COMPATIBILITY STUDY OF FOUR BINARY COMBINATIONS OF ACTIVE INGREDIENTS FOR DERMAL FILM FORMING SYSTEMS

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Abstract

Adapalene (ADA), levofloxacin (LEV), meloxicam (MEL) and miconazole nitrate (MIC) were selected as potential active ingredients for film systems with dermal or transdermal delivery. This study aimed to evaluate the compatibility of four binary mixtures - ADA and LEV (M1), ADA and MIC (M2), LEV and MEL (M3), and LEV and MIC (M4) – combinations for new bioadhesive polymeric matrix dermal systems produced by the casting solvent evaporation technique. The Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and molecular modelling (MM) were used to characterise and evaluate the compatibility between the selected active substances. In the FTIR data, M1, M2 and M4 mixtures presented a good compatibility. DSC revealed different chemical interactions as the temperature rose above 160°C, especially for M3 and M4. Through MM technique, interactions were found on the M2 and M3 mixtures. Following the correlation of all obtained results, the best compatibility was found to be on M1 and M4 combinations.

Keywords: dermal and transdermal therapeutic systems, infrared spectroscopy, differential scanning calorimetry, molecular modelling

Introduction

In healthcare, there is ongoing research for the development of new drug delivery systems, with release profiles that are able to improve the performance of the drug in terms of efficacy and safety. The classic topical forms are represented by ointments, emulsions and creams, products with conventional skin release. Significant innovation has occurred with the development of transdermal therapeutic systems - TTSs ("patches") as an attractive alternative to oral administration of drugs, discrete dosage forms that deliver the drug through the skin at a controlled rate to the systemic circulation (transdermal delivery). In the last decades, transdermal delivery has been and still is one of the most promising methods for the systemic delivery of drugs, being easy to administer and effective for the patient, which simultaneously improves the patient’s compliance. Additionally, it is possible to associate different drugs in these easy-to-use devices. These aspects improve bioavailability, as they result in more uniform plasma levels, with a longer duration of action, which will reduce dose frequency [3]. Although transdermal therapy has real benefits for the patient, over the last four decades, the Food and Drug Administration (FDA) has only approved a small number of these
The development of these devices is focused on the quick and accurate release of active substances, controlled by physiological signals such as pH, blood glucose level and enzyme activity [92]. The emergence of new forms of drug administration is dependent on the discovery of new methods to increase the skin permeability for macromolecules [46]. Thus, the production of TTSs is based on a number of physicochemical techniques such as lasers, microjets, electroporation, sonophoresis and iontophoresis, microneedles and use of chemical penetration enhancers [35, 57, 74]. Recently, in situ film-forming systems (on the skin, after spreading in a thin layer) - FFSs which form a film on the skin after spreading in a thin layer, have been developed as a new approach to be used as an alternative to both conventional and transdermal topical formulations [12]. In this paper, four pharmaceutical substances were selected as potential active ingredients (Figure I) that could be useful in the form of film-forming systems with dermal and/or transdermal delivery: adapalene (ADA) [23, 84], levofloxacin (LEV) [36, 69], meloxicam (MEL) [16], and miconazole nitrate (MIC) [10]. These therapeutic agents have some significant physicochemical properties (Table I) based on which they can be individually used to treat various skin diseases: skin candidiasis, acne vulgaris, other skin infections, etc. [4, 8, 31, 78].

In the absence of any activation mechanism, penetration of the skin by the therapeutic agent released from the dermal delivery system is determined by its solubility and diffusivity through the stratum corneum [91], it depends on certain intrinsic properties of the molecule. For example, the aqueous solubility of a non-ionized organic compound is related to its octanol-water partition coefficient (P) [43], and is correlated with its melting point (MP) [62]. It is already known that a low MP will result in better absorption of the compounds [17]. Also, the diffusivity in stratum corneum is correlated with the molecular weight (MW); thus, the compound should not have high molecular weight and size [16, 62].

### Table I

Physicochemical properties of selected compounds [1, 7, 9, 14, 20, 25, 27, 51, 55, 63, 70, 78, 81]

| Compound/Therapeutical class | MW (g/mol) | Solubility | pKₐ | LogP | MP (°C) |
|-----------------------------|------------|------------|-----|------|---------|
| ADA/Retinoids; NSAIDs       | 412.52     | Soluble in THF, DMSO and DMF; sparingly soluble in ethanol, and practically insoluble in water | 4.23 | 8.04; 8.60 | 319 - 322 |
| LEV/Fluoroquinolones       | 361.37     | Freely soluble in glacial acetic acid, chloroform, sparingly soluble in water | (HA) 5.59 | 1.27 | 225 - 227 (with decomposition) |
| MEL/NSAIDs                 | 351.4      | Insoluble in water, soluble in DMF, very slightly in methanol; 1.736 M x 10⁻³ (in 0.2 M phosphate buffer pH 7.4 at 37°C) | (HA) 4.5 | 2.71 | 257 - 260 |
| MIC/Antifungals            | 479.1      | Very slightly soluble in water (1 in 6250) and isopropanol (1 in 1408), slightly soluble in chloroform (1 in 525) and ethanol (1 in 312), sparingly soluble in propylene glycol (1 in 119) and methanol (1 in 75), freely soluble in DMSO | 6.7 | 5.96 | 178 - 184 |

THF – tetrahydrofuran, DMSO – dimethylsulfoxide and DMF – dimethylformamide, MP – melting point

### Table II

Fulfilling the general requirements for selected drug candidates to be comprised in TTSs [13, 21, 31, 52, 47, 59, 78, 86, 87]

| Requirements                      | ADA | LEV | MEL | MIC |
|-----------------------------------|-----|-----|-----|-----|
| MW < 400                          | +   | +   | +   | -   |
| Log P 1.0 - 4 (octanol-water)     | -   | +   | +   | -   |
| Low MP (< 200°C)                  | +   | -   | +   | -   |
| Low dose (< 20 mg/day)            | +   | -   | +   | -   |
| Half-life ≤ 10 hours              | +   | +   | -   | -   |
| Non-irritating and non-sensitizing to the skin properties | + | no data | no data | + |
| Low oral bioavailability          | +   | -   | +   | +   |

+ = fulfil the requirement, +/- = fulfil the requirement partially, - = do not fulfil the requirement

Additionally, a series of other biopharmaceutical parameters are known to influence the selection of a drug candidate for passive skin penetration: dose, half-life, log P, skin permeability coefficient, oral bioavailability, therapeutic index, and non-irritating and non-sensitizing properties [33]. None of the substances studied in this work has all the intrinsic biopharmaceutical parameters to fulfill all the necessary conditions for transdermal penetration after skin application (Table II). Thus, their bio-distribution on/in the skin and deeper will also have to be modulated by...
extrinsic factors such as excipients, system structure and the biological variability. Consequently, the factors that influence the release and level of activity of the drug into the skin depend not only on the intrinsic properties but also on the excipients and system structure and not least on the biological variability [62]. Moreover, combinations of these different drugs, which have different permeation pathways and mechanisms of action, could have better therapeutic outcomes than the drugs used individually, as evidenced by some published studies summarized below

Adapalene (ADA), like other retinoids, is considered by the Global Alliance on Improving Acne Outcomes as the first-line therapy in the treatment of acne, being recommended to be used either independently in cases of mild acne, or together with a broad-spectrum antibiotic in cases of other forms of acne. In this line, since 2011, several retinoids were investigated as combinations with classical topical antibiotics (clindamycin, erythromycin) [30]. Also, the new combination between ADA and nadifloxacin (a fluoroquinolone) was clinically and microbiologically evaluated in topical products for the treatment of acne [83].

Levofloxacin (LEV), the pure levigire isomer of ofloxacin (a forerunner of nadifloxacin, recently introduced on a TTS device [42]), is used both systematically and topically [41, 49]. In addition to numerous ophthalmic formulations with LEV, other formulations such as emulsions, ointments, etc. have been studied [56-58]. In fact, LEV is currently widely used as an antibiotic for the skin and soft tissues. Due to the favourable results, LEV could be a candidate for insertion into binary TTSs, exhibiting much lower bacterial resistance than other antimicrobials [48, 66, 73]. Practically, ADA and LEV would be a unique combination of topical pharmaceutical preparations to be formulated.

Miconazole nitrate (MIC) is an antifungal used in many diseases, being known as a beneficial adjuvant in acne therapy, as a single compound or in combinations [28, 32]. Its significant advantage is that it remains cumulative in the stratum corneum for up to 4 days after application [11, 56]. Thus, combining ADA with an antifungal, e.g. MIC, could potentially minimise complications of chronic acne. Furthermore, combinations could cumulate the antifungal effect of MIC and antimicrobial effect of LEV or anti-inflammatory effect of ADA, respectively. The combination of LEV with MIC could be considered optimal because the action of LEV is potentiated when is used with MIC [18, 65].

Meloxicam (MEL) belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) without any topical form present on the market. The cutaneous combinations could take advantage of the potential synergism between an anti-inflammatory (MEL, or ADA) associated with an antibiotic active on soft tissues (LEV), or an antifungal (MIC), thus being potentially useful for patients, without any known incompatibility issue [24, 39, 50].

The compatibility studies between the associated substances represent a compulsory stage in the formulation of TTSs (or FFSs) in form of bioadhesive polymeric matrix produced by the casting solvent evaporation technique [85] because they will interact at the molecular level both in the fusion stage based on dissolution of ingredients and in the evaporation of the solvent stage, during the formation of the dermal films. Therefore, as the first stage of preformulation studies, the aim of this study was to evaluate the chemical compatibility of four binary combinations of drugs - ADA and LEV (M1), ADA and MIC (M2), LEV and MEL (M3), and LEV and MIC (M4) - using 2 methods: computational chemistry by molecular modelling analysis (MM), followed by correlation of the virtual results with the experimental data obtained by Fourier transform infrared (FTIR) spectroscopy and Differential scanning calorimetry (DSC) analysis, respectively.

Materials and Methods

**MM - software and method**

HyperChem® software (version 8, Hypercube Inc., USA) was used to compute, based on their known chemical structures and physicochemical properties, the chemical structures of the studied compounds (ADA, LEV, MEL, MIC) and then to establish their most stable conformation in binary mixture (M1-M4), both in the 1:1 molar ratio and in the 1:1 mass ratio (corresponding to the molar ratio of 7ADA/6LEV – M1, 7ADA/6MIC – M2, 1LEV/1MEL – M3 and 4LEV/3MIC – M4).

MM2 calculations were performed to evaluate the mechanical molecular compatibility of binary mixtures [40]. MM2 force field simulations in vacuum were carried out, using the Polak-Ribiere minimisation algorithm and 0.01 RMS gradients. The structures of the four pharmaceutical compounds were generated through SMILES (Simplified Molecular Input Line Entries), and the geometry of ADA, LEV, MEL, MIC and their four studied binary combinations were then minimised using the parameters mentioned above in Hyperchem. The binding energies (E) of the binary combinations were calculated using the equation (1):

\[ E_{\text{binding}} = E_{\text{complex}} - (E_{\text{compound}1} + E_{\text{compound}2}) \]

where \( E_{\text{binding}} \) represents the supposed binding energy between the two active ingredients, \( E_{\text{complex}} \) and \( E_{\text{compound}1} \) and \( E_{\text{compound}2} \) represents the energy after minimisation is performed. Negative binding energy shows a thermodynamically favoured complex [40].

**Chemicals, apparatus and analysis methods**

Selected compounds were purchased in the form of the reference standard, as follows: ADA, LEV and
MIC from Sigma Aldrich, USA, and MEL from Techno Drugs & Intermediates Ltd., India. Each of them was analysed individually and in binary combinations (M1-M4) prepared by mixing the compounds in a mass ratio of 1:1. The Fourier-transform infrared spectroscopy (FTIR) spectra were registered by FT/IR-470Plus Jasco spectrometer, Japan, and processed using the Jasco Spectra Manager for Windows XP. The samples (binary mixture in 1:1 mass ratio) were prepared as KBr pellets in the range of 4000 cm\(^{-1}\) - 600 cm\(^{-1}\), 16 scans at 4 cm\(^{-1}\) resolution. Differential Scanning Calorimetry (DSC) analysis was performed in a DSC 60 Shimadzu apparatus. The samples were weighted (3 mg) (binary mixture in 1:1 mass ratio) and placed in aluminum crucibles. The DSC curves were registered in the range of 40 - 400\(^{\circ}\)C, with a temperature increase rate of 10\(^{\circ}\)C/min, in an air atmosphere, using reference material.

**Results and Discussion**

*Compatibility study by MM analysis*

MM is a relatively new method to predict the compatibility of different substances for the screening on design pharmaceutical form and HyperChem, used in this study, is a software that offers quantum chemical calculations, molecular mechanics, and dynamics. Based on E binding values, it is possible to predict if combinations of two substances are (if E > 0) or are not (if E < 0) thermodynamically compatible [45, 58, 64, 75, 76, 82, 93].

**Figure 1.**

Molecules of the studied compounds computed by MM, based on their known chemical structure:

1) Adapalene (ADA), 2) Levofloxacin (LEV), 3) Meloxicam (MEL), 4) Miconazole nitrate (MIC)

It was verified the molecular mechanics' compatibility between the associated compounds in the four studied mixture (M1-M4), calculations were performed for both 1:1 molar ratio - to identify the most favourable complex, and 1:1 mass ratio - the form in which the samples are usually prepared in experimental studies. The calculated values of E binding and the specific energies are shown in Table III.

**Binary mixtures 1:1 molar ratio** (Table III, a). The negative values of the binding energy predicted for M1 and M4 mixtures indicate that complexes were thermodynamically favoured between their two compounds (Figure 2). For M1 and M4 mixtures indicate that complexes formed between their two components were thermodynamically favoured. In the case of the M1 complex, the LEV molecule is situated close to the methoxyphenyl-naphthoic acid moiety of ADA, while on the M4 complex, the piperazinyl group of the LEV is oriented toward the imidazole group of the MIC. Consequently, based on the analysis of the detailed energy data, the drug-drug interactions were due to van der Waals and bond angle forces, with no hydrogen-bonds being detected.

**Binary mixtures 1:1 mass ratio** (Table III, b). Forcing several molecules to approach by mechanical, spatial constraints cause thermodynamic changes, observed especially in the case of M2 (7ADA/6MIC mole ratio), case in which the value of E binding became negative due to the release of a very weak electrostatic energy. In the case of M3, the 1:1 molar ratio corresponds to a mass ratio of 1.027 LEV: 0.93 MEL which can be considered to be sufficiently close to the 1:1 mass ratio, given that it was not possible to adjust by
increasing the number of molecules in the computed mixture.

| Energies (kcal/mol) | a) Molar ratio 1:1 | b) Mass ratio 1:1 |
|---------------------|-------------------|------------------|
| Bond                | 5.39              | 32.83            |
| Angle               | 4.37              | 39.93            |
| Dihedral            | 4.84              | 42.87            |
| Van der Waals       | 33.29             | 61.78            |
| Stretch-bend        | 0.39              | 1.95             |
| Electrostatic       | 0                 | 0.00036          |
| E binding           | -12.58            | -284.10          |

The most stable conformations of M1 and M4
(legend of atoms: light blue – C; dark blue – N; red – O; grey – H; yellow – F)

Compatibility study by FTIR analysis
FTIR analysis is a sensitive spectroscopic technique often used as a compatibility screening. The vibrational changes of registered spectra offer information regarding potential intermolecular interactions among the selected binary mixture of active substances. Advantages of FTIR spectroscopy comprise small samples size, detection of incompatibility (the shifts of unique bands), rapid data acquisition, availability of databases and accessibility of instruments [15].

The FTIR spectra of pure compounds and their binary mixtures (1:1 mass ratio) had been registered. The most relevant comparisons of FTIR spectra of active substances with the spectra of their mixtures are presented in Figure 3.

The FTIR spectra of the M1-M4 stored for one month in the refrigerator (8°C) did not reveal any parameter changes.

M1 vs. ADA and LEV. Regarding M1 FTIR spectra, the strongest band at 2901.38 cm\(^{-1}\) assigned to OH (carboxyl) stretching vibrations and 2848.35 cm\(^{-1}\), assigned to (-CH\(_2\)-) stretching vibrations of ADA [37], remained unchanged, while a particularly strong band of ADA spectra at 1686.44 cm\(^{-1}\) assigned to C=O (carboxyl) stretching vibration [22, 60] slightly increased to 1689.34 cm\(^{-1}\), in the mixture with LEV. The band at 1236.15 cm\(^{-1}\) assigned to R-O-Ar (aromatic ether) appears as comprised in the band at 1240.97 cm\(^{-1}\) of LEV spectra, which is assigned to R-O-R (ether) [77]. Thus, on LEV spectra the peaks at 2973.7 cm\(^{-1}\), 2936.09 cm\(^{-1}\) and 2802.06 cm\(^{-1}\), assigned to C–H stretching vibrations of methyl radical at the N4\(^{+}\) nitrogen atom in the piperazinyl moiety [20] remain unchanged in the mixture with ADA. The band at 1453.1 cm\(^{-1}\), assigned to δ(CH\(_3\)) and C=C stretching aromatic ring [26, 77], also appeared unchanged, while the absorption band at 1135.87 cm\(^{-1}\), assigned to δ(OH) [77], was slightly shifted to 1134.9 cm\(^{-1}\). A strong absorption band at 1089.58 cm\(^{-1}\) assigned to R-O-R [54] moiety of LEV was not shifted in the M1 spectra. In fact, the characteristic bands of both ADA and LEV were not changed significantly in their mixture - M1.
Examples of recorded FTIR spectra of M - mass ratio 1: 1 in binary mixtures (above) and the overlay spectra of their individual compounds (below): a) M2 and ADA/MIC spectra; b) M3 and LEV/MEL spectra

M2 vs. ADA and MIC (Figure 3a). All the characteristic registered frequencies of ADA spectra were not changed in the M2 spectra, except for the band at 1686.44 cm$^{-1}$, assigned to C=O (carboxyl) stretching vibration [22, 54, 60] which was slightly increased to 1689.34 cm$^{-1}$. Also, there were no changes regarding significant bands of MIC spectra in its mixture with ADA. The bands at 3182.93 cm$^{-1}$ assigned to C-N imidazole stretch vibration and 3106.76 cm$^{-1}$ assigned to C-H aromatic stretching vibration [38, 67] were not shifted. Other registered bands of MIC spectra were at 1588.09 cm$^{-1}$, 1561.09 cm$^{-1}$ and 1546.6 cm$^{-1}$ assigned to C-C stretching vibration and C=C aromatic vibration; 1475.28 cm$^{-1}$ assigned to C-H stretching vibration; 1329.68 cm$^{-1}$ assigned to C-O stretching vibration, and 1015.34 cm$^{-1}$ assigned to C-C stretching vibration [5, 38, 67, 80, 89]. Also, the strong absorption band at 2961.16 cm$^{-1}$ (MIC spectra) to 2962.13 cm$^{-1}$ (M2 spectra), which can be assigned to C-H aliphatic stretching vibration (C2 spectra) [38, 67]. Thereby, it can be considered that the FTIR spectra of ADA and MIC did not change significantly in their mixture - M2.

M3 vs. LEV and MEL (Figure 3b). The FTIR bands of LEV in its mixture with MEL presented several slight shifts when compared to the bands of LEV spectra. For example, the band at 2936.09 cm$^{-1}$ and 2847.38 cm$^{-1}$, assigned to C-H stretching vibrations of methyl radical at the N4' nitrogen atom in the piperazinyl moiety [20], was slightly shifted to 2937.06 cm$^{-1}$ and 2848.35 cm$^{-1}$ in M3 spectra. Thus, the significant bands of LEV spectra were not influenced by combination with MEL. Also, in the spectra of M3, several slight shifts of MEL characteristic frequencies occurred. The most representative shifts were from 1456.96 cm$^{-1}$ to 1455.99 cm$^{-1}$ (δ(CH$_3$), C=C stretching aromatic ring), 1347.03 cm$^{-1}$ to 1346.07 cm$^{-1}$ and 1161.9 cm$^{-1}$ to 1162.87 cm$^{-1}$ (S=O stretching vibration), 1044.26 cm$^{-1}$ to 1046.19 cm$^{-1}$ (aromatic C-H vibration), and 714.497 cm$^{-1}$ to 713.533 cm$^{-1}$ (aliphatic C-H vibration) [26, 77, 94]. Overall, however, it may be considered that the FTIR spectra of LEV and MEL were not changed significantly in their mixture M3.
which was slightly shifted to 1331.61 cm$^{-1}$. However, the characteristic bands of both LEV and MIC were not changed significantly in their mixture M4. Analysing the FTIR recorded data punctually for each compound of the samples, at each binary mixture, a common peak was identified with both components of the mixture, these being as follows: 1540.85 cm$^{-1}$ – M1, 1141.65 cm$^{-1}$ – M2, 1619.91 cm$^{-1}$ – M3 and 963.27 cm$^{-1}$ – M4. Also, it appears that the most significant changes determined in the mixtures occur in the regions of the 3900 to 2600 cm$^{-1}$ frequencies (Figure 4), intervals for specific absorption and vibrations of -OH (alcohol-free or intermolecular bonded), N-H (primary or secondary amine, amine salt), -OH from carboxylic acid (usually centred on 3000 cm$^{-1}$), C-H (aldehyde) and S-H (thiol) [15, 54, 80].

**Correlation of the results obtained by the MM and FTIR analyses**

**LEV and its binary mixtures.** The data computed and calculated by MM showed that M1 (with ADA) and M4 (with MIC) have thermodynamically compatible chemical structures both in 1:1 molar ratio and in 1:1 mass ratio, as opposed to M3 (with MEL) in which the two components have potential for chemical interactions. These behaviours were confirmed by FTIR data.

**ADA, MIC, MEL, and their binary mixtures.** The data recorded by FTIR confirms that ADA has better compatibility in M1 (with LEV) than in M2 (with MIC); MIC shows better compatibility in M2 (with ADA) than in M4 (with LEV), and MEL (with LEV in M3) shows the weakest compatibility of all studied compounds. It should be noted that MIC was used in the form of nitrate (salt) anion which could explain the thermodynamic changes favourable to compatibility and highlight by MM in the case of M2 in 1:1 mass ratio versus M2 in 1:1 molar ratio.

**Compatibility study by DSC analysis**

The DSC is a thermal analysis method commonly used for the screening of incompatibilities between active substances to be included in pharmaceutical formulations [15, 29, 90]. This technique has several advantages such as being a rapid method that requires a small amount of sample, which allows simple detection of physical interactions (polymorphic form changes, crystalline to amorphous form conversion) [15]. The registered DSC curves are graphically presented in Figure 5. Analysed samples contained pure substances and physical mixtures in mass ratio 1:1. The DSC curves were analysed and compared in terms of MPs and changes in enthalpy (Table IV). If there is high compatibility of the compounds in the binary mixture, then a sum of the individual components is expected on the DSC curves of mixtures. On the contrary, incompatibility of components is related to the presence of significant shifts on MP values of the compound, the occurrence of a new peak (exo- or endothermic) and modification of enthalpies [15, 90].

**M1 vs. ADA and LEV (Figure 5a).** DSC curve of LEV showed a dehydration process (endothermic transition) [34] also maintained in the mixture with ADA, which proves that the LEV sample is a hydrated compound. No significant changes were noticed until the mixture sample began to degrade at 217.93°C [72] similar to

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**Figure 4.**

The registered FTIR peaks (cm$^{-1}$) of individually compounds (1 - ADA, 2 - LEV, 3 - MEL and 4 - MIC) overlay on peaks of their binary mixtures (1:1 mass ratio), in the frequency regions of 3900 to 2600 (cm$^{-1}$)
another fluoroquinolone previously analysed [71].
The MP of LEV was slightly shifted from 227.72°C,
most probably characteristic to β anhydrous crystallisation
form [34], to 224.66°C. Although the DSC curve of
ADA did not show any phase transition peak until
328.28°C (MP), in mixture with LEV, several peaks
appeared between 280-350°C and the MP was
probably shifted to 300.27°C. This modification of

M1 - DSC curve shape could be attributed to possible
hydrogen bonds between the –COOH groups of both
compounds (ADA and LEV), decarboxylation processes,
and a degradation process at high temperature. All
these can be considered significant changes in the
thermal behaviour in the mixture M1, compared to
the pure ADA and LEV.

Figure 5.
DSC curves of M1-M4 1:1 mass ratio binary mixtures (below) and their individually analysed compounds
(above): a) M1 and ADA/LEV; b) M2 and ADA/MIC; c) M3 and LEV/MEL; d) M4 and LEV/MIC

M2 vs ADA and MIC (Figure 5b). The DSC curve of
M2 is very similar to the DSC curve of MIC, with no
ADA characteristic properties registered. The recorded
MP value of MIC was 186.98°C [27]. In M2 the MP
of MIC was slightly shifted to 187.98°C. A recent thermal study highlights that the MIC molecules cannot be involved in hydrogen bonds and show high glass-forming ability and also high glass stability [68]. An aspect of the two DSC curves of binary mixtures with ADA (M1 and M2) was that the characteristic properties of ADA were not cumulated on DSC curves of mixtures with LEV or MIC. As an excipient can solubilise an active substance [19], probably a similar phenomenon was possible on M1 and M2 mixtures. The ADA-peaks disappearance in both mixtures can be explained by thermally induced drug-drug interaction or dissolution of ADA in the melted LEV or MIC during the DSC scan [53].

**Conclusions**

The therapeutic compounds LEV, ADA and MIC associated in binary 1:1 mass mixtures (M1, M2 and M4) proved to be compatible, in contrast to MEL in mixture with LEV (M3), by FTIR analyses method.

**Table IV**

| Characteristics of the thermal behaviour determined in the samples analysed by DSC |
|---|
| **DSC parameter** | **a) Individual compound** | **b) Binary mixture (1:1 mass ratio)** |
|  | ADA | LEV | MEL | MIC | M1 | M2 | M3 | M4 |
| **0 → 40 min; 10°C/min rate** | | | | | | | | |
| Signal difference mW | -3.8 | -0.6 | -3.4 | - | - | 2.4 | -4.3 | -3.5 |
| Heat kJ/g | -1.2 | -3.5 | -3.3 | -1.7 | -4.7 | -4.7 | -2.1 | -1.7 |
| Peak °C | 328.2 | 227.7 | 264.4 | 186.9 | 224.6 | 142.0 | 343.0 | 182.8 |
| Peak height: mV | -29.2 | 19.3 | 21.9 | 15.8 | 18.01 | 1.45 | 29.7 | 12.9 |
| M4 vs LEV and MEL (Figure 5c). The DSC curve of M3 recorded many changes compared to the pure compounds. Thus, both substances had shifted MPs, LEV from 227.72°C [34] to 219.65°C and MEL from 264.43°C to 278.60°C, represented by an endothermic peak with low amplitude [6]. In M3 there were possible interactions between –COOH group of LEV and secondary –NH- group of MEL, and also dipole forces may occur between fluorine-carbon of LEV and the hydroxyl-carbon bonds of MEL. These significant changes suggest the incompatibility of LEV and MEL in the mixture – M3, in the conditions of increasing temperature.

These experimental findings confirm the predictions of the mechanical-chemical compounds molecular compatibility calculated by molecular modelling, expressed in terms of thermodynamic binding energy which generates the ability of the associated compounds to form molecular complexes in binary mixtures. The used computational method is able to generate data closer to the experimentally measured ones, especially for 1:1 mass ratios binary mixtures, than for 1:1 molar ratio.

The thermal behaviour during DSC proved that different chemical interactions occur as the temperature rises above 160 °C, especially for LEV associated with MEL (M3) and with MIC (M4). The binary combinations ADA/LEV, ADA/MIC and LEV/MIC, could be considered as therapeutic ingredients for systems with dermal and/or transdermal delivery, prepared in the form of bio-adhesive dermal films produced by techniques that do not use or use moderate heat, such as the casting solvent evaporation technique.
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Conflict of interest
The authors declare no conflict of interest.

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