



Article

The Study of Amorphous Kaempferol Dispersions Involving FT-IR Spectroscopy

Natalia Rosiak ¹, Ewa Tykarska ² and Judyta Cielecka-Piontek ^{1,*}

¹ Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznan University of Medical Sciences, 3 Rokietnicka St., 60-806 Poznan, Poland; nrosiak@ump.edu.pl

² Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, 6 Grunwaldzka St., 60-780 Poznan, Poland; etykarsk@ump.edu.pl

* Correspondence: jpiontek@ump.edu.pl; Tel.: +48-61-641-83-95

Abstract: Attenuated total reflection-Mid-Fourier transform-infrared (ATR-Mid-FT-IR) spectroscopy combined with principal component analysis (PCA) has been applied for the discrimination of amorphous solid dispersion (ASD) of kaempferol with different types of Eudragit (L100, L100-55, EPO). The ASD samples were prepared by ball milling. Training and test sets for PCA consisted of a pure compound, physical mixture, and incomplete/complete amorphous solid dispersion. The obtained results confirmed that the range 400–1700 cm^{−1} was the major contributor to the variance described by PC1 and PC2, which are the fingerprint region. The obtained PCA model selected fully amorphous samples as follows: five for KMP-EL100, two for KMP-EL100-55, and six for KMP-EPO (which was confirmed by the XRPD analysis). DSC analysis confirmed full miscibility of all ASDs (one glass transition temperature). FT-IR analysis confirmed the formation of hydrogen bonds between the –OH and/or –CH groups of KMP and the C=O group of Eudragits. Amorphization improved the solubility of kaempferol in pH 6.8, pH 5.5, and HCl 0.1 N.

Keywords: amorphous solid dispersion; kaempferol; FT-IR; PCA



Citation: Rosiak, N.; Tykarska, E.; Cielecka-Piontek, J. The Study of Amorphous Kaempferol Dispersions Involving FT-IR Spectroscopy. *Int. J. Mol. Sci.* **2023**, *24*, 17155. <https://doi.org/10.3390/ijms242417155>

Academic Editor: Simona Sabbatini

Received: 9 October 2023

Revised: 27 November 2023

Accepted: 3 December 2023

Published: 5 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Kaempferol (KMP) is a flavonoid found in various edible plants (red fruits, grapes, citrus fruits), and it has also been identified in several medicinal plants (*Hippophae rhamnoides*, *Tilia* spp., *Ginkgo biloba*). As confirmed by numerous preclinical and clinical studies, KMP has been noted for its ability to exhibit antioxidant, anti-inflammatory, anticancer, and neuroprotective properties [1–5]. Nevertheless, KMP struggles to reach the necessary therapeutic concentrations. This is related to the limited water solubility of KMP, which results in poor bioavailability after oral administration [6,7]. Studies have shown that upon absorption KMP is frequently metabolized into sulfate, methyl, or glucuronide forms [7,8]. To boost absorption and improve the clinical response, increasing the solubility of KMP is essential.

Obtaining amorphous solid dispersion (ASD) is a potent method for enhancing the solubility and bioavailability of poorly water-soluble compounds [9–13]. ASD involves the dispersion of the drug (polyphenol) within an amorphous matrix, typically a hydrophilic polymer or a combination of polymers. Transforming the crystalline polyphenol into an amorphous form significantly enhances the dissolution rate and solubility, resulting in improved absorption and therapeutic effects. The amorphous matrix provides stability and prevents the polyphenol from reverting to its crystalline form, ensuring sustained supersaturation and improved drug release [9–12,14,15]. Obtaining ASD can be achieved through various techniques such as hot melt extrusion [11], spray drying [16,17], solvent evaporation [18], and ball milling [9,10,19]. These methods allow for achieving a molecular-level dispersion of polyphenols within the matrix, creating a highly homogeneous and amorphous system. In recent years, it has been demonstrated that compounds such as

sinapic acid [18], resveratrol [17], pterostilbene [10], curcumin [17,20,21], hesperidin [9,22], daidzein [16], genistein [19,23], hesperetin [12,22], naringenin [24], and quercetin [25–27] can be formulated using ASD. To date, no ASDs of KMP have been reported.

For ASD analysis, X-ray powder diffraction (XRPD) is the method of choice, confirming the absence of crystallinity through the amorphous halo in the X-ray powder diffraction pattern [9–12,28]. Another useful technique is differential scanning calorimetry (DSC), where the disappearance of the compound's melting point indicates its amorphous state. Nevertheless, efforts are being made to identify a rapid analytical method capable of distinguishing both crystalline and amorphous forms of active pharmaceutical ingredients (APIs) simultaneously.

Recently, vibrational spectroscopic techniques have shown promise as fast, nondestructive, and simple methods [29,30]. According to the literature, FT-IR spectroscopy combined with multivariate analysis (especially principal component analysis, PCA) was confirmed to be effective in classifying, among others, the style and quality of sparkling wines [31], saffron samples in terms of geographical origin [32], authentication and adulteration detection of Iranian saffron samples [33], differences in ewe's milk [34], detection of the adulteration of cocoa content in chocolates [35], and identify different parts and harvests time of *Dendrobium officinale* [36].

Culbert et al. [31] demonstrated the effective classification of sparkling wine style and quality by employing ATR-MIR spectroscopy in conjunction with PCA. The models developed in this study exhibited high accuracy and good predictive ability for both style and quality ratings. Maamouri et al. [34] studied the effect of the lactation period and feed type on the quality of ewe's milk. In their study, applying PCA to the spectral ranges of 2800–3000 cm^{-1} and 900–1500 cm^{-1} enabled effective visual discrimination of milk samples based on the feed.

Therefore, our research aimed to employ infrared analysis combined with chemometric methods (i.e., PCA) to develop a model for detecting the amorphous state of KMP within the Eudragit matrix.

2. Results and Discussion

This paper shows the screening of ASD of KMP with different types of Eudragit (EL100, EL100-55, EPO). To the best of our knowledge, this work is the first report using PCA combined with FT-IR spectroscopy as an efficient method to confirm the amorphous state of KMP in a polymer matrix.

The first stage of the study focused on preparing training and test sets for PCA analysis. Incomplete (NA) and complete (A or ASD) amorphous solid dispersions (confirmed by XRPD) were obtained by the milling method. Physical mixtures (PM or ph.m.) were prepared using the vortex method. The total amount of twenty-five samples were prepared and analyzed by XRPD and FT-IR. From the registered FT-IR spectra, 18 constituted a training set, while 7 constituted a test set (see Table 1).

Table 1. Principal component analysis (PCA); the samples used in the training set and test set.

KMP Content	Training Set			Test Set		
	EL100	EL100-55	EPO	EL100	EL100-55	EPO
20%	A	A/PM	A/PM	ph.m.1		
30%	A/PM	NA/PM	PM	ASD2	ASD5	
40%	PM	NA	A/PM	ASD3	NA4	
50%	NA/PM	PM		ASD		
60%	NA					ph.m.9

Legend: A/ASD—amorphous solid dispersion (confirmed by XRPD); PM/ph.m.—physical mixture; NA/NA4—incomplete amorphous solid dispersion (confirmed by XRPD).

In the next step, the obtained spectra data were analyzed using PCA. A loading plot of the first and second principal component (PC1 and PC2) for the full MIR range

(400–4000 cm^{-1}) is given in Figure 1 and divided into three regions (R) as follows: R1: 400–1700 cm^{-1} , R2: 1750–2750 cm^{-1} , and R3: 2750–3500 cm^{-1} .

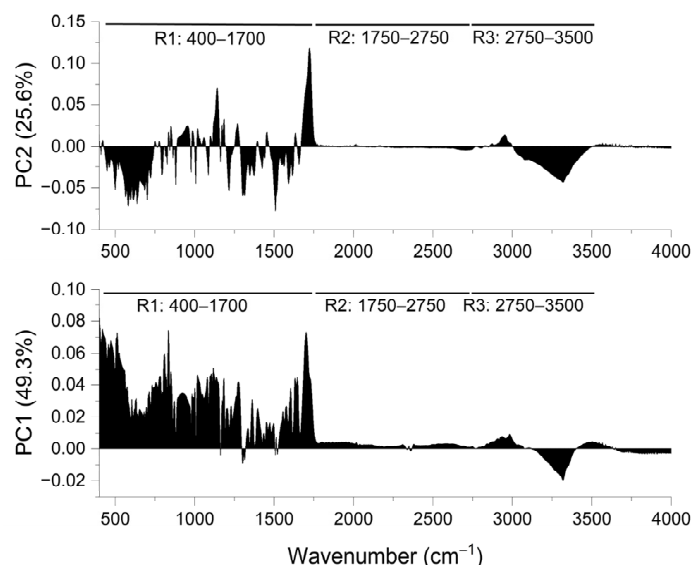


Figure 1. Loading plot of PC1 and PC2. R1, R2, R3—range of the wavenumber.

In the fingerprint region (R1), PC1 showed a positive correlation, whereas PC2 showed a positive and negative correlation. The R2 region did not significantly influence the differentiation of KMP–Eudragit samples since no significant peaks were detected in that region. In the R3 region, a negative correlation prevails for PC1 and PC2. The obtained results confirmed that the major contributor to the variance described by PC1 and PC2 was the R1 region (400–1700 cm^{-1}), which constitutes a fingerprint region characterized by a highly complex but unique absorption pattern for each organic structure.

FT-IR spectra of the training set and test set (wavenumbers from the fingerprint region) were used to generate a PCA score plot. The PCA score plot of the first two principal components (PC) derived from the R1 range of all KMP–Eudragit samples is shown in Figure 2a,b.

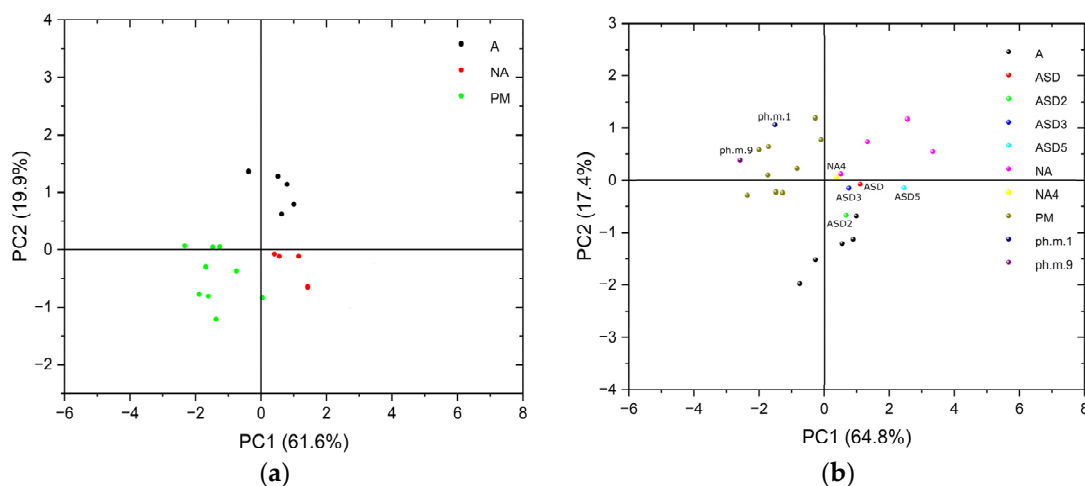


Figure 2. PCA score plot of the kaempferol–Eudragit samples from the wavenumber (400–1700 cm^{-1}) from the (a) training set and (b) the training and test set. Samples of the “training set”: A—amorphous solid dispersion; NA—incomplete amorphous solid dispersion; PM—physical mixture. Samples of the “test set”: ASD (ASD, ASD2, ASD3, ASD5)—amorphous solid dispersion; NA (NA4)—incomplete amorphous solid dispersion; ph.m. (ph.m.1, ph.m.9)—physical mixture.

For the training set (Figure 2a), PC1 explains 61.6% of the variation observed and resulted in a clear separation of the amorphous solid dispersions (A, black dot) from the other samples. For the training and test set (Figure 2b), PC1 explains 64.8% of the variation observed and resulted in a clear separation of the amorphous solid dispersions (A/ASD, lower right quadrant) from the other samples. Separation of physical mixtures (upper left quadrant) and incomplete amorphization (upper right quadrant) were also observed. Several outliers were observed, i.e., three physical mixtures and two amorphous solid dispersions in the lower left quadrant. Nevertheless, they are located very close to the quarters they should belong to (see Figure 2b).

The obtained PCA model was employed in the subsequent part of the research to select ASDs among the samples obtained from the milling process. The diffractograms of samples selected on the basis of PCA are presented in Figure 3.

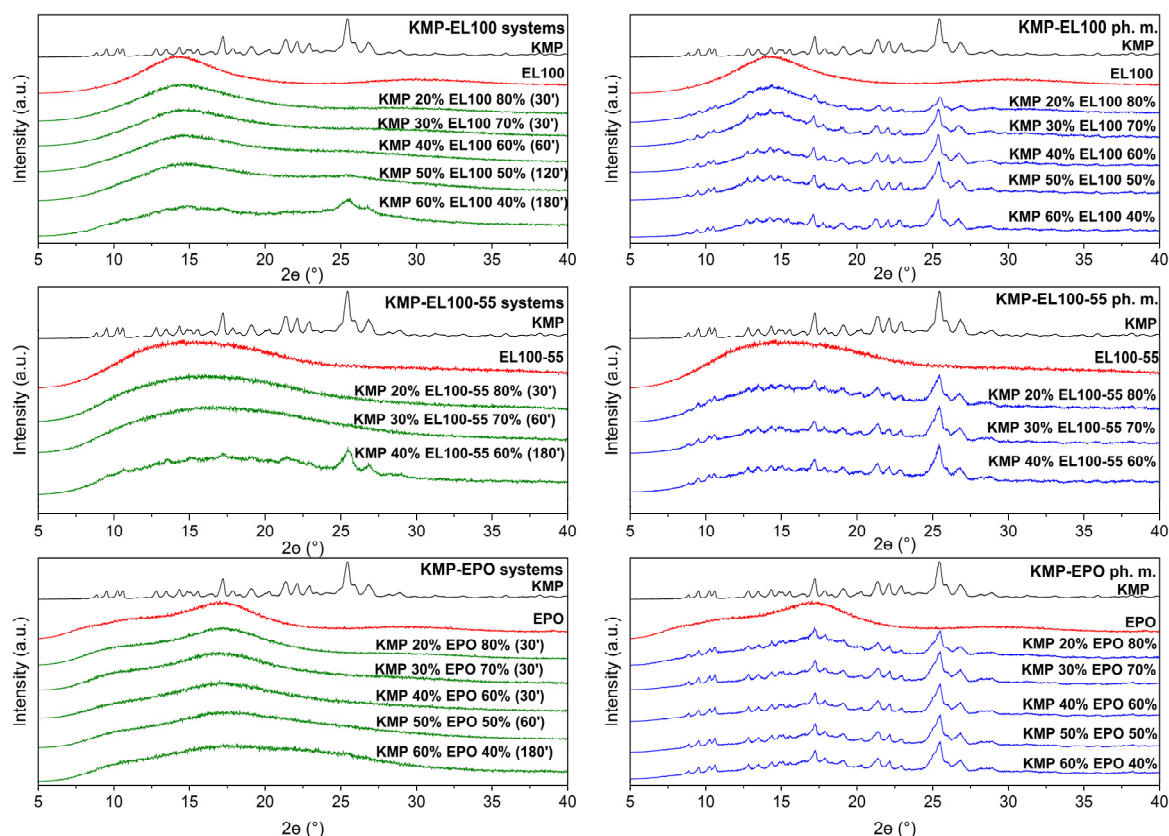


Figure 3. XRPD analysis: kaempferol crystalline form (black), Eudragit (red), and kaempferol–Eudragit samples after the milling process (green) and the physical mixtures (blue). Legend: KMP—kaempferol; EL100—Eudragit L100; EL100-55—Eudragit L100-55; EPO—Eudragit EPO; KMP 20–60%—content of kaempferol in the samples; 30'–180'—time of the milling process.

The crystalline KMP is characterized by a crystal pattern consisting of a series of well-defined sharp peaks between 8° 2θ and 30° 2θ . The diffractogram of L100, L100-55, and EPO (Figure 3, red line) displayed no peaks due to its amorphous nature [37–39]. KMP–Eudragit physical mixtures are the sum of diffraction patterns of KMP and Eudragit.

The samples for which a complete disappearance of the Bragg peaks (“halo effect”) was observed in the diffractograms of KMP–Eudragit systems (Figure 3, green line) will be referred to as ASD. The highest efficiency of amorphization was observed in the following order: EPO, L100, and L100-55. ASDs with KMP–EPO were obtained after 30, 60, and 180 min of milling, allowing for the highest KMP loading in the polymer matrix (60% KMP in ASD). Eudragit L100 allowed the amorphous KMP to be obtained after 30, 60, and 120 min; however, it was possible to load up to 50% of the compound into the polymer matrix. The lowest amorphization efficiency was shown by EL100-55, for which ASDs were

obtained for samples with a KMP content of 20% (after 30 min) and 30% (after 60 min). Regarding the systems with higher KMP loading values, incomplete amorphousness was still observed even after three hours of milling.

The obtained results confirm that Eudragits are suitable polymers for achieving the amorphous state of the drug, which is consistent in the literature. The completely amorphous “halo” of the X-ray diffraction patterns in compound-Eudragit systems were also observed by Chenchen et al. [15], Wang et al. [40], Zong et al. [14], and Alsayad et al. [41]. In addition, evidence was provided that the use of PCA together with FT-IR spectroscopy is an effective technique for confirming the amorphous state of KMPs.

Subsequently, thermal characterization of KMP, EL100, EL100-55, and EPO, and their ASDs was carried out using thermogravimetric (TG) and differential scanning calorimetry (DSC) methods. The TG analysis was carried out to determine the moisture content and sample stability upon heating. KMP was stable up to ~ 315 °C (moisture loss: 1.31%), EL100 to 176 °C (moisture loss: 1.45%), EL100-55 to 176 °C (moisture loss: 0.86%), and EPO to 205 °C (moisture loss: none) (Figure S1, Supplementary Materials).

TG analysis of the ASD of KMP–Eudragit confirmed that all samples degraded in the range where the melting point of kaempferol was observed (Figure S2a–c, Supplementary Materials). For this reason, in a further part of this study, the DSC analysis was carried out in terms of the stability of all ASDs and was aimed at recording the glass transition.

The DSC thermogram of KMP reveals a sharp endothermic peak at its melting point of 287 °C, indicating its crystalline nature (Figure S3, Supplementary Materials). This is consistent with reports in the literature. Colombo et al. observed the melting point of KMP at about 287 °C [42], Tzeng et al. at 285.5 °C [43], and Zhou et al. at 288.1 °C [44].

The heating and cooling method allowed us to observe the glass transition of raw samples and ASDs (Figure 4a–d).

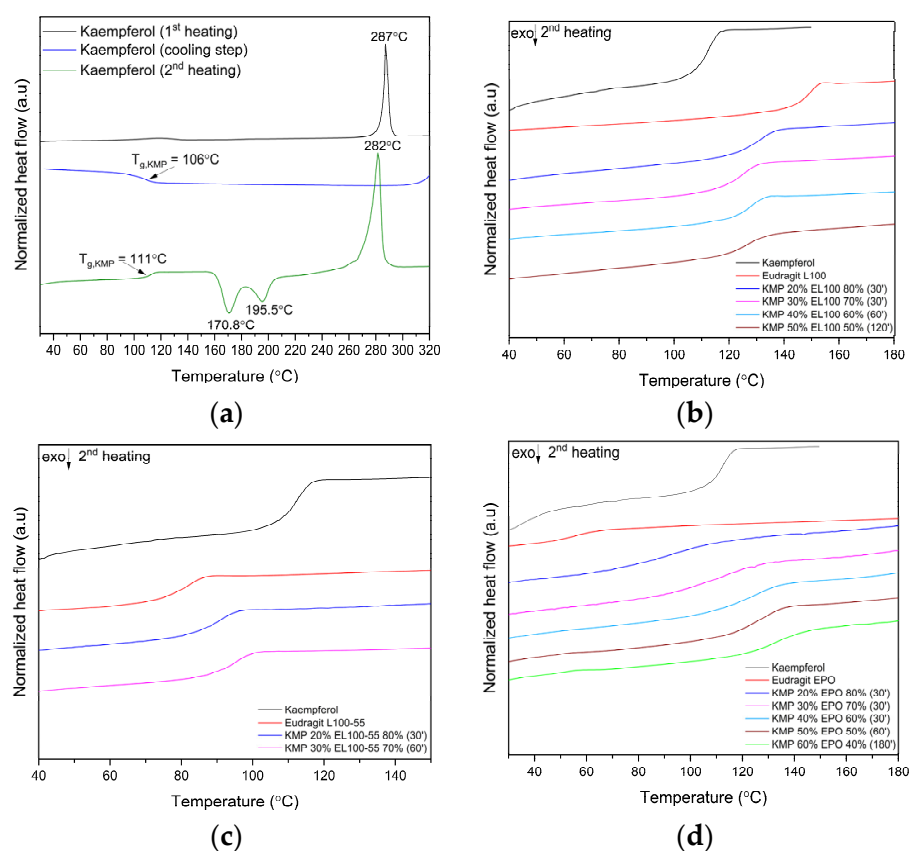


Figure 4. DSC analysis: (a) melted kaempferol; (b) Eudragit L100 and amorphous solid dispersion of kaempferol–Eudragit L100; (c) Eudragit L100-55 and amorphous solid dispersion of kaempferol–Eudragit L100-55; (d) Eudragit EPO and amorphous solid dispersion of kaempferol–Eudragit EPO.

In the cooling step, a glass transition temperature (T_g) of melted KMP was noted at 106 °C (Figure 4a, blue line). In the second heating step, three thermal effects were visible on the thermogram of the melted KMP: T_g at 111 °C, wide exothermic effect with maxima at 170.8 °C and 195.5 °C (probably corresponding to the cold crystallization [45]), and melting point (T_m) at 281.8 °C (Figure 4a). The percentage of KMP crystallinity after the melting process was determined to be about 6%. The crystallinity percent was determined using the following equation (Equation (1)):

$$\% \text{ Crystallinity} = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_{m^\circ}} \times 100\%, \quad (1)$$

In this equation, the heats of melting (ΔH_m) and cold crystallization (ΔH_{cc}) are in terms of $J \cdot (g \cdot ^\circ C)^{-1}$. ΔH_{m° stands as a reference value and represents the heat of melting in $J \cdot (g \cdot ^\circ C)^{-1}$ if the KMP was 100% crystalline.

It is suggested that the observed glass transition is related to the presence of ~94% of the KMP amorphous phase.

Amorphous KMP produced by heat quenching in the DSC cycle exhibited a T_g of 111.0 °C and the amorphous polymers displayed T_g values of 147.1 °C, 79.7 °C and 55.4 °C for EL100, EL100-55, and EPO, respectively. Based on the obtained DSC results for raw samples, theoretical studies (see Section 3.4.2) were conducted to predict the T_g values of amorphous KMP–Eudragit systems.

Both the Gordon–Taylor (G–T) and Couchman–Karasz (C–K) models rely on the free volume theory. These mathematical models are applicable under the assumption that the mixing partners are perfectly blended and share similar forms and sizes. The absence of interactions between the mixing partners and the additive behavior of the partners in their free volume are indicated by these models [46]. The differences observed between the experimental ($T_{g,exp}$) and theoretical ($T_{g,theo}$) values are related to positive ($T_{g,exp} > T_{g,theo}$) or negative deviations ($T_{g,exp} < T_{g,theo}$) [10].

The experimental and predicted values for T_g using the G–T ($T_{g,G-T}$) and C–K ($T_{g,C-K}$) equations are shown in Table 2.

Table 2. Summary of the most important parameters of the thermal analysis of the KMP–Eudragit ASD; the experimental and theoretical T_g values of KMP–Eudragit ASD.

	Mass (mg)	ΔC_p ($J \cdot (G \cdot ^\circ C)^{-1}$)	$T_{g,exp}$ ($^\circ C$)	$T_{g,G-T}$ ($^\circ C$)	$T_{g,C-K}$ ($^\circ C$)	Deviation
KMP	6.52	0.519	111.0			
EL100	7.08	0.329	147.1			
EL100-55	5.44	0.336	79.7			
EPO	3.86	0.201	55.4			
KMP 20% EL100	8.28	0.334	128.2	139.4	136.9	N
KMP 30% EL100	5.26	0.391	122.9	135.7	132.5	N
KMP 40% EL100	6.44	0.349	125.7	132.0	128.6	N
KMP 50% EL100	5.28	0.299	126.3	143.6	142.2	N
KMP 20% EL100-55	6.24	0.329	88.9	142.6	137.0	N
KMP 30% EL100-55	6.71	0.334	94.1	140.0	132.7	N
KMP 20% EPO	6.25	0.091	69.1	144.1	132.9	N
KMP 30% EPO	4.63	0.024	78.5	142.3	128.1	N
KMP 40% EPO	3.91	0.122	99.2	140.1	124.3	N
KMP 50% EPO	4.66	0.051	109.3	137.5	121.1	N
KMP 60% EPO	5.18	0.006	117.4	134.4	118.4	N

ΔC_p —heat capacity; $T_{g,exp}$ —glass transition temperature (experimental); $T_{g,G-T}$ —glass transition temperature (calculated by Gordon–Taylor equation); $T_{g,C-K}$ —glass transition temperature (calculated by Couchman–Karasz equation); N—negative deviation.

The fundamental determinant of amorphous pharmaceutical solid dispersion system stability is the drug–polymer miscibility; partial miscibility can lead to the creation of concentrated drug domains that may be susceptible to recrystallization after manufacture and during storage [47,48]. A single T_g was observed for all of the ratios of KMP–Eudragit ASDs. This suggests miscibility of KMP and polymer in the given ratios and the presence of a single phase in all of the systems. Lee et al. [49] confirmed the miscibility of ternary blends (biodegradable polymers with natural polyphenol) based on a single T_g value. Additionally, the literature has confirmed, based on DSC analysis, the formation of miscible systems with Eudragit EPO involving drugs such as ibuprofen, naproxen, efavirenz, and indomethacin, among others [47,50,51]. In other studies, full miscibility has been demonstrated based on the observation of a single T_g value for pterostilbene–Soluplus® systems [10], hesperidin–Soluplus®/hesperidin–HPMC systems [9], curcumin–piperine–PVPVA 64 systems [11], genistein–amino acid systems [19], and hesperidin–PVP dispersions [52].

As shown in Table 2, the experimentally determined T_g values for all KMP–EL100 ASDs are below the T_g of the polymer, suggesting a plasticization effect of the KMP on the EL100. Similar results were obtained by Sathigari et al. for efavirenz–Plasdone S-630 and efavirenz–EPO binary mixtures [47]. In another study, Kanaze et al. showed that the observed T_g values for hesperidin–PVP dispersions were lower than that recorded for the pure polymer. They proposed that the low-molecular-mass compound acted as a plasticizer, reducing the T_g value of the polymer and/or some weak interaction was involved between the PVP and hesperidin, causing the formation of amorphous solid dispersion systems [52].

In contrast, the T_g values for all ASDs of KMP–EL100-55 and KMP–EPO are higher than that of pure polymer. In the literature, this is often referred to as the antiplasticization effect [53,54]. Moreover, all $T_{g,exp}$ values for the KMP–Eudragit ASDs are less than the theoretical values. Sathigari et al. reported similar results for efavirenz–EPO systems. They suggested that the free volume in the homogenous phase is larger than that in the ideal mixture assumed by the G-T and C-K models [47].

Inquiry into the interaction and/or compatibility of polymer–polymer and polymer–drug systems have been conducted by numerous researchers using FT-IR [9,10,49,55,56]. In order to confirm the molecular interaction between KMP and Eudragits, we performed spectroscopic analyses on both pure compounds as well as KMP–Eudragit physical mixtures and KMP–Eudragit ASDs.

The experimental bands of KMP were compared with the theoretical FT-IR spectrum, which was calculated using the B3LYP/6-31G(d,p) level of theory (see Figure S4). The most characteristic bands of the crystalline KMP were observed in the FT-IR–ATR spectra at about 400–1800 cm^{-1} and 3000–3500 cm^{-1} . In the first range, predominant bands correspond to the OH, CCOH, CCOC, CCO, CH, OH, and C=O vibrations. In the second range, one broad band is visible at 3321 cm^{-1} , corresponding to OH groups (see Table S1, Supplementary Materials).

Assignment bands for Eudragit L100, L100-55, and EPO (Figure 5) were made based on values report in the literature (see Table S2, Supplementary Materials).

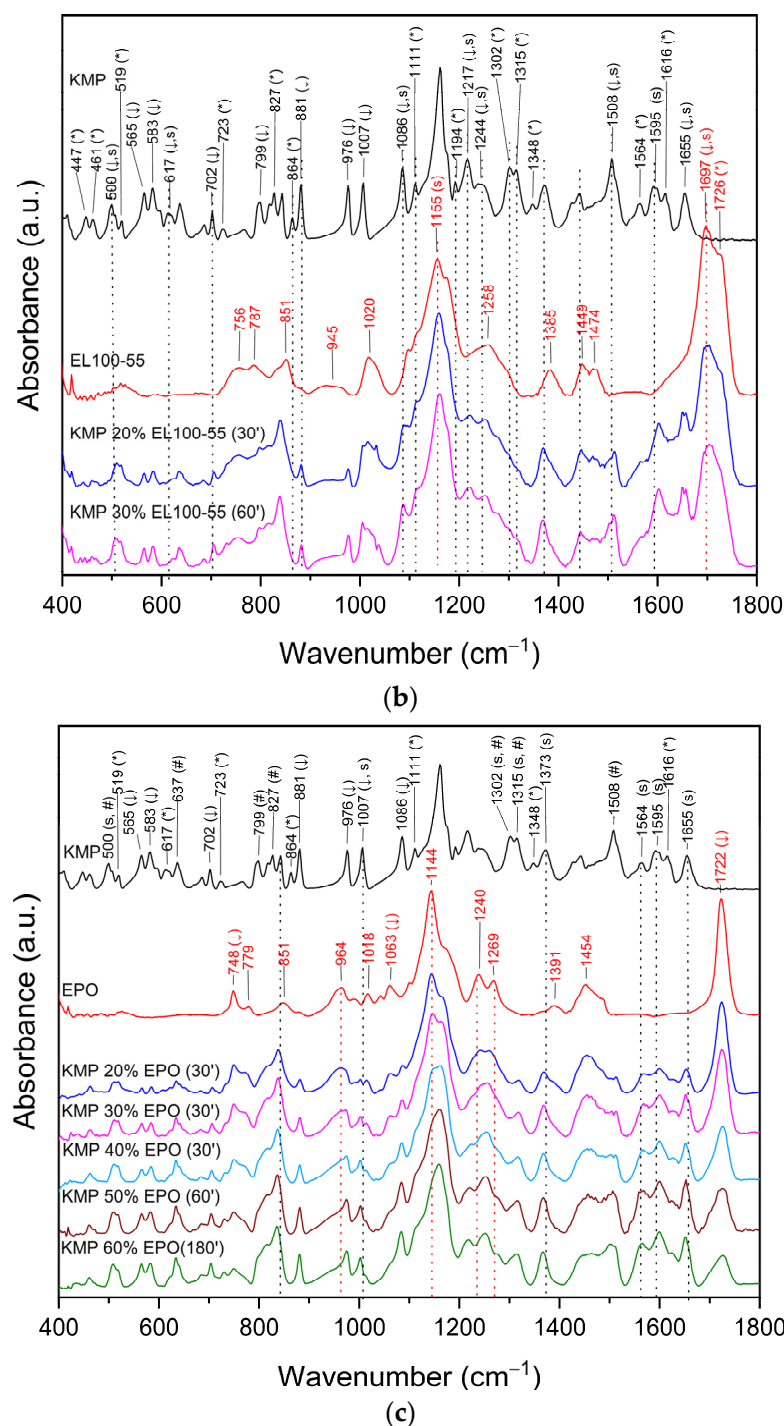


Figure 6. FT-IR-ATR analysis, range 400–1800 cm^{-1} : (a) kaempferol–Eudragit L100 amorphous solid dispersion; (b) kaempferol–Eudragit L100-55 amorphous solid dispersion; (c) kaempferol–Eudragit EPO amorphous solid dispersion. Legend: kaempferol (black line, KMP); Eudragit L100/L100-55/EPO (red line, EL100/EL100-55/EPO); the percentage of KMP in the system (KMP 20%–KMP 60%); shape change (#); band disappearance (*); intensity decrease (↓); band shift (s).

In the ranges of 400–1800 cm^{-1} (Figure 6a–c) and 2600–4000 cm^{-1} (Figure S5a–c), numerous changes in the characters of bands from both KMP and Eudragits were observed (shape change—#; band disappearance—*; intensity decrease—↓; band shift—s). The observed changes are summarized in Tables S3–S5 for KMP-EL100, KMP-EL100-55, and KMP-EPO, respectively, along with the assignment of the corresponding bands. The

shifting of the characteristic peaks of KMP and Eudragits in the FT-IR suggests that the chemical environment of the molecules has changed. In addition, these shifts indicate the formation of new bonds or interactions between the KMP and the polymer. A decrease in the intensity of certain FT-IR bands could indicate a reduction in the concentration of specific functional groups, whereas disappearance is a strong indicator of a chemical change or interaction. These peaks could represent specific functional groups that have interacted with the Eudragits.

In all ASDs, the most important changes involve bands corresponding to -CH (KMP-EL100: 799, 843, 1217 cm^{-1} ; KMP-EL100-55: 799, 827, 1111, 1217 cm^{-1}), -CO (KMP-EL100/-EL100-55/-EPO: 1302, 1315, $\sim 1600\text{--}1655$ cm^{-1}), and -OH (KMP-EL100: 461, 864, 3321 cm^{-1} ; KMP-EL100-55: $\sim 460\text{--}519$, 864, 881, 1194, 3321 cm^{-1} ; KMP-EPO: $\sim 500\text{--}519$, 864, 881, 3321 cm^{-1}) bonds in KMP, indicating that they may be involved in the formation of hydrogen bonds with Eudragits.

In the case of KMP-EL100, a disappearance of the characteristic EL100 bands were recorded at 966 cm^{-1} (C-O or O-H) [57,58], 1063 cm^{-1} (C=O) [59], 1192 cm^{-1} (C-O) [70], and 1389 cm^{-1} (CH_x) [63]. A decrease in intensity was observed for bands at 1481 cm^{-1} (CH_x) [63], 1705 cm^{-1} (C-O) [67], and 1724 cm^{-1} (C=O) [12,58,65], among others. In addition, band shifts were applied to peaks at 1705 cm^{-1} and 1724 cm^{-1} . On the other hand, for KMP-EL100-55, there were shifts in the bands recorded at 1155 cm^{-1} (-C-O-C) [62], 1697 cm^{-1} (COO or -C=O) [62,65], and 3215 cm^{-1} (O-H) [61]; there was a decrease in band intensity at 1697 cm^{-1} and an increase in band intensity at 3215 cm^{-1} . For KMP-EPO, a decrease in intensity or a disappearance of the characteristic EPO bands were observed at 1144 cm^{-1} (C-N or C-O) [60], 1240 cm^{-1} (C-O) [71], 1269 cm^{-1} (C-O) [71], 1722 cm^{-1} (C=O) [68], 2770 cm^{-1} (dimethylamino group) [66], and 2822 cm^{-1} (dimethylamino group) [66].

The obtained results confirm the formation of hydrogen bonds between the -O-H and/or -CH groups of KMP and the C=O group of Eudragits. In addition, in the case of KMP-EPO, changes in the dimethylamino groups in EPO suggest that these groups form hydrogen bonds with the C=O/C-O/C-O-C groups of KMP.

These interactions made it feasible for the formation of an amorphous KMP-Eudragit solid dispersion (confirmed by XRPD analysis). This aligns with other research indicating that the disappearance or shift in vibration peaks is often associated with crystallization or the formation of intermolecular hydrogen bonds [72,73]. Liu et al. [51] proposed a peak shift around 1700 cm^{-1} (C=O), confirming hydrogen bonding between the carbonyl group of EPO and the hydroxyl group of the drug. Similarly, Zong et al. [14] proposed that the ASD of curcumin with Eudragit RS PO and Eudragit RL PO formed a hydrogen bond between the -OH group of curcumin and the C=O group of the polymer. Furthermore, for the curcumin-EPO ASD, the authors suggested that curcumin may donate one hydrogen to the dimethylaminoethyl methacrylate group of Eudragit EPO [14].

The interaction between KMP and Eudragits might affect its solubility. Eudragits are known for their pH-dependent behavior. Specifically, for targeted drug administration in the jejunum, Eudragit L100 is soluble at a pH over 6.0. Eudragit L100-55 is insoluble below pH 5 and soluble above 5.5. Eudragit EPO, on the other hand, is soluble up to pH 5.0 and becomes insoluble above pH 5.0 [74].

The screening involved testing the solubility of the obtained ASDs in four media: water, 0.1 N HCl, pH 5.5 buffer, and pH 6.8 buffer.

It was confirmed that the ASDs of KMP-EL100 exhibited improved solubility in pH 6.8 buffer, and KMP-EPO in pH 5.5 buffer and HCl 0.1 N. Conversely, KMP-EL100-55 showed no improvement in solubility in any of the considered media. Subsequent tests were conducted for selected media.

Crystalline KMP in pH 6.8, pH 5.5, and HCl 0.1 N buffers was practically insoluble, consistent with reports in the literature [75]. Notably, for KMP-EL100 in pH 6.8 and KMP-EPO in pH 5.5 and HCl 0.1 N, an improvement in solubility was observed compared to pure KMP (Table S6).

The obtained results show that KMP 40% EL100 exhibited the maximum solubility in pH 6.8, reaching $113.3 \pm 2.3 \mu\text{g}\cdot\text{mL}^{-1}$. On the other hand, KMP 50% EPO demonstrated the highest solubility in pH 5.5 ($95.1 \pm 1.2 \mu\text{g}\cdot\text{mL}^{-1}$), while KMP 30% EPO exhibited the most significant improvement in solubility in HCl 0.1 N ($43.5 \pm 0.8 \mu\text{g}\cdot\text{mL}^{-1}$).

3. Materials and Methods

3.1. Materials

Kaempferol (purity > 98%) was purchased from Xi'an Tian Guangyuan Biotech Co., Ltd. (Xi'an, China). Eudragit L100, L100-55, and EPO were purchased from Röhm Pharma (Weierstadt, Germany). Hydrochloric acid, dimethyl sulfoxide, sodium chloride, and potassium dihydrogen phosphate were obtained from Avantor Performance Materials (Gliwice, Poland). Formic acid 98–100% was purchased from POCH (Gliwice, Poland). A Direct-Q 3 UV purification system (Millipore, Molsheim, France; model Exil SA 67120) was used to produce high-quality clean water.

3.2. Preparation of Amorphous Solid Dispersion (ASD) and Physical Mixtures

Amorphous solid dispersions were prepared by the ball milling method at room temperature. Firstly, 500 mg of KMP–Eudragit physical mixtures with different percent content of KMP were obtained by vortexing for 60 s. Next, physical mixtures and three stainless steel balls (diameter 12 mm) were added to a 50 mL stainless steel jar that fit the MIXER MILL MM 400 (Retsch, Haan, Germany). The milling frequency was set to 30 Hz. The milling time was between 30 and 180 min. The total grinding time depended on the success of the KMP amorphization process. The acquired systems had the appearance of being a uniform, fine powder. For further research, the powders were kept in a desiccator.

3.3. Determination of the Physical State of Kaempferol in ASDs

3.3.1. X-ray Powder Diffraction (XRPD)

The verification of the kaempferol physical state for (i) the pure compound, (ii) the physical mixture, and (iii) the prepared ASDs was made via powder X-ray diffractometry using a Bruker D2 Phaser diffractometer (Bruker, Germany). The diffraction patterns were captured using CuK α radiation ($1.54060 \text{ \AA}$) at tube voltages of 30 kV and tube currents of 10 mA. With a step size of $0.02^\circ 2\Theta$ and a counting rate of $2 \text{ s}\cdot\text{step}^{-1}$, the angular range was 5° to $40^\circ 2\Theta$. The obtained data were analyzed using Origin 2021b software (OriginLab Corporation, Northampton, MA, USA).

3.3.2. ATR-FTIR Spectroscopy Supported by Density Functional Theory (DFT) Calculations

ATR-FTIR spectra in the MIR region ($400\text{--}4000 \text{ cm}^{-1}$) were obtained using an IRTracer-100 spectrophotometer with a QATR that holds a diamond ATR system (Shimadzu, Kyoto, Japan). The resolution was 4 cm^{-1} , while 100 scans over the selected wavenumber range were averaged for each sample. All infrared spectra were collected using LabSolution IR software (version 1.86 SP2, Shimadzu, Kyoto, Japan).

The molecular geometries of KMP were optimized using the density functional theory (DFT) method, employing Becke's three-parameter hybrid functional (B3LYP) and the standard 6–311G(d,p) basic set. Additionally, calculations for normal mode frequencies and intensities were conducted. The DFT calculations were performed on the PL-Grid platform (website: www.plgrid.pl, accessed on 11 March 2021), utilizing the Gaussian 09 package from Wallingford, CT, USA. The GaussView program (Wallingford, CT, USA, Version E01) was employed to propose the initial geometry of the investigated molecules and visually inspect the normal modes.

The obtained data were analyzed using the Origin 2021b software (OriginLab Corporation, Northampton, MA, USA).

3.3.3. Principal Component Analysis (PCA)

LabSolution IR software (version 1.86 SP2, Shimadzu, Kyoto, Japan) was used for spectra data preprocessing. Chemometric methods were applied for the purpose of sample discrimination. In the present study, PCA was performed using Origin 2021b software with Principal Component Analysis for Spectroscopy tools (OriginLab Corporation, Northampton, MA, USA).

3.4. Thermal Analysis of KMP—Eudragit Amorphous Solid Dispersions

3.4.1. Thermogravimetric Analysis (TG)

In a TG 209 F3 Tarsus[®] micro-thermogravimetric balance (Netzsch, Selb, Germany), kaempferol, Eudragit L100, Eudragit L100-55, Eudragit EPO, and amorphous solid dispersions were tested for thermal stability. Then, 6–11 mg of the sample powder was added to an 85 μ L open Al_2O_3 crucible. The TG study's temperature ranged from 25 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$, with a continuous heating rate of 10 $^{\circ}\text{C}$ per minute in a nitrogen atmosphere (flow rate 250 $\text{mL}\cdot\text{min}^{-1}$). Proteus 8.0 was used to assess the TG data once collected (Netzsch, Selb, Germany). Origin 2021b software (OriginLab Corporation, Northampton, MA, USA) was used to display the results.

3.4.2. Differential Scanning Calorimetry (DSC)

The differential scanning calorimeter, model DSC 214 Polyma (Netzsch, Selb, Germany), was used to conduct the DSC investigations. Powdered samples weighing 3.9–10.0 mg were placed in sealed pans with lid holes, with the reference sample being a blank sealed aluminum DSC pan with a cover.

The melting point of KMP in the neat compound was observed using a single heating mode at a temperature range of 30–320 $^{\circ}\text{C}$ with a scanning rate of 10 $^{\circ}\text{C}$ per minute. The glass transition (T_g) of KMP, EL100, EL100-55 and EPO were observed in melting and cooling modes. A flow rate of 250 $\text{mL}\cdot\text{min}^{-1}$ was chosen for the nitrogen atmosphere. Proteus 8.0 was used to analyze the acquired DSC data (Netzsch, Selb, Germany). Origin 2021b software (OriginLab Corporation, Northampton, MA, USA) was used to display the results.

Based on the obtained glass transition temperature (T_g) for KMP, EL100, EL100-55, and EPO, theoretical studies due to the Gordon–Taylor (G-T, Equation (2)) and Couchman–Karasz (C-K, Equation (4)) equations aimed to predict the glass transition value of amorphous KMP–Eudragit systems.

The Gordon–Taylor equation:

$$T_{g,G-T} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2}, \quad (2)$$

where w_1 , w_2 —weight fraction of kaempferol and polymer, respectively. $T_{g,G-T}$, T_{g1} , T_{g2} —predicted glass transition temperature of a binary system; glass transition temperature of kaempferol, and glass transition temperature of the polymer, respectively. K —constant indicates a measure of interaction between two components.

K can be expressed mathematically as follows (Equation (3)):

$$K = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}}, \quad (3)$$

where ρ_1 , ρ_2 —the densities of two components (KMP: $1.5803 \pm 0.0021 \text{ g}\cdot\text{cm}^{-3}$, L100: $1.2887 \pm 0.0004 \text{ g}\cdot\text{cm}^{-3}$, L100-55: $1.2601 \pm 0.0083 \text{ g}\cdot\text{cm}^{-3}$, EPO: $1.1424 \pm 0.0006 \text{ g}\cdot\text{cm}^{-3}$). The density of KMP, L100, L100-55, and EPO was measured experimentally with a helium gas pycnometer (Accupyc 1340, Micrometrics Instrument Corporation, Norcross, GA, USA). T_{g1} , T_{g2} —glass transition temperature of kaempferol and polymer, respectively.

Couchman–Karasz equation:

$$T_{g,C-K} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2}, \quad (4)$$

where w_1 , w_2 —weight fraction of kaempferol and polymer, respectively. $T_{g,C-K}$, T_{g1} , T_{g2} —predicted glass transition temperature of a binary system; the glass transition temperature of kaempferol, and the glass transition temperature of the polymer, respectively. K —constant indicates a measure of interaction between two components.

K can be expressed mathematically as follows (Equation (5)):

$$K = \frac{\Delta c_{p2}}{\Delta c_{p1}} \quad (5)$$

where Δc_{p1} and Δc_{p2} is the change in the heat capacity at T_{g1} and T_{g2} , respectively.

3.5. HPLC Analysis

All HPLC assays were performed on a Shimadzu Nexera (Shimadzu Corp., Kyoto, Japan) [10]. For the stationary phase, a Dr. Maisch ReproSil-Pur Basic-C18 100 column with particle sizes of 5 μm and 100×4.60 mm (Dr. Maisch, Ammerbuch-Entringen, Germany) was used. The mobile phase was methanol/0.1% formic acid (70:30 *v/v*). A 0.45 μm nylon filter was used to vacuum filter the mobile phase. The experimental conditions were as follows: 0.8 $\text{mL} \cdot \text{min}^{-1}$ flow rate, 366 nm wavelength, and 35 $^{\circ}\text{C}$ column temperature.

3.6. The Solubility Studies of Kaempferol

In this experiment, surplus KMP and ASDs were put into glass vials together with 4 mL of distilled water, pH 6.8 buffer, pH 5.5 buffer, and HCl 0.1 N buffer (pH 1.2). A vortex mixer was used to mix all samples for 30 s (time T0). The samples were then incubated in a lab incubator, the MaxQ 4450 (Thermo Scientific, Waltham, MA, USA), for 24 h at 298.15 K at a steady speed of 75 rotations per minute (rpm). The suspensions were then filtered via a 0.22 μm filter and put through an HPLC analysis procedure. Each measurement was made in triplicate.

4. Conclusions

In this study, amorphous dispersions of kaempferol (KMP) with Eudragits were successfully obtained through the milling method. XRPD analysis confirmed that Fourier-transform infrared (FT-IR) spectroscopy combined with principal component analysis (PCA) may serve as an effective analytical tool for verifying the amorphous state of KMP in binary Eudragit dispersions.

Thermal analysis confirmed the complete miscibility of the obtained amorphous dispersions. FT-IR observations indicated the formation of hydrogen bonds between the $-\text{O}-\text{H}$ and/or $-\text{CH}$ groups in KMP and the $\text{C}=\text{O}$ group in Eudragit.

The amorphization process had a positive impact on the solubility of KMP under different pH conditions. The highest solubility was achieved at pH 6.8 for the dispersion with Eudragit L100 (40% KMP content), at pH 5.5 for the dispersion with Eudragit EPO (50% KMP content), and in 0.1 N HCl for the dispersion with Eudragit EPO (30% KMP content).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms242417155/s1>.

Author Contributions: Conceptualization, N.R. and J.C.-P.; methodology, N.R., E.T. and J.C.-P.; software, N.R., E.T. and J.C.-P.; validation, N.R., E.T. and J.C.-P.; formal analysis, N.R., E.T. and J.C.-P.; investigation, N.R., E.T. and J.C.-P.; resources, J.C.-P.; data curation, N.R., E.T. and J.C.-P.; writing—original draft preparation, N.R., E.T. and J.C.-P.; writing—review and editing, N.R. and J.C.-P.; visualization, N.R.; supervision, J.C.-P.; project administration, N.R.; funding acquisition, J.C.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the grant OPUS from the National Science Centre Poland UMO-2020/37/B/NZ7/03975.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kubatka, P.; Kapinová, A.; Kello, M.; Kruzliak, P.; Kajo, K.; Výbohová, D.; Mahmood, S.; Murin, R.; Viera, T.; Mojžiš, J. Fruit peel polyphenols demonstrate substantial anti-tumour effects in the model of breast cancer. *Eur. J. Nutr.* **2016**, *55*, 955–965. [\[CrossRef\]](#)
2. Gutiérrez-del-Río, I.; Villar, C.J.; Lombó, F. Therapeutic uses of kaempferol: Anticancer and antiinflammatory activity. *Biosynth. Food Sources Ther. Uses* **2016**, *15*, 71.
3. Navarro, S.L.; Schwarz, Y.; Song, X.; Wang, C.-Y.; Chen, C.; Trudo, S.P.; Kristal, A.R.; Kratz, M.; Eaton, D.L.; Lampe, J.W. Cruciferous Vegetables Have Variable Effects on Biomarkers of Systemic Inflammation in a Randomized Controlled Trial in Healthy Young Adults. *J. Nutr.* **2014**, *144*, 1850–1857. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Hosseinpour-Niazi, S.; Mirmiran, P.; Fallah-Ghohroudi, A.; Azizi, F. Non-soya legume-based therapeutic lifestyle change diet reduces inflammatory status in diabetic patients: A randomised cross-over clinical trial. *Br. J. Nutr.* **2015**, *114*, 213–219. [\[CrossRef\]](#)
5. Wang, X.; Yang, Y.; An, Y.; Fang, G. The mechanism of anticancer action and potential clinical use of kaempferol in the treatment of breast cancer. *Biomed. Pharmacother.* **2019**, *117*, 109086. [\[CrossRef\]](#) [\[PubMed\]](#)
6. López-Lázaro, M.; Calderón-Montaña, J.; Burgos-Morón, E.; Pérez-Guerrero, C. A review on the dietary flavonoid kaempferol. *Mini Rev. Med. Chem* **2011**, *11*, 298–344.
7. Devi, K.P.; Malar, D.S.; Nabavi, S.F.; Sureda, A.; Xiao, J.; Nabavi, S.M.; Daglia, M. Kaempferol and inflammation: From chemistry to medicine. *Pharmacol. Res.* **2015**, *99*, 1–10. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Xie, Y.; Luo, H.; Duan, J.; Hong, C.; Ma, P.; Li, G.; Zhang, T.; Wu, T.; Ji, G. Phytic acid enhances the oral absorption of isorhamnetin, quercetin, and kaempferol in total flavones of *Hippophae rhamnoides* L. *Fitoterapia* **2014**, *93*, 216–225. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Rosiak, N.; Wdowiak, K.; Tykarska, E.; Cielecka-Piontek, J. Amorphous Solid Dispersion of Hesperidin with Polymer Excipients for Enhanced Apparent Solubility as a More Effective Approach to the Treatment of Civilization Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 15198. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Rosiak, N.; Tykarska, E.; Cielecka-Piontek, J. Amorphous Pterostilbene Delivery Systems Preparation—Innovative Approach to Preparation Optimization. *Pharmaceutics* **2023**, *15*, 1231. [\[CrossRef\]](#)
11. Wdowiak, K.; Pietrzak, R.; Tykarska, E.; Cielecka-Piontek, J. Hot-Melt Extrusion as an Effective Technique for Obtaining an Amorphous System of Curcumin and Piperine with Improved Properties Essential for Their Better Biological Activities. *Molecules* **2023**, *28*, 3848. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Wdowiak, K.; Miklaszewski, A.; Pietrzak, R.; Cielecka-Piontek, J. Amorphous System of Hesperetin and Piperine—Improvement of Apparent Solubility, Permeability, and Biological Activities. *Int. J. Mol. Sci.* **2023**, *24*, 4859. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Bhujbal, S.V.; Mitra, B.; Jain, U.; Gong, Y.; Agrawal, A.; Karki, S.; Taylor, L.S.; Kumar, S.; Zhou, Q.T. Pharmaceutical amorphous solid dispersion: A review of manufacturing strategies. *Acta Pharm. Sin. B* **2021**, *11*, 2505–2536. [\[CrossRef\]](#)
14. Zong, S.; Liu, Y.; Park, H.J.; Ye, M.; Li, J. Curcumin solid dispersion based on three model acrylic polymers: Formulation and release properties. *Braz. J. Pharm. Sci.* **2022**, *58*, e18946. [\[CrossRef\]](#)
15. Yu, C.; Zhang, C.; Guan, X.; Yuan, D. The solid dispersion of resveratrol with enhanced dissolution and good system physical stability. *J. Drug Deliv. Sci. Technol.* **2023**, *84*, 104507. [\[CrossRef\]](#)
16. Panizzon, G.P.; Giacomini Bueno, F.; Ueda-Nakamura, T.; Nakamura, C.V.; Dias Filho, B.P. Manufacturing Different Types of Solid Dispersions of BCS Class IV Polyphenol (Daidzein) by Spray Drying: Formulation and Bioavailability. *Pharmaceutics* **2019**, *11*, 492. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Ha, E.-S.; Choi, D.H.; Baek, I.; Park, H.; Kim, M.-S. Enhanced Oral Bioavailability of Resveratrol by Using Neutralized Eudragit E Solid Dispersion Prepared via Spray Drying. *Antioxidants* **2021**, *10*, 90. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Garbiec, E.; Rosiak, N.; Tykarska, E.; Zalewski, P.; Cielecka-Piontek, J. Sinapic Acid Co-Amorphous Systems with Amino Acids for Improved Solubility and Antioxidant Activity. *Int. J. Mol. Sci.* **2023**, *24*, 5533. [\[CrossRef\]](#)
19. Garbiec, E.; Rosiak, N.; Zalewski, P.; Tajber, L.; Cielecka-Piontek, J. Genistein Co-Amorphous Systems with Amino Acids: An Investigation into Enhanced Solubility and Biological Activity. *Pharmaceutics* **2023**, *15*, 2653. [\[CrossRef\]](#)
20. Mai, N.N.S.; Otsuka, Y.; Kawano, Y.; Hanawa, T. Preparation and Characterization of Solid Dispersions Composed of Curcumin, Hydroxypropyl Cellulose and/or Sodium Dodecyl Sulfate by Grinding with Vibrational Ball Milling. *Pharmaceutics* **2020**, *13*, 383. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Elbadawy, M.; Hayashi, K.; Ayame, H.; Ishihara, Y.; Abugomaa, A.; Shibutani, M.; Hayashi, S.-M.; Hazama, S.; Takenouchi, H.; Nakajima, M.; et al. Anti-cancer activity of amorphous curcumin preparation in patient-derived colorectal cancer organoids. *Biomed. Pharmacother.* **2021**, *142*, 112043. [\[CrossRef\]](#) [\[PubMed\]](#)

22. Wdowiak, K.; Rosiak, N.; Tykarska, E.; Żarowski, M.; Płazińska, A.; Płaziński, W.; Cielecka-Piontek, J. Amorphous Inclusion Complexes: Molecular Interactions of Hesperidin and Hesperetin with HP- β -CD and Their Biological Effects. *Int. J. Mol. Sci.* **2022**, *23*, 4000. [[CrossRef](#)] [[PubMed](#)]
23. Zaini, E.; Putri, V.Z.; Octavia, M.D.; Ismed, F. Peningkatan Laju Disolusi Dispersi Padat Amorf Genistein dengan PVP K-30. *J. Sains Farm. Klin.* **2017**, *4*, 67. [[CrossRef](#)]
24. Ishimoto, K.; Shimada, Y.; Ohno, A.; Otani, S.; Ago, Y.; Maeda, S.; Lin, B.; Nunomura, K.; Hino, N.; Suzuki, M.; et al. Physicochemical and Biochemical Evaluation of Amorphous Solid Dispersion of Naringenin Prepared Using Hot-Melt Extrusion. *Front. Nutr.* **2022**, *9*, 850103. [[CrossRef](#)]
25. Shi, X.; Fan, N.; Zhang, G.; Sun, J.; He, Z.; Li, J. Quercetin amorphous solid dispersions prepared by hot melt extrusion with enhanced solubility and intestinal absorption. *Pharm. Dev. Technol.* **2020**, *25*, 472–481. [[CrossRef](#)] [[PubMed](#)]
26. Wang, Y.; Fang, Y.; Zhou, F.; Liang, Q.; Deng, Y. The amorphous quercetin/hydroxypropylmethylcellulose acetate succinate solid dispersions prepared by co-precipitation method to enhance quercetin dissolution. *J. Pharm. Sci.* **2021**, *110*, 3230–3237. [[CrossRef](#)]
27. Hatwar, P.; Pathan, I.B.; Chishti, N.A.H.; Ambekar, W. Pellets containing quercetin amino acid co-amorphous mixture for the treatment of pain: Formulation, optimization, in-vitro and in-vivo study. *J. Drug Deliv. Sci. Technol.* **2021**, *62*, 102350. [[CrossRef](#)]
28. Ahmed, S.; Sheraz, M.A.; Ahmad, I. Tolfenamic Acid. In *Profiles of Drug Substances, Excipients and Related Methodology*; Elsevier: Amsterdam, The Netherlands, 2018; Volume 43, pp. 255–319, ISBN 1871-5125.
29. Moros, J.; Garrigues, S.; de la Guardia, M. Vibrational spectroscopy provides a green tool for multi-component analysis. *TrAC Trends Anal. Chem.* **2010**, *29*, 578–591. [[CrossRef](#)]
30. Lin, X.; Sun, D.-W. Recent developments in vibrational spectroscopic techniques for tea quality and safety analyses. *Trends Food Sci. Technol.* **2020**, *104*, 163–176. [[CrossRef](#)]
31. Culbert, J.; Cozzolino, D.; Ristic, R.; Wilkinson, K. Classification of Sparkling Wine Style and Quality by MIR Spectroscopy. *Molecules* **2015**, *20*, 8341–8356. [[CrossRef](#)] [[PubMed](#)]
32. Anastasaki, E.; Kanakis, C.; Pappas, C.; Maggi, L.; del Campo, C.P.; Carmona, M.; Alonso, G.L.; Polissiou, M.G. Differentiation of saffron from four countries by mid-infrared spectroscopy and multivariate analysis. *Eur. Food Res. Technol.* **2010**, *230*, 571–577. [[CrossRef](#)]
33. Amirvaresi, A.; Nikounezhad, N.; Amirahmadi, M.; Daraei, B.; Parastar, H. Comparison of near-infrared (NIR) and mid-infrared (MIR) spectroscopy based on chemometrics for saffron authentication and adulteration detection. *Food Chem.* **2021**, *344*, 128647. [[CrossRef](#)]
34. Maâmour, O.; Rouissi, H.; Dridi, S.; Kammoun, M.; De Baerdemaeker, J.; Karoui, R. Mid infrared attenuated total reflection spectroscopy as a rapid tool to assess the quality of Sicilo-Sarde ewe's milk during the lactation period after replacing soybean meal with scotch bean in the feed ration. *Food Chem.* **2008**, *106*, 361–368. [[CrossRef](#)]
35. Santos, I.A.; Conceição, D.G.; Viana, M.B.; Silva, G.d.J.; Santos, L.S.; Ferrão, S.P.B. NIR and MIR spectroscopy for quick detection of the adulteration of cocoa content in chocolates. *Food Chem.* **2021**, *349*, 129095. [[CrossRef](#)] [[PubMed](#)]
36. Li, L.; Zhao, Y.; Li, Z.; Wang, Y. Multi-information based on ATR-FTIR and FT-NIR for identification and evaluation for different parts and harvest time of *Dendrobium officinale* with chemometrics. *Microchem. J.* **2022**, *178*, 107430. [[CrossRef](#)]
37. Inam, S.; Irfan, M.; Lali, N.U.A.; Khalid Syed, H.; Asghar, S.; Khan, I.U.; Khan, S.-U.-D.; Iqbal, M.S.; Zaheer, I.; Khames, A.; et al. Development and Characterization of Eudragit® EPO-Based Solid Dispersion of Rosuvastatin Calcium to Foresee the Impact on Solubility, Dissolution and Antihyperlipidemic Activity. *Pharmaceuticals* **2022**, *15*, 492. [[CrossRef](#)] [[PubMed](#)]
38. Wang, X.; Wang, M.; Wang, Q.; Yuan, Y.; Hao, Q.; Bi, Y.; He, Y.; Zhao, J.; Hao, J. Fabrication and in vitro/in vivo characterization of Eudragit enteric nanoparticles loaded with indomethacin. *Chem. Pap.* **2022**, *76*, 1119–1133. [[CrossRef](#)]
39. Hashemi, S.; Mortazavi, S.A.; Moghimi, H.R.; Darbasizadeh, B. Development and evaluation of a novel methotrexate-loaded electrospun patch to alleviate psoriasis plaques. *Drug Dev. Ind. Pharm.* **2022**, *48*, 355–366. [[CrossRef](#)]
40. Wang, X.; Zhu, Y.; Zhao, X.; Zhang, S.; Cao, M.; Wang, X.; Li, W. Development and characterization of an amorphous curcumin-Eudragit® E100 solid dispersions with improved solubility, stability, and pharmacokinetic properties. *Pharm. Dev. Technol.* **2022**, *27*, 965–974. [[CrossRef](#)] [[PubMed](#)]
41. Alsayad, R. Preparation and in vitro evaluation for amorphous solid dispersion of azithromycin. *Res. Sq.* **2023**, preprint. [[CrossRef](#)]
42. Colombo, M.; de Lima Melchades, G.; Michels, L.R.; Figueiró, F.; Bassani, V.L.; Teixeira, H.F.; Koester, L.S. Solid Dispersion of Kaempferol: Formulation Development, Characterization, and Oral Bioavailability Assessment. *AAPS PharmSciTech* **2019**, *20*, 106. [[CrossRef](#)] [[PubMed](#)]
43. Tzeng, C.-W.; Yen, F.-L.; Wu, T.-H.; Ko, H.-H.; Lee, C.-W.; Tzeng, W.-S.; Lin, C.-C. Enhancement of Dissolution and Antioxidant Activity of Kaempferol Using a Nanoparticle Engineering Process. *J. Agric. Food Chem.* **2011**, *59*, 5073–5080. [[CrossRef](#)] [[PubMed](#)]
44. Zhou, H.; Duan, C.; Qin, H.; Huang, C.; Hou, J.; Chen, Y.; Zhu, J.; Xu, C.; Jin, J.; Zhuang, T. Synthesis and structural characterization of a novel palbociclib-kaempferol cocrystal with improved tabletability and synergistic antitumor activity. *J. Mol. Struct.* **2023**, *1281*, 135101. [[CrossRef](#)]
45. Schammé, B.; Couvrat, N.; Malpeli, P.; Delbreilh, L.; Dupray, V.; Dargent, É.; Coquerel, G. Crystallization kinetics and molecular mobility of an amorphous active pharmaceutical ingredient: A case study with Biclotymol. *Int. J. Pharm.* **2015**, *490*, 248–257. [[CrossRef](#)] [[PubMed](#)]
46. Schugmann, M.; Foerst, P. Systematic Investigation on the Glass Transition Temperature of Binary and Ternary Sugar Mixtures and the Applicability of Gordon–Taylor and Couchman–Karasz Equation. *Foods* **2022**, *11*, 1679. [[CrossRef](#)] [[PubMed](#)]

47. Sathigari, S.K.; Radhakrishnan, V.K.; Davis, V.A.; Parsons, D.L.; Babu, R.J. Amorphous-state characterization of efavirenz–polymer hot-melt extrusion systems for dissolution enhancement. *J. Pharm. Sci.* **2012**, *101*, 3456–3464. [[CrossRef](#)] [[PubMed](#)]
48. Gordon, M.; Taylor, J.S. Ideal copolymers and the second-order transitions of synthetic rubbers. i. non-crystalline copolymers. *J. Appl. Chem.* **2007**, *2*, 493–500. [[CrossRef](#)]
49. Lee, L.-T.; Chien, H.-R.; Yang, C.-T. Ternary miscibility with specific interactions in novel blends comprising biodegradable polymers and a natural polyphenol. *J. Polym. Res.* **2016**, *23*, 1. [[CrossRef](#)]
50. Tian, Y.; Jacobs, E.; Jones, D.S.; McCoy, C.P.; Wu, H.; Andrews, G.P. The design and development of high drug loading amorphous solid dispersion for hot-melt extrusion platform. *Int. J. Pharm.* **2020**, *586*, 119545. [[CrossRef](#)]
51. Liu, H.; Zhang, X.; Suwardie, H.; Wang, P.; Gogos, C.G. Miscibility Studies of Indomethacin and Eudragit® E PO by Thermal, Rheological, and Spectroscopic Analysis. *J. Pharm. Sci.* **2012**, *101*, 2204–2212. [[CrossRef](#)]
52. Kanaze, F.I.; Kokkalou, E.; Niopas, I.; Georgarakis, M.; Stergiou, A.; Bikiaris, D. Thermal analysis study of flavonoid solid dispersions having enhanced solubility. *J. Therm. Anal. Calorim.* **2006**, *83*, 283–290. [[CrossRef](#)]
53. Painter, P.C.; Coleman, M.M. *Fundamentals of Polymer Science: An Introductory Text*; CRC Press: Boca Raton, FL, USA, 1997.
54. Li, Y.; Pang, H.; Guo, Z.; Lin, L.; Dong, Y.; Li, G.; Lu, M.; Wu, C. Interactions between drugs and polymers influencing hot melt extrusion. *J. Pharm. Pharmacol.* **2014**, *66*, 148–166. [[CrossRef](#)]
55. Puttipatkhachorn, S.; Nunthanid, J.; Yamamoto, K.; Peck, G. Drug physical state and drug–polymer interaction on drug release from chitosan matrix films. *J. Control. Release* **2001**, *75*, 143–153. [[CrossRef](#)] [[PubMed](#)]
56. Di, R.; Liu, J.; Grohgan, H.; Rades, T. A Multivariate Approach for the Determination of the Optimal Mixing Ratio of the Non-Strong Interacting Co-Amorphous System Carvedilol-Tryptophan. *Molecules* **2021**, *26*, 801. [[CrossRef](#)]
57. Dupeyrón, D.; Kawakami, M.; Ferreira, A.M.; Caceres, P.R.; Rieumont, J.; Azevedo, R.B.; Carvalho, J.C.T. Design of indomethacin-loaded nanoparticles: Effect of polymer matrix and surfactant. *Int. J. Nanomed.* **2013**, *8*, 3467–3477. [[CrossRef](#)] [[PubMed](#)]
58. Nikam, A.; Sahoo, P.R.; Musale, S.; Pagar, R.R.; Paiva-Santos, A.C.; Giram, P.S. A Systematic Overview of Eudragit® Based Copolymer for Smart Healthcare. *Pharmaceutics* **2023**, *15*, 587. [[CrossRef](#)] [[PubMed](#)]
59. Janakidevi, S.; Ramanamurthy, K.V. Design of a Novel Colon Targeted Microsponges Loaded with Diclofenac Sodium Using Three Different Polymers. *Int. Res. J. Pharm.* **2018**, *9*, 10–19. [[CrossRef](#)]
60. Lin, S.-Y.; Cheng, W.-T.; Wei, Y.-S.; Lin, H.-L. DSC-FTIR microspectroscopy used to investigate the heat-induced intramolecular cyclic anhydride formation between Eudragit E and PVA copolymer. *Polym. J.* **2011**, *43*, 577–580. [[CrossRef](#)]
61. Mohammadi, G.; Mirzaeei, S.; Taghe, S.; Mohammadi, P. Preparation and Evaluation of Eudragit® L100 Nanoparticles Loaded Impregnated with KT Tromethamine Loaded PVA -HEC Insertions for Ophthalmic Drug Delivery. *Adv. Pharm. Bull.* **2019**, *9*, 593–600. [[CrossRef](#)]
62. Vlachou, M.; Kikionis, S.; Siamidi, A.; Kyriakou, S.; Tsotinis, A.; Ioannou, E.; Roussis, V. Development and Characterization of Eudragit®-Based Electrospun Nanofibrous Mats and Their Formulation into Nanofiber Tablets for the Modified Release of Furosemide. *Pharmaceutics* **2019**, *11*, 480. [[CrossRef](#)] [[PubMed](#)]
63. Franco, P.; De Marco, I. Eudragit: A Novel Carrier for Controlled Drug Delivery in Supercritical Antisolvent Coprecipitation. *Polymers* **2020**, *12*, 234. [[CrossRef](#)]
64. Kumar, N.; Aggarwal, R.; Chauhan, M.K. Extended levobunolol release from Eudragit nanoparticle-laden contact lenses for glaucoma therapy. *Futur. J. Pharm. Sci.* **2020**, *6*, 109. [[CrossRef](#)]
65. Rezazadeh, M.; Safaran, R.; Minaiyan, M.; Mostafavi, A. Preparation and characterization of Eudragit L 100-55/chitosan enteric nanoparticles containing omeprazole using general factorial design: In vitro/in vivo study. *Res. Pharm. Sci.* **2021**, *16*, 358. [[CrossRef](#)]
66. Sutar, Y.; Nabeela, S.; Singh, S.; Alqarihi, A.; Solis, N.; Ghebremariam, T.; Filler, S.; Ibrahim, A.S.; Date, A.; Uppuluri, P. Niclosamide-loaded nanoparticles disrupt Candida biofilms and protect mice from mucosal candidiasis. *PLoS Biol.* **2022**, *20*, e3001762. [[CrossRef](#)] [[PubMed](#)]
67. Abdi, M.; Zakeri-Milani, P.; Ghorbani, M. Designing and Evaluating pH-Responsive Electrospun Eudragit® L-100/Hydroxypropyl Methyl Cellulose Composite Mats for Release of Propolis as a Novel Wound Dressing. *J. Polym. Environ.* **2023**, *31*, 3215–3229. [[CrossRef](#)]
68. Alshehri, S.M. Versatility of Hot-Melt Extrusion for Dosage form Design. Ph.D. Thesis, University of Mississippi, Oxford, MI, USA, 2015.
69. Adibkia, K.; Javadzadeh, Y.; Dastmalchi, S.; Mohammadi, G.; Niri, F.K.; Alaei-Beirami, M. Naproxen–eudragit® RS100 nanoparticles: Preparation and physicochemical characterization. *Colloids Surf. B Biointerfaces* **2011**, *83*, 155–159. [[CrossRef](#)] [[PubMed](#)]
70. Santos, T.M.M.; Oliveira, P.H., Jr.; Ribeiro, L.A.A.; Oliveira, H.P. Drug/magnetite-loaded enteric particles: The influence of localized magnetic field on controlled release of nifedipine. *Asian J. Biochem. Pharm. Res.* **2014**, *4*, 63–71.
71. Abdelhakim, H.E.; Coupe, A.; Tuleu, C.; Edirisinghe, M.; Craig, D.Q.M. Utilising Co-Axial Electrospinning as a Taste-Masking Technology for Paediatric Drug Delivery. *Pharmaceutics* **2021**, *13*, 1665. [[CrossRef](#)]
72. Jia, S.; Ning, S.; Leng, Y.; Jing, Q.; Xu, Z.; Ren, F. Stabilizing Effect of Soluplus on Erlotinib Metastable Crystal form in Microparticles and Amorphous Solid Dispersions. *Polymers* **2022**, *14*, 1241. [[CrossRef](#)]
73. Karavas, E.; Koutris, E.; Papadopoulos, A.G.; Sigalas, M.P.; Nanaki, S.; Papageorgiou, G.Z.; Achilias, D.Z.; Bikiaris, D.N. Application of density functional theory in combination with FTIR and DSC to characterise polymer drug interactions for the preparation of sustained release formulations between fluvastatin and carrageenans. *Int. J. Pharm.* **2014**, *466*, 211–222. [[CrossRef](#)]

74. Sakhno, T.V.; Barashkov, N.N.; Irgibaeva, I.S.; Pustovit, S.V.; Sakhno, Y.E. Polymer Coatings for Protection of Wood and Wood-Based Materials. *Adv. Chem. Eng. Sci.* **2016**, *6*, 93–110. [[CrossRef](#)]
75. Trendafilova, I.; Lazarova, H.; Chimshirova, R.; Trusheva, B.; Koseva, N.; Popova, M. Novel kaempferol delivery systems based on Mg-containing MCM-41 mesoporous silicas. *J. Solid State Chem.* **2021**, *301*, 122323. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.