LA-KET mixtures imply the presence of new and stronger molecular interactions between the components, in contrast with an ideal eutectic system whereby the strength of intermolecular interactions is comparable with those in the pure components. The experimental and theoretical liquidus curves for all tested systems do not coincide with each other, confirming the occurrence of intermolecular interactions such as H-bonding between the condensed phases of the APIs. The presence of peaks of parent compounds were confirmed in XRD patterns of the freshly prepared binary mixtures at room temperature (Fig. 3), but no new Bragg peaks were detected.

The behaviour of powders during mixing of equimolar LA-KET systems was analysed by XRD and also visually. The equimolar BUP-KET and MEP-KET powder mixtures did not change appearance during mixing, however after 2 weeks of storage at 25°C (ambient relative humidity) the powder agglomerated (Fig. 3) and became sticky. The XRD studies indicated a decrease in intensity of Bragg peaks after 14 days of storage for both, BUP-KET and MEP-KET systems. The equimolar LID-KET mixture became sticky during mixing and complete liquefaction was observed within 2 days. This is consistent with XRD results, whereby a decrease in intensity of the diffraction peaks was observed after a few hours of storage and the sample stored for 2 days at 25°C showed no peaks in the X-ray diffractogram. This observation may suggest a moisture-mediated phase transformation of the samples.

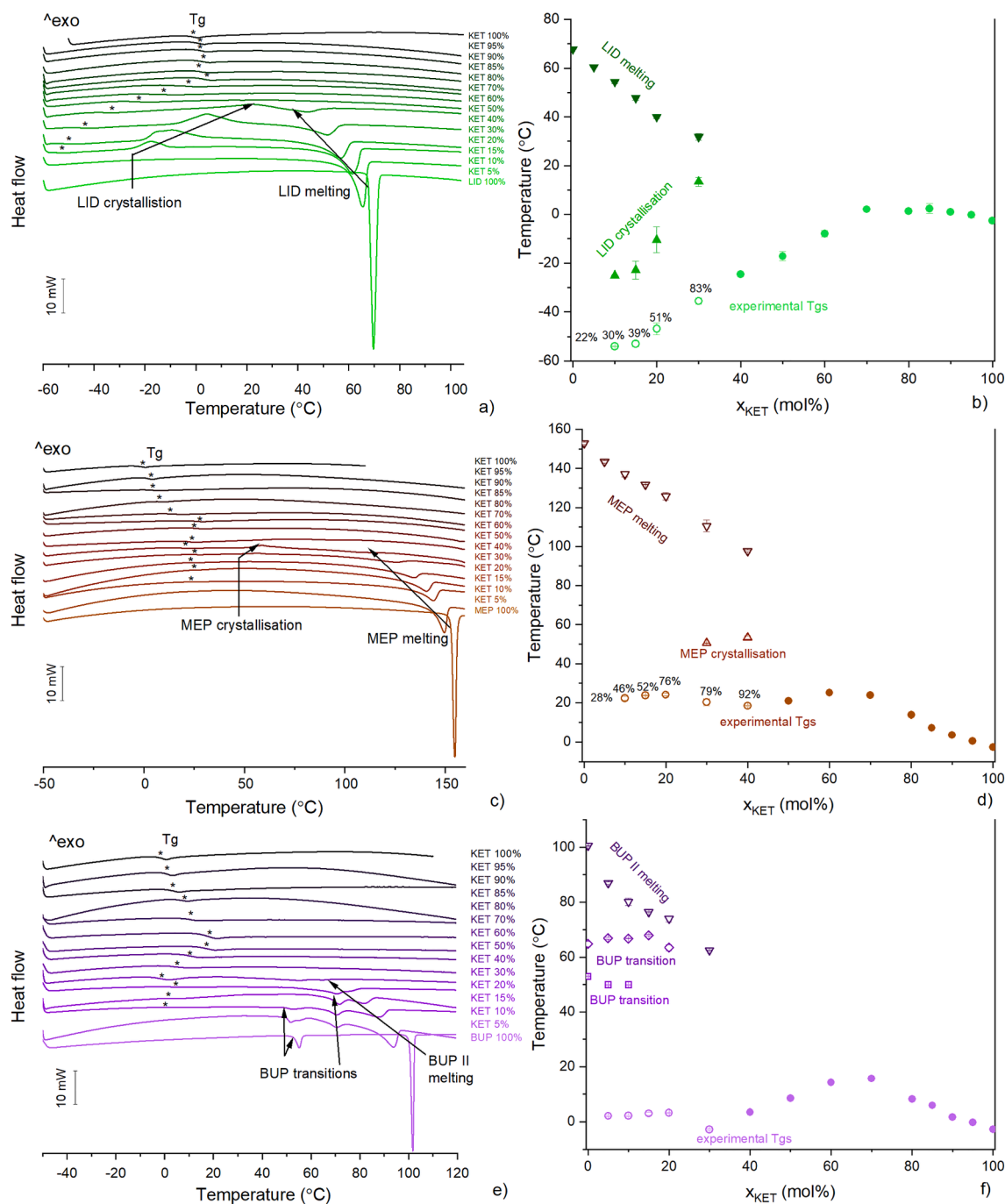


Fig. 4. Thermal analysis of LA-KET systems; DSC thermograms from second heating of (a) LID-KET mixtures, (c) MEP-KET mixtures and (e) BUP-KET mixtures and phase diagrams based on thermal analysis of second DSC heating of (b) LID-KET mixtures, (d) MEP-KET mixtures and (f) BUP-KET mixtures. The samples were first heated above the melting point at 10 °C, quench cooled at a nominal rate of 300 °C/min, and reheated at 10 °C/min. In the thermograms asterisks indicate glass transition while the arrows show the position of API melting peaks. Open circles in phase diagrams indicate T_g s of samples that were not fully disordered after quench cooling; the % value indicates the content of disordered phase calculated using Eq. (2).

3.3. Thermal behaviour of quench cooled KET-LA mixtures

In addition to mechanosynthesis, quench cooling of mixtures was examined to obtain supercooled samples and remove residual crystallinity. The DSC thermograms and phase diagrams of quench cooled (QC) KET, LA and LA-KET melts are shown in Fig. 4. KET formed a supercooled liquid with a T_g of -2.7 ± 0.5 °C, in agreement with previously reported data (Umerska et al., 2020a). According to Baird et al. (2010), KET is a class III molecule, which does not crystallise from the supercooled melt (Baird et al., 2010, Umerska et al., 2020a).

LID is a class I molecule with high crystallisation tendency that does not form a glass (Baird et al., 2010, Wojnarowska et al., 2018). It was observed here that LID crystallised during the cooling step and the enthalpy of melting on second heating was 98.5% of that obtained from the first heating. Interestingly, addition of only 5 mol% of KET prevented some LID crystallisation and the degree of crystallinity of this mix was 78%. T_g s were detected for QC mixtures containing at least 10 mol % of KET and ranged from -54.1 ± 0.1 (90 mol% of LID) to $+2.1 \pm 0.1$ °C (30 mol% of LID) (Fig. 4a). Deviations from linearity between the compositions and T_g s were observed in KET-rich samples (≥ 80 mol% of

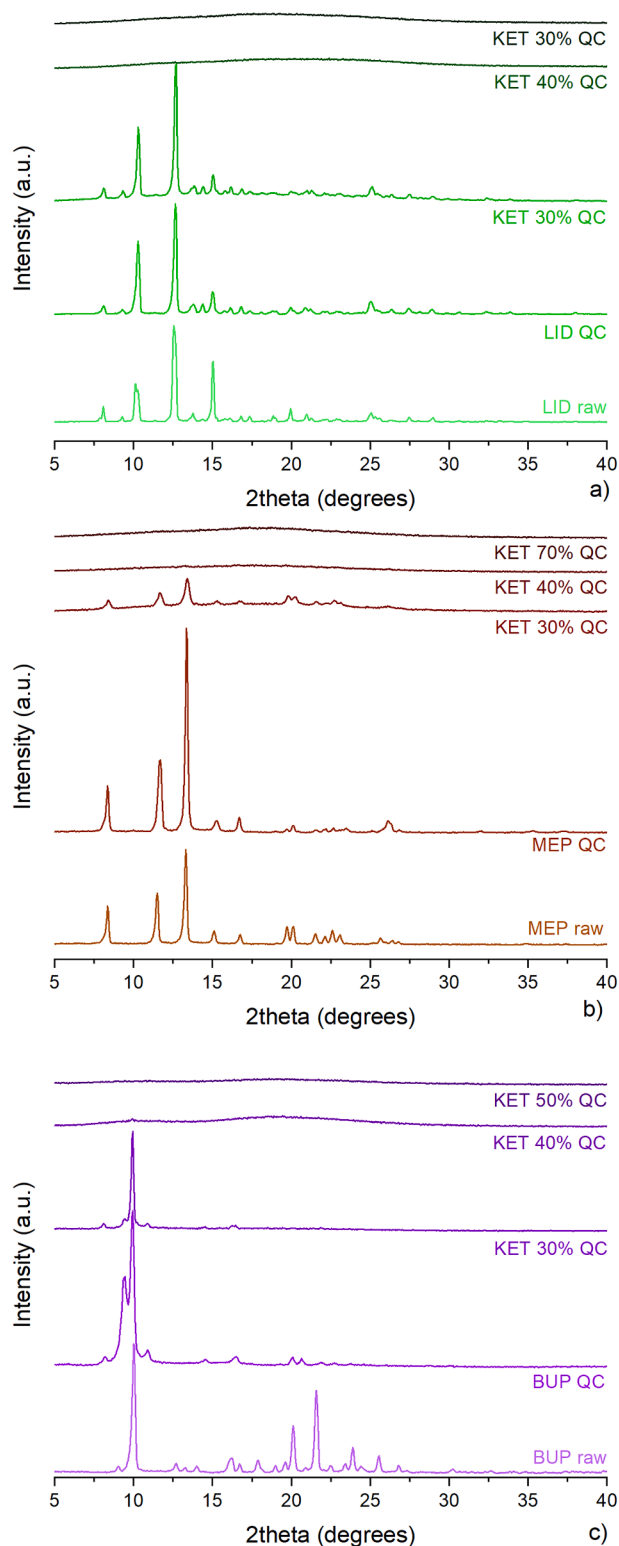


Fig. 5. XRD of quench cooled (QC) LAs and LA-KET mixtures (a) LID-KET, (b) MEP-KET and (c) BUP-KET systems.

KET) (Fig. 4b). In samples containing 10–30 mol% of KET the Tg was followed by LID crystallisation with the onset increasing from -25.1 ± 0.1 to 13.4 ± 1.8 °C when KET content increased from 10 to 30 mol%. The crystallisation event was followed by LID melting at onset temperatures decreasing with an increasing KET content (Fig. 4a). The % of crystalline LID decreased from 70% to 17% when the KET content increased from 10 to 30 mol%. Samples containing at least 40 mol% of

KET showed only one phase transformation, a Tg, during the second heating and the presence of crystalline LID was not detected by DSC and XRD. It means that the addition of at least 40 mol% of KET to LID increased thermal stability of the QC mix and prevented crystallisation of LID. The highest Tg of approximately 2 °C were observed in samples containing 70 and 85 mol% of KET. X-ray diffractograms of samples containing 30 mol% of KET and less had the same pattern as that of LID, thereby confirming that it is the LA that crystallises, whereas samples containing at least 40 mol% of KET showed a pattern characteristic of non-crystalline, disordered materials (Fig. 5a).

In the second heating of MEP neither Tg nor crystallisation was observed, but the sample melted at 153 °C. This sample was approximately 97% crystalline when compared with the starting material. XRD experiments confirmed that MEP crystallised to the same polymorphic form as the starting material (not shown). Hence, MEP can be categorised as a class I molecule with high crystallisation tendency and no glass forming ability. Neither Tg nor crystallisation was detected in the second heating of 5 mol% KET MEP-KET mixture, suggesting a similar behaviour to LID. In the samples containing 10–20 mol% of KET a Tg at 22–24 °C was detected, that was followed by a melting endotherm. The degree of MEP crystallinity was 54, 48 and 24% for samples containing 10, 15 and 20 mol% of KET, respectively (Fig. 4c). In samples containing 30 and 40 mol% of KET the Tg occurred at approximately 20 °C, then a crystallisation event with the onset at 50.5 ± 0.1 °C and 53.4 ± 0.8 °C, respectively, was observed, which was followed by melting of MEP. Melting points of MEP decreased corresponding to an increasing KET content and were the same as those from the first heating, but in the first cycle melting was not detected in the 40 mol% of KET mixture with MEP. In the samples containing at least 50 mol% of KET only the Tg was observed, with the highest Tg (25.1 ± 0.5 °C) detected for the sample containing 60 mol% of KET (Fig. 4d). XRD data are in agreement with DSC results as the XRD patterns of samples containing at least 50 mol% of KET did not show Bragg peaks and were typical of disordered samples, whereas in samples containing 30 mol% and less KET the diffraction patterns were identical to that of MEP, confirming crystallisation of LA in excess (Fig. 5b). The sample containing 40 mol% of MEP did not clearly show the presence of MEP, only trace amounts of crystalline material were detected (peaks at approximately 13–17° 2theta with very low intensity), consistent with low MEP crystallinity by DSC, that is close to detection limit of XRD.

Three endothermic transitions were observed in QC BUP during the second heating with the onset at 53.0 ± 0.1 , 64.8 ± 1.0 and 100.5 ± 0.1 °C (Fig. 4e). These events occurred at lower temperatures than melting of the starting material BUP. Neither crystallisation nor Tg were observed, thereby indicating that BUP crystallised on cooling and can be assigned as a class I molecule. XRD indicated that a different polymorphic form of this drug, referred to as BUP II, was obtained by quench cooling (Fig. 5c). Hot stage microscopy experiments were performed to visualise the BUP transitions during the reheating. It can be observed that the material was dominated by the phase that melted at 100.5 °C and little changes were seen below this temperature (Fig. 6). A Tg was detected in all tested BUP-KET mixtures except for 5 mol% KET, this transition was more difficult to be observed in BUP-rich samples (10–20 mol% of KET) and it was followed by BUP crystallisation (Fig. 4e). The other BUP transitions in the 5–20 mol% samples were similar to those seen in BUP on its own, but in the BUP-KET samples the endotherms were broader and closer to one another. The sample containing 30 mol% of KET showed a pronounced Tg, followed by transitions difficult to be assigned due to peak overlapping. Samples containing at least 40 mol% of KET showed only one phase transition, a Tg, in the second heating and the XRD pattern was characteristic of non-crystalline, disordered materials (Fig. 5c). The highest Tg (15.7 ± 0.5 °C) was observed in the sample containing 70 mol% of KET (Fig. 4f).

In the LA-KET systems investigated, only one Tg was observed in samples containing at least 10 mol% of KET, indicating mixing of components at the molecular level. In LA-rich samples the excess of LA, a

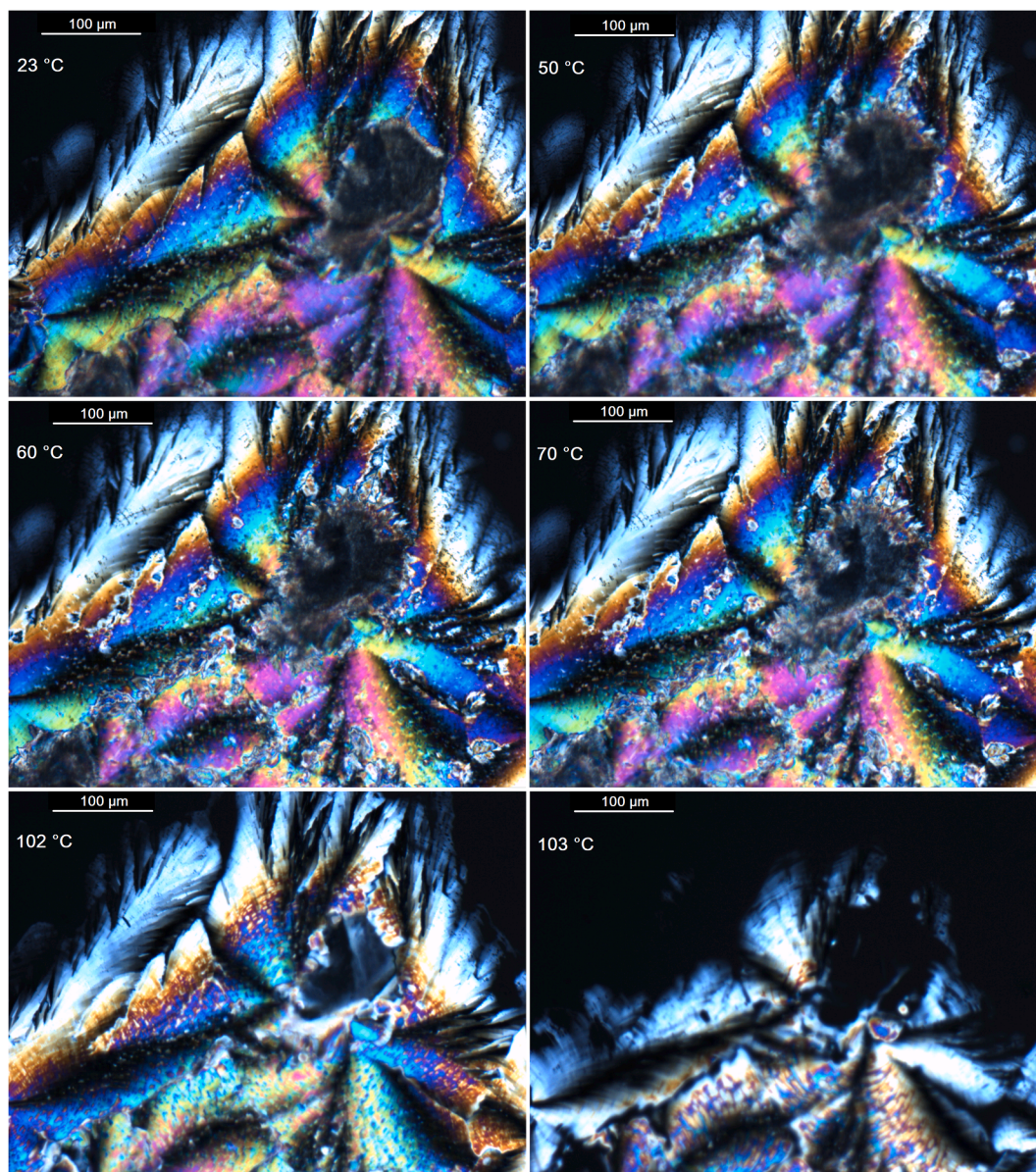


Fig. 6. Polarized light hot stage microscopy of BUP in the second heating cycle at (a) 23 °C, (b) 50 °C, (c) 60 °C, (d) 70 °C, (e) 102 °C and (f) 103 °C.

compound with a high crystallisation tendency, crystallised because there were not enough KET molecules to efficiently interact with all LA molecules present in the system. However, LID and MEP crystallised to the same polymorph as the starting material, whereas in the case of BUP a different polymorphic form (BUP II) was obtained. The presence of KET increased the glass forming ability and enabled the formation of disordered LAs, which otherwise show very high tendency to crystallise. The mixtures of LID and BUP containing 5 mol% of KET can be considered as class I regarding their crystallisation tendency, similarly to LAs. MEP mixtures containing 5–20 mol% of KET can also be categorised as class I. LID and BUP mixtures containing at least 40 mol% of KET and MEP mixtures containing at least 50 mol% of KET belong to class III, and the remaining mixtures can be categorised as class II. Hence, KET was less effective at inhibiting crystallisation of MEP.

In the previous study, at least 50 mol% of KET was necessary to decrease the crystallisation tendency of benzocaine (class I molecule), but only 15 mol% of KET reduced the crystallisation of tetracaine (which is a class II molecule) (Umerska et al., 2020a). The difference between tetracaine and LAs described in this study may be attributed to the lower crystallisation tendency of the former, but the fact that complexes of

different stoichiometry may form is also substantial.

3.4. Spectroscopic evaluation of LA-KET interactions

Liquefaction of solid acids and bases can occur not only due to proton transfer, but also by hydrogen bonding (Bica et al., 2011). FTIR spectroscopy was used to analyse the interactions between KET and the LAs. The spectra of QC LID-KET, BUP-KET and MEP-KET are shown in Fig. 7. The spectrum of QC KET is presented for comparison, as in all tested samples crystalline KET was not detected, evidenced by the lack of vibrations characteristic for centrosymmetric acid-acid dimers. QC KET showed carboxylic acid bands, with a stretching C=O peak at 1704 cm^{-1} and a shoulder at 1737 cm^{-1} (Umerska et al., 2020a). The stretching of ketone C=O group gave a peak at 1656 cm^{-1} (Champeau et al., 2015, Umerska et al., 2020a). The peaks at 1663 cm^{-1} (LID), 1649 cm^{-1} (BUP polymorph I), 1650 cm^{-1} (BUP polymorph II) and 1650 cm^{-1} (MEP) can be attributed to the C=O stretch of amide groups of LAs (Wang et al., 2014, Miwa et al., 2016, Martins et al., 2017). In all systems the intensity of the carboxyl C=O stretching peak at 1704 cm^{-1} decreased markedly in samples containing 90 mol% of KET, and it was

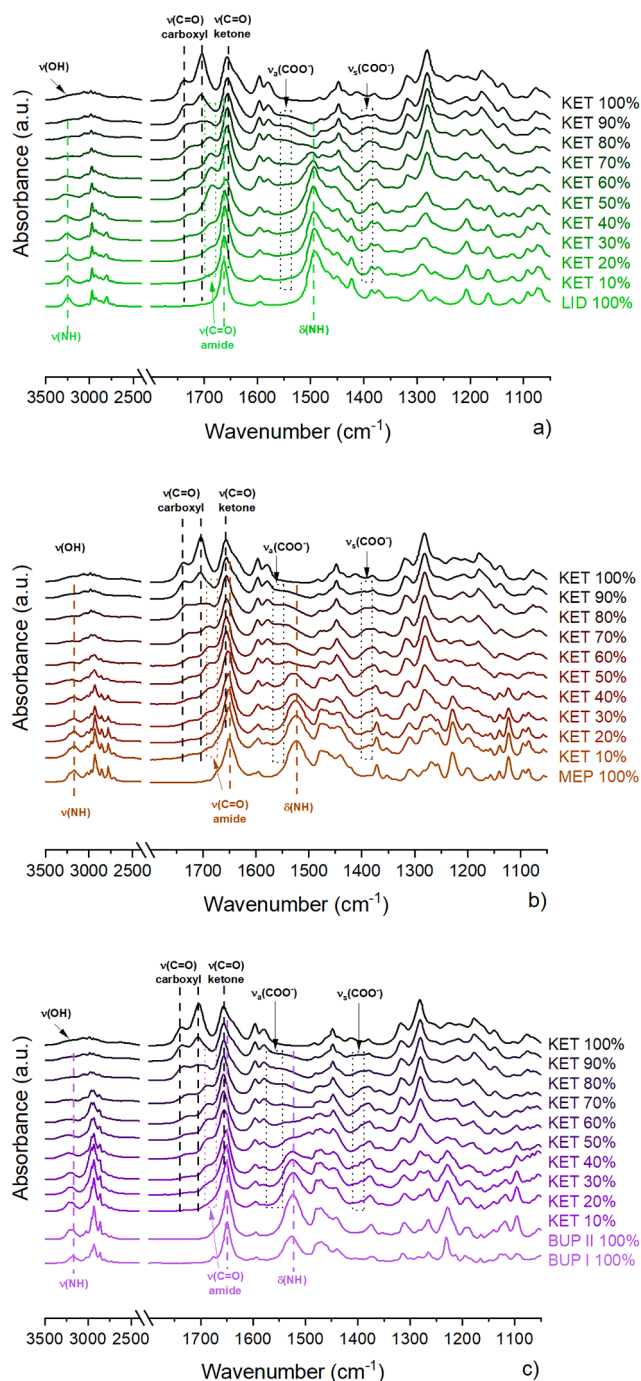


Fig. 7. Infrared spectra of (a) quench cooled KET, LID and LID-KET mixtures, (b) quench cooled KET, MEP and MEP-KET mixtures and (c) quench cooled KET, BUP and BUP-KET mixtures. N – stretching, ν_a – asymmetric stretching, ν_s – symmetric stretching, δ – bending vibrations.

hardly visible in samples containing 80 mol% of KET. Also, the intensity of the shoulder decreased corresponding to a decreasing KET concentration and the peak moved towards lower wavenumbers. However, a shoulder at approximately 1686, 1684 and 1682 cm^{-1} for LID-KET, MEP-KET and BUP-KET samples, respectively, appeared on the left-hand side of the amide C=O stretching band. The intensity of this shoulder/band correlated well with the decreasing crystallinity of the LA determined by DSC, hence the shift towards higher wavenumber is most likely due to the release of LA molecules from the crystal lattice and a change in the hydrogen bonding environment of the C=O part of the amide group. The ‘amorphous’ amide C=O stretching shoulder

overlapped with the carboxyl C=O stretching band of KET in the KET-rich samples. It was impossible to obtain FTIR spectra of non-crystalline LAs and the LA-rich samples because of their high crystallisation tendency. The amide II band, present in crystalline LAs at 1491 cm^{-1} (LID), 1523 cm^{-1} (MEP), 1526 cm^{-1} (BUP polymorph I) and 1526 cm^{-1} (BUP polymorph II), shifted towards higher wavenumbers, became less sharp in samples containing non-crystalline LAs and it was hardly visible in the KET-rich samples (70 mol% of KET). This may be the dilution effect, but also due to hydrogen bonding with the carboxyl group of KET molecules. Absence of this peak was not observed in samples containing ester LAs (Umerska et al., 2020a). Similarly, the N-H stretching band appearing in crystalline LAs at 3248 cm^{-1} (LID), 3167 cm^{-1} (MEP), 3171 cm^{-1} (BUP polymorph I) and 3180 cm^{-1} (BUP polymorph II) shifted towards higher wavenumbers corresponding to a decreasing content of crystalline LA and overlapped with the OH stretching band of KET (approximately 3200 cm^{-1}) in the KET-rich samples.

It is expected that carboxylate anion bands should appear at 1650–1550 cm^{-1} and 1400 cm^{-1} , arising from asymmetric and symmetric stretching, respectively, if ionisation is to occur between KET and the LA. The former band should be intense and the latter low in magnitude (Umerska et al., 2020a). An increased absorbance in these regions was observed in all the KET-LA systems, particularly in the KET-rich samples, indicating that a fraction of KET molecules has ionised in those mixtures and the proton is transferred probably to tertiary amino groups of LAs. It can be concluded that the carboxyl group of KET interacts with the amide group of the LA primarily by strong hydrogen bonding with possibly a small contribution of ionic interactions.

LID has been shown to form crystalline complexes with NSAIDs such as naproxen (Fiandaca et al., 2020) and indomethacin (Umeda et al., 2009) at a molar ratio of 1:2. The crystal complex described by Umeda et al., (2009) was grown by a slow evaporation from solution in ethanol and contained two different, crystallographically independent indomethacin molecules showing different conformations. Proton transfer from the carboxyl group of one of indomethacin molecules to an amino group of LID, intermolecular hydrogen bonds and CH/ π interactions were observed by single crystal analysis (Umeda et al., 2009). In the study by Fiandaca et al., (2020) the LID-naproxen sample was obtained from tetrahydrofuran and partial ionisation of naproxen based on a decreased intensity of the C=O carboxylic stretch peak was observed by the authors.

The above studies of Umeda et al., (2009) and Fiandaca et al., (2020) refer to crystals, whereas the systems described in our study are disordered, hence they may not show such a precise stoichiometry and ordering as crystals. Since BUP and MEP are chemically related to LID, it is likely that they could form complexes similar to those formed by LID. The maximum Tg values observed for all disordered LA-KET systems described in this study are in favour of the hypothesis of formation of complexes with a 2:1 stoichiometry between KET and those LAs. However, for instance, Moreira et al. (2015) obtained a solid state complex containing flurbiprofen and prilocaine at equimolar ratio. Interestingly, prilocaine is chemically related to LAs investigated in this study, because it is an amide local anaesthetic, but not a xylylidine derivative as LID, BUP and MEP.

The ΔpK_a difference between acid and base greater than 3 would normally be sufficient to generate a substantial proton transfer in aqueous solutions (Stoimenovski et al., 2010; Bica et al., 2011). However, it has been shown that tertiary amines tend to form mixtures with a low degree of proton transfer, in contrast with primary amines that produce highly ionised ILs (Stoimenovski et al., 2010). The systems described in this paper were mixtures of the parent acid and parent base plus rather small amounts of ionised species. The low degree of proton transfer between the KET and LA molecules is in agreement with other previously described disordered NSAID-LA mixtures, such as KET-tetracaine (Umerska et al., 2020a), KET-procaine (Umerska et al., 2020a) and ibuprofen-lidocaine (Wang et al., 2014). The important

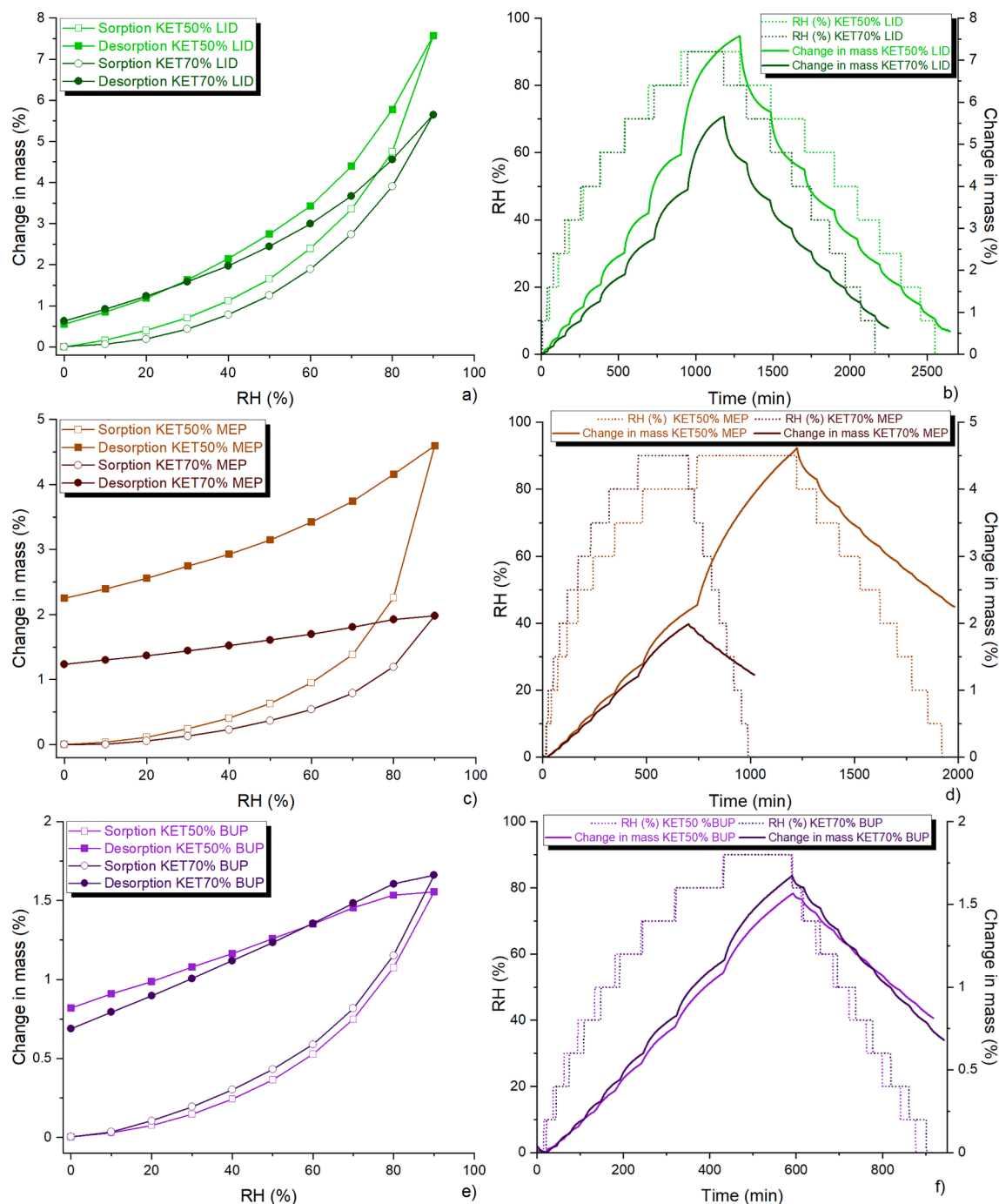


Fig. 8. Moisture sorption and desorption isotherm plots at 25 °C for quench cooled (a) LID-KET, (c) MEP-KET, (e) BUP-KET mixtures. Moisture sorption and desorption kinetic plots at 25 °C for quench cooled (b) LID-KET, (d) MEP-KET, (f) BUP-KET mixtures.

advantage of DEMs is that they can be prepared at various stoichiometry, in contrast with solid co-crystals, which are of a defined stoichiometry (Bica et al., 2011). The stoichiometry ratio of DEMs may be adjusted to achieve an optimal therapeutic effect, an aspect very important for pharmaceutical combination products. The constraint might be crystallisation of the parent molecules in excess if they have a high crystallisation tendency.

3.5. Water sorption studies

The moisture sorption properties are recognised as a critical factor in determining storage, stability, processing and application performance

of pharmaceutical systems. The stability of the QC samples containing 50 and 70 mol% of KET (equivalents of 1:1 and 1:2 stoichiometry) after exposure to various humidity levels was evaluated by DVS. In all samples a hysteresis, i.e. a difference in water vapour uptake between the sorption and desorption isotherms was observed (Fig. 8). The hysteresis appears due to quicker water diffusion into the sample bulk compared with its return to the surface, and consequently at the same RH level a greater amount of moisture will be present during desorption than sorption (Mesallati et al., 2019).

The binary systems can be considered as either slightly or moderately hygroscopic (0.2–2% w/w or 2–15% w/w of water uptake at 25 °C/80% RH, respectively) (Newman et al., 2008). The amount of water sorbed by

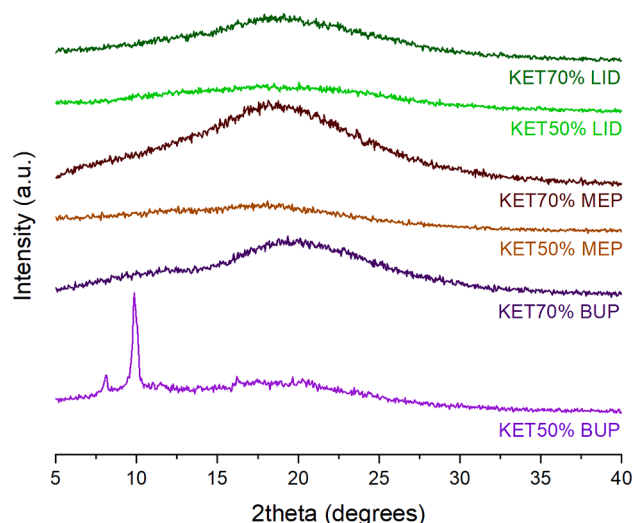


Fig. 9. XRD of LID-KET, MEP-KET and BUP-KET samples after the sorption-desorption DVS cycle.

the BUP samples at 90% RH was smaller than for either MEP or LID samples (Fig. 8). A certain amount of water was retained by all systems following the DVS study, approximately or less than 1% for the LID and BUP systems, except for the KET 70 mol% MEP system, which retained approximately 2% of water. Even though the LID samples showed the greatest water sorption abilities, the quantity of water retained in the systems at the end of the study was the smallest despite retaining the disordered character (Fig. 9). It may be due to higher mobility of molecules in these samples, as reflected by the lowest T_g values, which may have facilitated desorption of water molecules. Samples containing a smaller quantity of KET (50 mol%) sorbed higher amounts of water than the 70 mol% KET samples. The hygroscopicity of tested DEMs can be explained by their disordered character, and consequently random orientation of their molecules, which enables penetration of water into the samples. There were no obvious signs of crystallisation visible in the isotherms or the change in mass vs. time plots (Fig. 8). The water retained in the systems may be due to water dissolving in the super-cooled system as no hydrate formation was detected by XRD. After exposure from 0 to 90% at all RH steps the equilibrium was established within the maximum time limit for the step (480 min) in tested systems with exception of the equimolar KET-MEP sample, where the equilibrium was not reached within 480 min at 90% RH.

The permeation of water molecules into the bulk of a disordered system can increase the molecular mobility and thus induce crystallisation and/or increase its rate. As stated above, none of the samples tested by DVS (containing 50 and 70 mol% of KET) showed crystallisation on DSC and all of these mixtures can be categorised as class III. The crystallisation tendency evaluated by DSC is done under dry conditions, whereby the effects such as moisture sorption is minimised. Although KET has a high glass forming ability as is a class III molecule, upon exposure to humidity in DVS crystallisation was observed and confirmed by XRD (not shown). Interestingly, all systems subjected to DVS analysis, apart from the equimolar BUP-KET sample, remained disordered after DVS analysis (Fig. 9). Hence, the KET-LA interactions, as suggested based on the results of FTIR and DSC analysis, stabilise the systems and prevent crystallisation of components not only in the dry conditions of thermal analysis, but also upon exposure to moisture. Strong hydrogen bonds between KET and LAs reduce mobility of the complexed molecules rendering the orientation that creates steric hindrance and prevent nucleation and crystal growth of parent molecules. The Bragg peaks observed in the equimolar BUP-KET sample can be attributed to BUP II. This could be due to the excess of BUP molecules able to nucleate and crystallise. Higher percentage of KET in the 2:1

mixture (two molecules of KET per one molecule of BUP) effectively prevented crystallisation of the LA.

4. Conclusions

The xylylidine-derived amide LA-KET systems investigated in this work can be described as deep eutectics with strong interactions between the acid and base molecules resulting in a sudden decrease in melting temperature and deviations from the theoretical Schröder van Laar predictions. LID, BUP and MEP formed disordered complexes with KET and the T_g values suggested the tendency to form LA-KET complexes with approximately 1:2 stoichiometry. The electrostatic interactions existing between KET and LA molecules were found to reduce the crystallisation tendency of binary mixtures. One KET molecule per one LA molecule completely eliminated/suppressed the immense crystallisation tendency of LID, BUP and MEP in dry conditions. This effect was maintained in the presence of moisture, where one molecule of KET per one molecule of LA was sufficient to prevent the crystallisation of either KET or LID or MEP, whereas in the BUP-KET system two molecules of KET effectively prevented BUP crystallisation. Interestingly, despite the pK_a difference of 3 between KET and LAs, infrared spectroscopy indicated the formation of DEMs rather than ILs, with only a small amount of carboxylate anions/salt, although this pK_a difference would normally be sufficient to generate a substantial proton transfer in aqueous solutions. Hence the drastic reduction or elimination of LA melting and a decrease in the LA crystallisation tendency can be explained by the presence of non-ionic interaction between KET and LA molecules. Furthermore, the low ionicity observed in described LA-KET systems in comparison to the fully ionised salts provides the possibility for the LA-KET mixtures to penetrate membranes more efficiently and a possible use in transdermal drug delivery and local anaesthesia.

CRediT authorship contribution statement

Anita Umerska: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Julija Zotova:** Methodology, Formal analysis, Investigation, Visualization. **Lidia Tajber:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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