Acta Pharm. **73** (2023) 385–404
https://doi.org/10.2478/acph-2023-0030

Original research paper

Insights into the formulation properties, biocompatibility, and permeability of poorly water-soluble methoxyflavones with PEG400 and propylene glycol

FREDRICK NWUDE EZE[1]*
CHAWEEWAN JANSAKUL[3,4]
TEERAPOL SRICHANA[1,2]*

[1] *Drug Delivery System Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University Hat Yai, Songkhla 90110, Thailand*

[2] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University Hat Yai, Songkhla 90110, Thailand*

[3] *Natural Product Research Centre of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand*

[4] *Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand*

Accepted May 17, 2023
Published online May 18, 2023

ABSTRACT

Herein, thermal and non-thermal techniques were used to elucidate the putative physical and chemical interactions between poorly water-soluble *Kaempferia* methoxyflavones and PEG400/propylene glycol. Additionally, the biocompatibility of methoxyflavone-glycol solutions was evaluated using Caco-2 cells whereas the absorptive transport was investigated by measuring the apparent permeability coefficient (P_{app}) of the methoxyflavones and transepithelial electrical resistance (TEER) of the Caco-2 cell monolayer. Data from differential scanning calorimetry, Fourier-transform infrared (FTIR), and proton nuclear magnetic resonance (^{1}H NMR) spectroscopic analysis revealed physicochemical compatibility between the three methoxyflavones and PEG400/propylene glycol. Furthermore, PEG400 and propylene glycol solutions of the methoxyflavones were shown to be compatible with Caco-2 cells at pharmacologically effective concentrations. *In vitro* transport studies across the Caco-2 cell monolayer revealed high P_{app} values of 24.07×10^{-6} to 19.63×10^{-6} cm s^{-1} for PEG400 solutions of the methoxyflavones. The TEER values of the Caco-2 cell monolayers indicated that the increased drug transport was partly due to increased tight junction openings, but without compromising the epithelial barrier integrity. The good pharmaceutical and biocompatibility profiles, as well as improved transport of the methoxyflavones in PEG400 and propylene glycol solutions, are suggestive of the worthiness of this approach for further consideration pertaining to the development of these drugs into oral liquid dosage forms.

Keywords: methoxyflavones, compatibility, permeability, PEG 400, pre-formulation study

Methoxyflavones are one of the most promising classes of natural flavonoids due to their many remarkable attributes. Methylation of flavonoids reportedly improves not only

*Correspondence; e-mails: fredrickeze10@gmail.com, teerapol.s@psu.ac.th

their biological and pharmacological properties over their unmethylated counterparts, but also enhances their hepatic stability, solubility, membrane permeability, and cytocompatibility. Recently, methoxyflavones from *Kaempferia parviflora* (KAPE methoxyflavones) have attracted much attention due to their diverse pharmacotherapeutic potentialities (1). Despite increasing pharmacological evidence of the beneficial effects of the methoxyflavones such as anticancer, antiinflammatory, antiosteoathritic, anti-obesity, neuroprotective, and cardioprotective properties (1, 2), their low oral bioavailability remains a major impediment towards their successful development into commercial oral drug formulations (3). Consequently, research that is aimed at improving the prospects of delivering KAPE methoxyflavone in oral dosage formulations is of interest.

The oral route represents the most common mode of administration for many drugs because of convenience, ease of compliance, and cost benefits to patients. While the oral bioavailability of methylated flavones is generally higher compared to unmethylated flavonoids, it is worth emphasizing that the absorbed amounts are still remarkably low compared to the amounts needed for effective cellular biological response. Consequently, various approaches have been adopted in the recent past to enhance the oral bioavailability of *K. parviflora* extract rich in methoxyflavones. These included nanosuspensions, self-microemulsifying and self-nanoemulsifying drug delivery systems, complexation with cyclodextrin (4, 5), and solid dispersion (6). Nonetheless, studies focusing on improving the oral delivery of pure methoxyflavones are quite scarce (7). It is generally understood that poor oral bioavailability of bioflavonoids arising from inadequate solubility in the gastrointestinal tract coupled with the presystemic metabolism plays a major role in their lack of clinical success (3, 8). Pertaining to the oral delivery of poorly water-soluble drugs, two formulations approaches can be adopted to enhance their bioavailability, *viz.* improve the dissolution rate of the drug by delivery in the form of solid dispersion, or by obviating the dissolution and disintegration step through direct delivery of the drug in liquid dosage form. In fact, for most drug products, the liquid dosage form is often the formulation of choice for first *in vivo* investigations. Water soluble organic solvents such as polyethylene glycol (PEG) 400, propylene glycol, glycerin, *etc*., are excipients often used as vehicles/enhancers for improving the solubility of hydrophobic drugs in liquid dosage forms. Thus, it is important to examine the compatibility between the drug and these excipients to unveil potential physical and/or chemical interactions in the drug-excipient binary mixtures and thereby reduce or minimize unwanted effects that may adversely impact the physicochemical properties, stability, efficacy, or overall performance of the dosage form. Such pre-formulation studies are important initial steps and integral for the rational development of any dosage form (9, 10).

Polyethylene glycol 400 (PEG400) and propylene glycol (PG) are two common solvents widely used in the pharmaceutical industry due to their low levels of toxicity. These glycols possess a high propensity to dissolve in water and are thus very useful in augmenting the solubility and bioavailable of poorly water-soluble drugs in pharmaceutical formulations. They are frequently employed not only as co-solvents to enhance the aqueous solubility of weakly water-soluble drugs, but also in the preparation of oral and parenteral drug concentrates and soft gel formulations (11). The counteractive properties of PEG against mould growth and rancidity make it a highly suitable excipient for liquid dosage forms (12). Although widely regarded as safe and approved for use in many pharmaceutical and cosmetic products, the influence of PEG400 on the biopharmaceutical and physicochemical profiles of drugs

is increasingly being recognised. For instance, PEG400 was used to improve to minimise hydrolysis and enhance the stability of the drug busulfan (Busulfex® and Busulvex®). On the other hand, soft gelatin capsules of the drug nimodipine in PEG400 solution (Nimodipine Capsule, Caraco Pharmaceutical Labs) were recalled due to crystallisation of the API in the solution, which raised concerns regarding its actual bioavailability (13). These examples illustrate why ascertaining drug-excipient compatibility is essential during formulation development. At the moment, there have been no reports on the pharmaceutical or biocompatibility of binary mixtures of methoxyflavones in PEG400 or PG.

The objective of this study was therefore to evaluate the compatibility between the major bioactive methoxyflavone compounds isolated from *K. parviflora* and polyethylene glycol (PEG) *viz*. 5,7-dimethoxyflavone (DMF), 5,7,4′-trimethoxyflavone (TMF), and 3,5,7,3′,4′-pentamethoyflavone (PMF) and PEG400/PG. In addition, the solubility, cytotoxicity, and *in vitro* permeability through Caco-2 cell monolayers were evaluated. It is expected that findings from this work will provide valuable insights into the development of KAPE methoxyflavones in liquid dosage forms.

EXPERIMENTAL

Source of KAPE methoxyflavones

K. parviflora rhizomes were obtained locally from Ampur Phurua in Thailand. The fresh rhizomes were authenticated at the National Herbarium domiciled at Prince of Songkla University, Hat Yai, where a voucher specimen (No. 2548-03) was deposited. Subsequently, the methoxyflavones were isolated from the dichloromethane extract of the rhizomes by open column chromatography using RP-18 as the stationary phase and a gradient system of methanol/water (2:8 to 4:6 *V*/*V*) as the mobile phase. The chemical identity of the isolated compounds (DMF, TMF, and PMF) was established by spectroscopic data, ^{1}H and ^{13}C NMR as previously reported (14). The purity of the compounds was determined using analytical HPLC on an HP1100 system coupled with a photodiode array detector as previously described (15). The eluents were monitored at a wavelength of 254 nm and UV-Vis spectra were obtained from 200 to 500 nm.

Differential scanning calorimetry (DSC) measurement

Thermal analysis of the samples was performed using DSC as previously described with minor modifications (3). Briefly, a sample (3 to 5 mg) was accurately weighed into a standard aluminum pan and hermetically sealed. DSC thermal curves were obtained using Perkin Elmer (USA) DSC 800 differential scanning calorimeter. The temperature was held for 5 min at 25 °C, then ramped up to 200 °C at 10 °C min^{-1}, and finally cooled from 200 to 45 °C at 10 °C min^{-1} under a flow of nitrogen gas.

Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR analysis of KAPE methoxyflavones, PEG400, and PG as well binary mixtures of the methoxyflavones in PEG400 and PG were performed on VERTEX 70v Fourier transform infrared spectrometer equipped with an A225/Q Platinum ATR unit with a single

reflection diamond crystal (Bruker, Germany), at a resolution of 4 and 128 total scans. IR spectra of the samples were recorded from 4000 to 400 cm^{-1}. For the solutions, the IR data were obtained in the attenuated reflectance mode.

^{1}H NMR spectroscopic analysis of KAPE methoxyflavones in glycols

KAPE methoxyflavones were solubilized in DMSO-d_6. PEG400, PG with or without the methoxyflavones were also solubilized in DMSO-d_6, briefly vortexed, and filtered into oven-dried standard 5 mm NMR tubes. Experiments were performed on Nanalysis Corp 60 MHz NMreadyPro benchtop NMR spectrometer equipped with a permanent magnet and operating at a ^{1}H frequency of 60 MHz. For spectral acquisition, 2048 scans were performed over a spectral width of 12 ppm, scan delay of 2.7 s, time per scan of 14.9 s, and pulse width of 15.0 μs. The instrument line width was ≤ 3 Hz and residual DMSO was employed as the internal standard. Spectral data generated in .dx files were imported to Mestrelab Research MestReNovax64 software for data processing and analysis.

Determination of dielectric constant

Dielectric characterization of methoxyflavones was determined by capacitance method using Agilent Technologies' 4285A Precision LCR meter (USA) as described previously (16). Methoxyflavone tablets measuring 10 mm in diameter and 0.8 mm in thickness were inserted into brass foils and fitted between the capacitor plates. The dielectric constant was obtained from the capacitance measured at room temperature using the equation below.

$$\varepsilon_r = (t \times C_p)/(\varepsilon_0 \times A)$$

Where ε_r is the dielectric constant, t is the sample thickness (m), Cp the capacitance (F), A is the area (m^2) and ε_0 is the vacuum permittivity (8.854×10^{-12} F m^{-1}). The dielectric constant of the solvents was obtained from the literature (17).

Solubility of KAPE methoxyflavones

The solubility of KAPE methoxyflavones in neat PEG400, PG, and water was determined as previously described (18). Excess amounts of the methoxyflavones were added to 1 mL of solvents and vortexed continuously to attain equilibrium. The solutions were left at room temperature for 24 h. After centrifuging at 12000 rpm for 5 min, supernatants were collected, appropriately diluted (1000-fold) using 70 % methanol and filtered through a 0.22 μm nylon membrane syringe filter. Concentrations of KAPE methoxyflavones were measured using the HPLC method as previously described (15). Succinctly, the samples were analyzed on an HP1100 system fitted with a photodiode array detector (Agilent Technologies). Samples were eluted using Symmetry® C18 column (5 μm, 3.9 × 150 mm *i.d.*; Waters Corporation) as stationary phase and a gradient of methanol/water acidified with 0.05 % trifluoroacetic acid (10:90→100:0) as the mobile phase. The sample injection volume was 10 μL and eluted at a flow rate of 1 mL min^{-1} and detected by UV at a wavelength of 254 nm. Chromatographic elution peaks were presented at retention times of 22.87, 23.33, and 22.25 min for DMF, TMF, and PMF, respectively. There were no interferences from the sample matrix or solvent as can be seen from the chromatograms. The HPLC system was

validated as per guidelines (ICH, 2005) (19), which resulted in the linear regression analysis of standard curves with the correlation coefficient (R^2) over 0.999 for all the methoxyflavones. The recovery was between 99 and 100 % and the precision were less than 2 % coefficient of variation for both intra- and inter-day runs. The LLOQ values were 0.61, 1.86 and 0.46 μg mL^{-1} for DMF, TMF, and PMF, respectively.

Cytocompatibility studies

The impact of PEG400 solutions of methoxyflavones on Caco-2 cell viability was determined as described previously (20) with minor modifications. The cells were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10 % fetal bovine serum, FBS (Gibco, USA) at 37 °C in a 5 % CO_2 incubator for 24 h. Cells were subsequently treated with serial dilutions of the samples in the medium and further incubated for 24 h. The cells treated with medium only served as control. The cell viability was estimated using MTT assay at an OD of 570 nm and the results were calculated as percentage cell viability with respect to the control.

Caco-2 cell monolayer permeability assay

In vitro drug transport across intestinal cellular barriers was performed using human intestinal Caco-2 cell monolayers permeability assay according to Kamiya *et al.* (21). Caco-2 cells from ATCC (American Type Culture Collection, USA) in fresh medium (MEM + 10 % FBS) were seeded at a density of 1.5×10^5 cells/cm^2 onto the apical compartment of the 6-well plates (Transwell, 24-mm diameter; USA) and 2.5 mL of medium was added to the basolateral chamber. The cells were cultured at 37 °C, 5 % CO_2, and 95 % humidity whilst replacing the medium in both chambers every 1–2 days. To examine the integrity of the cell monolayer, transepithelial electrical resistance (TEER) across the monolayer was measured using Millicell-ERS-2 Voltohmmeter (Merck, Germany). TEER values above 500 Ω cm^2 were deemed appropriate for the monolayer, this was often obtained within 15–20 days post-cell seeding (22, 23). The cell monolayer was washed and replaced with fresh medium (MEM alone) immediately before commencing transport studies. To evaluate the transport of methoxyflavones from the apical to the basolateral compartments, 1.5 mL of medium containing 30 μg mL^{-1} of compounds was added to the apical compartment followed by 2.5 mL of medium only (MEM alone) in the basolateral chamber. The Transwell plate was immediately incubated at 37 °C and 0.5 mL of medium aliquot was withdrawn from the basolateral chamber at 0, 0.5, 1, 2, 3, 4, 5, 6, and 8 h. Fresh medium was added to the basolateral side to replace the withdrawn aliquot. The amount of methoxyflavone in the collected aliquots was quantified by RP-HPLC analysis as described supra. Samples for the HPLC analysis were prepared by extraction using ethyl acetate. An equal volume of ethyl acetate was added to the collected sample, briefly vortexed, and left for a few minutes. The upper layer was collected. The lower layer (aqueous phase) was re-extracted one more time and both fractions obtained were combined and evaporated using nitrogen gas. The dry sample was reconstituted using 70 % methanol. The apparent permeability coefficients (P_{app} in cm s^{-1}) of the methoxyflavones in PEG400 were derived thusly.

$$P_{app} = (dC/dt) \times (1/A\ C_0)$$

where dC/dt is the change in the amount of compound permeated across the Caco-2 cell monolayer at steady state (μg s^{-1}), while A represents the membrane surface area (cm^2) and C_0 the initial concentration pipetted into the apical chamber (μg mL^{-1}), respectively.

Tight junction measurement after cells exposure to methoxyflavones

A strong correlation is known to exist between the electrical resistance of the epithelial barrier and the preponderance of tight junction strands. The impact of the methoxyflavone-glycol mixtures on the integrity of the Caco-2 cell monolayers was examined by measuring the transepithelial electrical resistance (TEER) of the cell monolayers using the Millicell-ERS-2 Voltohmmeter (Merck KGaA, Germany) (Kreft *et al*. 2015). When the TEER value was constant for 2 days, the experiment was performed by measurement of TEER values of the Caco-2 cell monolayers before and after loading methoxyflavones samples (DMF, TMF, and PMF) for 2 h compared to the control.

Statistical data analysis

Data obtained in the biocompatibility and permeability studies were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc., USA) and presented as the mean ± standard deviation (n = 3). The Turkey-Kramer test was used to determine the statistical significance between groups and a p-value of < 0.05 was considered significant.

RESULTS AND DISCUSSION

Oral dosage forms are predominantly in solid dosage formulations such as tablets and capsules; however, many are delivered in liquid forms. Polyethylene glycols and PGs are frequently used in the preparation of liquid dosage forms of poorly aqueous-soluble drugs. Methoxyflavones from *K. parviflora* have demonstrated remarkable pharmacological potential. Nonetheless, their pharmaceutical development into oral dosage forms has greatly been impeded due to poor physico-chemical and biopharmaceutical attributes related to low water solubility. Herein, we examine the impact of two aqueous soluble organic excipients commonly used in the pharmaceutical industry to promote the solubility of poorly water-soluble compounds on key methoxyflavones isolated from *K. parviflora*,

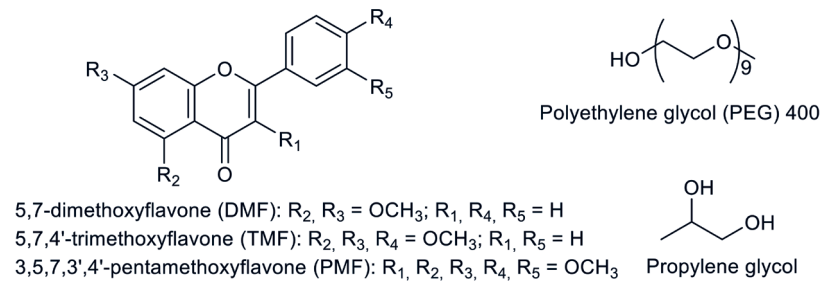

Fig. 1. Chemical structure of methoxyflavones isolated from *K. parviflora* and the glycols.

namely DMF, TMF, and PMF (Fig. 1). Particularly, API-excipient compatibility as well as *in vitro* cytotoxicity and absorption across intestinal cell barrier were examined. The findings obtained could be relevant in the future development of liquid dosage forms of the methoxyflavones and are presented thusly.

The HPLC elution profiles of DMF, TMF, PMF at 254 nm as well as their corresponding retention times are shown in Fig. 2. HPLC analysis of the obtained compounds presented exact retention times as previously reported (15). The purity of the obtained methoxyflavones was ascertained *via* quantitative analysis using HPLC chromatography. The absolute content of the obtained methoxyflavones was deduced from standard curves prepared from known concentrations of the pure compounds. The purity of the obtained methoxyflavones was about 99.9 %. It was essential to establish that the compounds obtained were in their pristine form given that the presence of impurities can alter the physicochemical

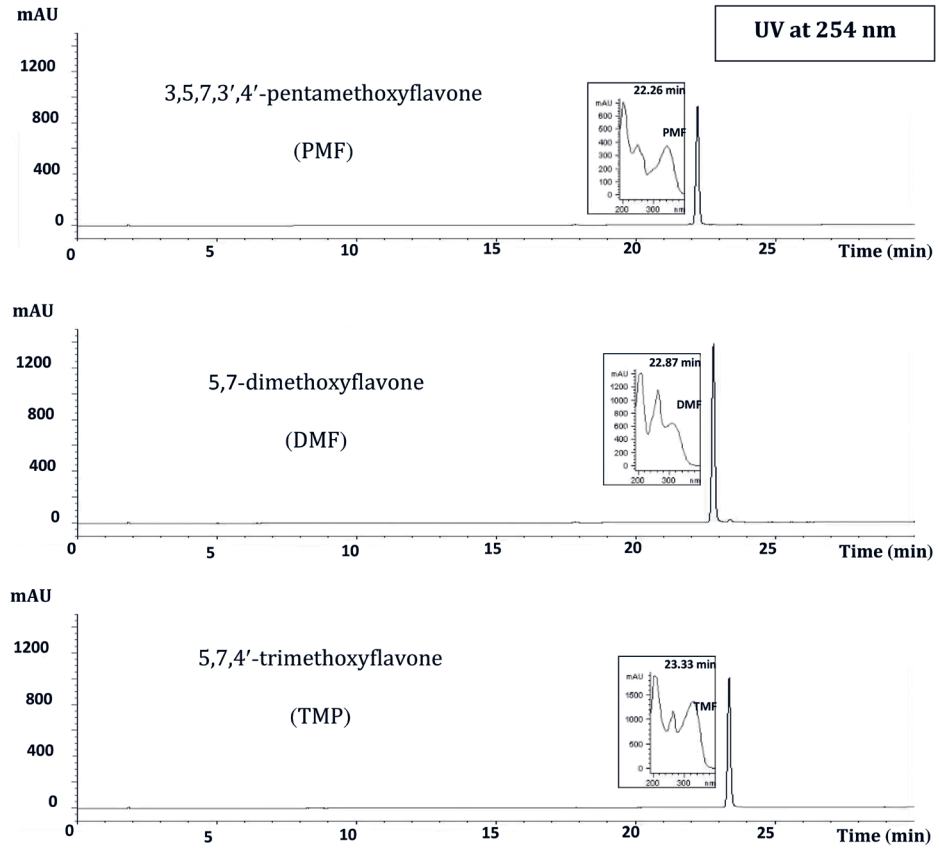

Fig. 2. HPLC chromatogram together with the UV spectrum of 3,5,7,3',4'-pentamethoxyflavone (PMF), 5,7-dimethoxyflavone (DMF) and 5,7,4'-trimethoxyflavone (TMP). The column eluent from PMF, DMF and TMF were scanned at wavelength of 254 nm.

properties as well as the biopharmaceutical profiles of the compounds being investigated. The HPLC analysis confirmed that the methoxyflavones used in this study were of high purity and therefore suitable for the intended studies.

Differential scanning calorimetry

DSC thermograms of KAPE methoxyflavones powder, liquid glycols, and mixtures of the methoxyflavones in glycols are presented in Fig. 3. Clearly-defined endothermic peaks were present in the solid methoxyflavones suggestive of their crystalline nature (Fig. 3a). The thermal events occurred between 145 to 155 °C and were comparable to the melting peaks of the methylated flavones (7, 24). The melting temperatures of PG and PEG 400 have been reported in the literature as –60 °C and 5.8 °C, respectively (25). In contrast, the DSC curves of the neat glycols from 25 to 200 °C did not feature any thermal peaks (Fig. 3). Similar DSC profiles had previously been observed for PEG 400 and neat PG (26–28). Noticeably, binary mixtures of the methoxyflavones and PEG 400 did not show any of the endothermic peaks present in the pure compounds and were actually similar to the thermal profiles of the neat

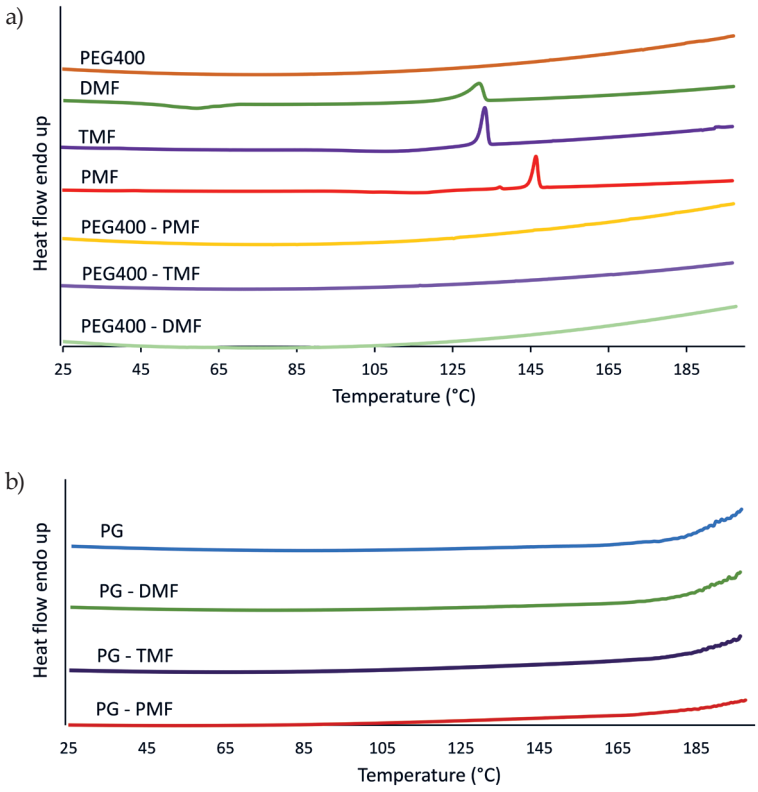

Fig. 3. Differential scanning calorimetry thermograms of KAPE methoxyflavones, neat glycols or solutions of the glycols with KAPE methoxyflavones.

glycol (Fig. 3a). Similar pattern was observed for the binary mixtures of the methoxyflavones and PG (Fig. 3b). The disappearance of the methoxyflavone peaks in the binary mixtures is suggestive that the compounds were in an amorphous state instead of the crystalline state of their pristine form (Fig. 3). The observed change in the thermal profile of the binary mixtures relative to the pure compounds is underpinned and indicative of the presence of physical interactions between the methoxyflavones and the glycols.

FTIR spectroscopy analysis

Further insights into the possible intermolecular interactions between KAPE methoxyflavones and the glycols were obtained *via* FTIR analysis. Fig. 4 displays the FTIR spectra of the powdered methoxyflavones, neat solvents as well as binary mixtures of the methoxyflavones and PEG 400 or PG. The characteristic vibrations of the asymmetric methoxy (C–H) of the flavones could be seen at 2836 cm^{-1} for DMF and at 2842 cm^{-1} for TMF and PMF. Additionally, a broad and distinct peak around 1600 cm^{-1} was observed in all the flavone compounds (Fig. 4a), and this could reasonably be attributed to the (C=C) and (C=O) stretching vibrations of the phenyl and carbonyl groups (29). In contrast, the glycols presented strong vibrations around 3300 to 3400 cm^{-1} and 2860 to 2880 cm^{-1}, corresponding to O–H stretching of the aliphatic hydroxyl groups and C–H stretching of the methylene groups, respectively. Also, the peaks at 1090 cm^{-1} and 1039 cm^{-1} are consistent with C–O–C stretching mode in PEG 400 (30) and the C–O–H stretching vibrations of PG (31).

In general, binary mixtures of the methoxyflavones and glycols display overlapping bands characteristic of the drug compounds and excipients (Fig. 4b,c). Here, the intense broad peaks of the pure methoxyflavone around 1600 cm^{-1} were reflected as minor peaks between 1620 and 1640 cm^{-1} in the IR spectra of their respective binary mixtures. Similarly, there was an overlap between the absorbance bands of the methylene groups of the glycols and those of the methoxy group of flavones (2800–2900 cm^{-1}). Another significant point was the slight shifts in the wavenumber of the glycols compared to methoxyflavones-glycol solutions. For instance, as shown in the position of the absorption band characteristic for hydroxyl group vibration (ca. 3320 cm^{-1}), the shift in peak position of the methoxyflavone/glycol mixtures though present, was almost unnoticeable and there was no peak broadening. This could be an indication of the presence of only a few intermolecular hydrogen bonding interactions between the methoxyflavones and glycol solutions. Also, neat PG yielded a peak at 2876 cm^{-1} which was shifted to 2877 cm^{-1} in PG-DMF and PG-TMF. Likewise, neat PEG400 gave a peak at 2868 cm^{-1} which was shifted to 2867 and 2866 cm^{-1} in PEG400-DMF, PEG400-TMF, and PEG400-PMF, respectively. These minor alterations in peak positions could be taken as an inference for the presence of non-specific interactions such as dipole-induced dipole and dispersion forces between the solutes and solvents. It should be emphasised that no new peaks were observed in the binary mixtures of all the samples evaluated, suggestive of the absence of new bond formations. Thus, the observed effects could be reasonably interpreted as the occurrence of physical interactions between the methoxyflavone compounds and the glycols.

Proton NMR spectroscopy

Furthermore, putative molecular interactions between KAPE methoxyflavones and the glycols were also investigated using ^{1}H NMR spectroscopy as previously reported (32). The

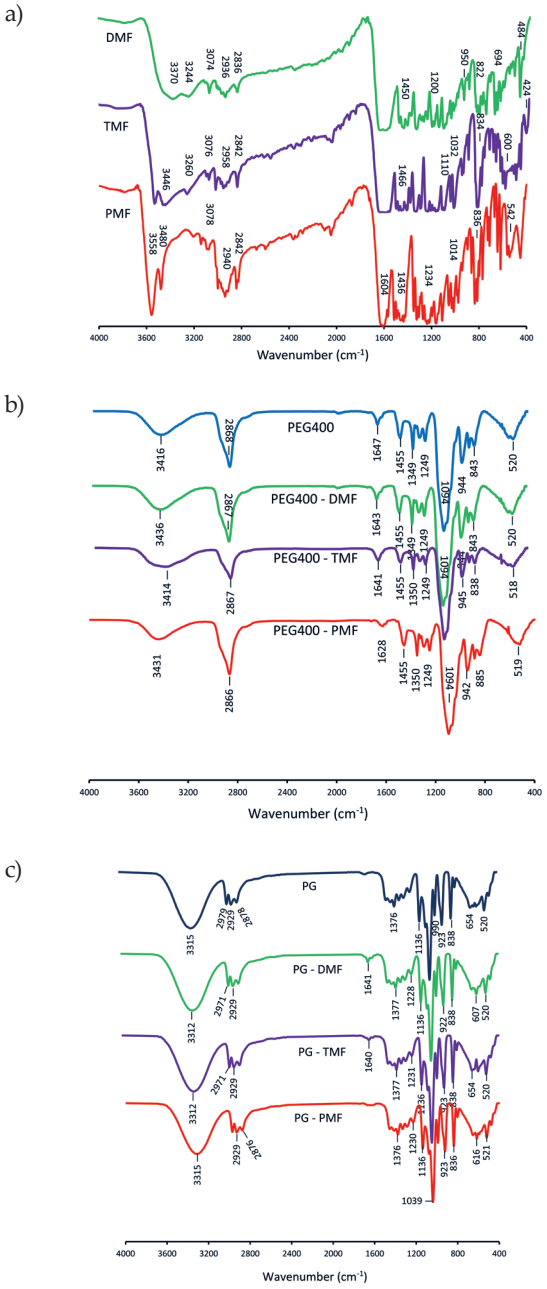

Fig. 4. FTIR spectra of: a) neat KAPE methoxyflavones; b) neat PEG400 as well as solutions of PEG400 with KAPE methoxyflavones; c) neat PEG400 as well as solutions of PEG400 with KAPE methoxyflavones.

^{1}H spectra of the neat solvents (PEG 400 and PG), methoxyflavones (DMF, TMF, and PMF) as well as solutions of the methoxyflavones in both solvents were obtained in DMSO-d_6 (Fig. 5). The ^{1}H NMR fingerprint of PEG400 revealed vibration signals at 4.06 and 3.45 ppm

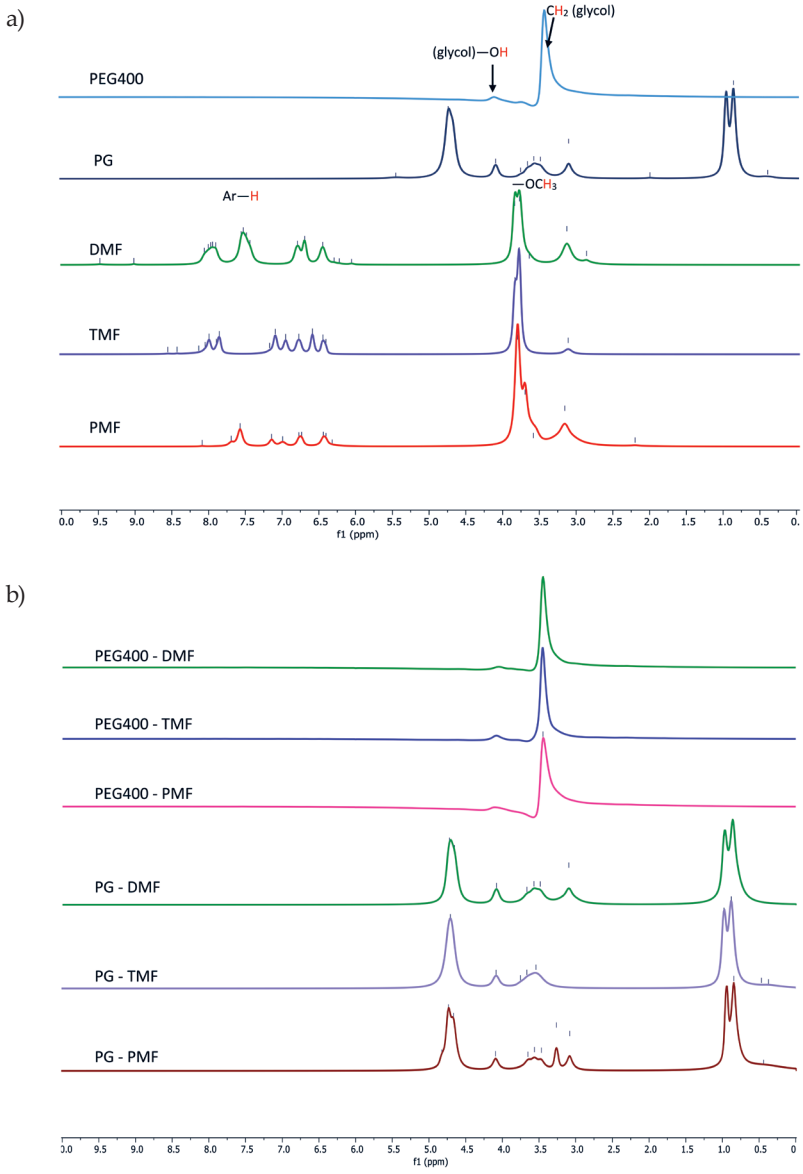

Fig. 5. ^{1}H NMR spectra of: a) neat KAPE methoxyflavones as well as neat solutions of PEG400 and propylene glycol; b) solutions of PEG400 or propylene glycol with KAPE methoxyflavones.

corresponding to the chemical shifts of the terminal hydroxyl and repeating ethylene oxide unit, respectively (33). The ^{1}H NMR spectrum of PG also displayed the ^{1}H vibrational signals representative of C_2 and C_3 at around 3 ppm and 4 ppm, respectively (Fig. 5a). Examination of KAPE methoxyflavones such as DMF revealed a band of vibrational signals at about 3 to 4 ppm and another at 6 to 8 ppm which had been attributed to protons of the methoxy and the 2-phenylchromone units, respectively (7). Surprisingly, the signals from the 2-chromone core of the methoxyflavones were completely absent in solutions of PEG400 and PG containing the solutes (Fig. 5b). According to ref. 34 the successful conjugation of the antibiotic, moxifloxacin to PEG was indicated by the clear appearance of peaks from both compounds in the proton NMR profile of the complex. In this context, the absence of the methoxyflavone signals suggests the absence of such chemical interactions.

Also, the lack of vibrational signals from the methoxyflavones whilst in solution could be interpreted as an inference of the extensive solvation effects of the glycols on the drug compounds. It is expected that successful molecular interactions between any two chemical species will alter the chemical environment of the compounds, which in turn would be reflected in their NMR fingerprint (35). In this work, minor shifts in the position of the proton signal from the PEG400 ethylene oxide extending unit were observed. That is, from 4.06 ppm in the spectrum of neat PEG400 to 4.02 ppm and 4.03 ppm in those of PEG400-DMF and PEG400-PMF, respectively (Table I). Clearly absent from the solute-solvent ^{1}H NMR fingerprints was the characteristic red shift in proton signals commonly associated with the formation of hydrogen bonds. In other words, the results from the low-resolution ^{1}H NMR analysis were consistent with those of the DSC and FTIR, indicating that no chemical interactions occurred between the bioflavonoids and the glycols. Based on the data from the thermal, FTIR, and ^{1}H NMR analysis, it can be concluded that the three methoxyflavones were compatible with PEG400 and PG.

Table I. Proton NMR chemical shifts of PEG400, PG with and without methoxyflavones

Sample	Chemical shift (δ ppm), coupling constants (Hz)
PEG400	4.06, 3.45
PG	3.80–3.35 (m, 4H), 3.08 (s, 7H), 0.89 (d, *J* = 6.0 Hz, 10 H), 0.37 (s, 7H)
DMF	8.21–7.76 (m, 1H), 7.48 (d, *J* = 4.9 Hz, 1H), 6.94–6.30 (m, 1H), 3.80 (d, *J* = 4.0 Hz, 2H), 3.13 (s, 5H)
TMF	7.95 (dd, *J* = 8.9, 3.0 Hz, 1H), 7.52–6.26 (m, 2H), 3.80 (d, *J* = 3.5 Hz, 2H), 3.11 (s, 1H)
PMF	7.63 (d, *J* = 7.3 Hz, 1H), 7.29–6.26 (m, 1H), 3.75 (d, *J* = 6.2 Hz, 6H), 3.15 (s, 9H)
PEG400-DMF	4.03, 3.42, 2.95 (s, 3H), 2.13 (s, 1H)
PEG400-TMF	4.06 (s, 1H)
PEG400-PMF	4.02, 2.96 (s, 1H)
PG-DMF	4.68 (d, *J* = 4.1 Hz, 6H), 4.08 (s, 1H), 3.56 (t, *J* = 5.6 Hz, 3H), 3.08 (s, 5H), 0.89 (d, *J* = 6.9 Hz, 8H)
PG-TMF	4.70 (s, 2H), 3.58 (d, *J* = 7.5 Hz, 1H), 3.09 (s, 2H), 0.89 (d, *J* = 6.1 Hz, 3H)
PG-PMF	4.74 (t, *J* = 4.8 Hz, 7H), 3.61 (q, *J* = 5.7 Hz, 4H), 3.17 (d, *J* = 10.9 Hz, 8H), 0.90 (d, *J* = 6.1 Hz 9H), 0.44 (s, 1H)

Solubility of KAPE methoxyflavones

DMF, TMF, and PMF revealed a significant increase in their solubility when they were dissolved in the glycols relative to water (Table II). The solubility of DMF, TMF, and PMF in PEG400, PG, and water was enhanced in the following direction: PEG400 > PG > water. The disparity in solubilizing power of the glycols compared to water may be accounted for by their differing solvation abilities which is associated with their polarities. This behavior may be explained by the fact that compounds that are alike tend to dissolve each other as articulated by Akerlof *et al.* (36). The dielectric constant (ε) is widely regarded as an acceptable index for material polarity. Highly polar materials like water yield a high dielectric constant, whereas compounds with low polarity have lower dielectric constant values. As the polarity of the solvent got closer to that of the methoxyflavones the better the solubility (Table III). The influence and importance of solvent polarity on the solubility of hydrophobic compounds were aptly demonstrated in the extraction of triterpenoids and non-polar flavonoids using subcritical water (37). As the polarity of water under subcritical conditions decreased from ε = 53 at 110 °C to ε = 36.5 at 190 °C (an ε value which is close to that of PG), its capacity to extract hydrophobic non-polar flavones and flavanones including apigenin, luteolin, naringenin, and hesperetin was dramatically increased (38). Similar behavior was observed here with regard to the solubility of KAPE methoxyflavones in PEG400, PG, and water. As the polarity of the neat solvent decreased, the solubility of the methoxyflavones in the corresponding solvent increased. About a six to forty-four-fold increase in solubility was observed when methoxyflavones were dissolved in glycols compared to water. Similar findings were previously reported for the methoxyflavone-rich *K. parviflora* dichloromethane extract (3).

With regards to solute-solvent interactions, it is well established that the main impact of polarity has to do with the strength of the van der Waals forces (39). However, other

Table II. Solubility of methoxyflavones in glycols and water measured at room temperature[a]

Material	PEG400 (mg mL^{-1})	PG (mg mL^{-1})	Water (μg mL^{-1})
DMF	11.76 ± 0.15	5.80 ± 0.07	14.71 ± 0.38
TMF	18.69 ± 0.18	5.84 ± 0.13	27.12 ± 0.84
PMF	11.80 ± 0.03	2.72 ± 0.02	30.22 ± 0.53

[a] Mean ± SD, n = 3.

Table III. Dielectric constant of methoxyflavones in glycols measured at room temperature

Material	Molecular formula	Molecular weight (g mol^{-1})	Dielectric constant (ε)
PEG400	$H(OCH_2CH_2)_nOH$	380–420	13.7
Propylene glycol	$C_3H_8O_2$	76.09	32.0
Water	H_2O	18.02	78.5
DMF	$C_{17}H_{14}O_4$	282.29	9.08×10^{-4}
TMF	$C_{18}H_{16}O_5$	312.30	8.94×10^{-4}
PMF	$C_{20}H_{20}O_7$	372.37	9.84×10^{-4}

molecular forces such as H-bonding and hydrophobic interactions may equally be consequential (10). For instance, the methoxyflavones harbor between 4 to 7 hydrogen bond acceptors, which could participate in hydrogen bonding with hydrogen donating groups of the glycols (-OH). However, due to the preponderance of the hydrocarbon moieties in the methoxyflavones, it is likely that their solubility in the glycols is mostly delineated by London dispersion forces.

Biocompatibility of KAPE methoxyflavones

Caco-2 cells were used as models to determine the cytotoxicity of the samples given its relevance in the *in vitro* prediction of oral drug bioavailability. The cells were treated

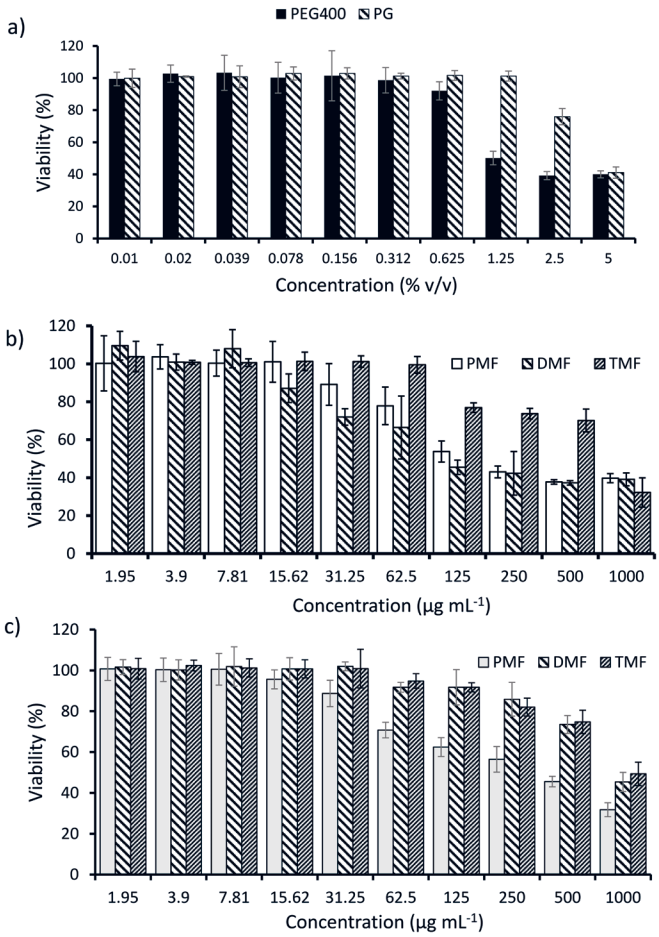

Fig. 6. Effect of KAPE methoxyflavones-PEG400 and: a) KAPE methoxyflavone-propylene glycol; b) solutions on the viability of Caco-2 cells.

with serial dilutions of the methoxyflavones in PEG400 (maximum concentration of PEG 400 was 5 %, *V*/*V*) Fig. 6a. The effects of PEG400-DMF, PEG400-TMF and PEG400-PMF on Caco-2 cell viability are presented in Fig. 6b. It was found that the cells were tolerant to plain PEG400 up to a concentration of 1.25 % (*V*/*V*) presenting viability of 84.74 %. On the other hand, PG was tolerant up to a concentration of 2.5 % (*V*/*V*), for which the cell viability was 96.95 %. Beyond these concentrations, the cell viability decreased drastically. These results were in agreement with some of the published data on PEG400 as a solubiliser or vehicle. For instance, MacDonald *et al*., noted that PEG400 (< 5.0 %) was suitable for *in vitro* drug delivery studies using respiratory epithelial (Calu-2) cells PEG400 (40). Studies examining the impact of solvents on the viability of human HepG2 cells by Le Hegarat *et al*., found that PEG400 at 1.25 % was tolerant to the cells, whereas higher concentrations of the solvent (≥ 2.5 %) were cytotoxic (41). Furthermore, our data revealed that, but for PMF, the methoxyflavones in PEG400 did not substantially affect the cell viability up to a concentration of 250 μg mL^{-1} (89 and 84 % for DMF and TMF, respectively). PMF, in contrast, exhibited a sharp decrease in cell viability at concentrations beyond 62.5 μg mL^{-1} (72 % viability). After 250 μg mL^{-1}, cell viability decreased in a concentration-dependent manner (Fig. 6b). Similar trend was observed for the methoxyflavones in PG (Fig. 6c). It should be mentioned that some studies had reported that KAPE methoxyflavones induce their biological activities at concentrations well below 250 μg mL^{-1} (42–44). Thus, 250 and 62.5 μg mL^{-1} could be considered as the safety thresholds for DMF, TMF, and PMF, respectively.

In vitro *transport through Caco-2 cell monolayers*

For a long time, pharmacological excipients were widely regarded as inert from a pharmaceutical perspective (45). Accumulating evidence in the previous few decades seems to suggest otherwise, especially with regard to oral absorption in some drugs (18). Cancer cell membrane fluidity was reportedly altered by cholesterol, a common emulsifier. In humans, low concentrations of polyethylene glycol 400 were shown to enhance the absorption of ranitidine whereas high concentrations exhibited an adverse effect by reducing the intestinal transit time (46). The solubility and systemic exposure of berberine as well as increased *via* the influence of PEG 400 acting as a chemical permeation enhancer which facilitated its reversible paracellular transport across the intestine (18). Thus, pharmaceutical excipients such as PEG400 could exert diverse attributes that could influence the therapeutic efficacy of drugs with poor water solubility.

Herein, the extent of intestinal absorption of KAPE methoxyflavones in PEG400 was estimated using the Caco-2 cell monolayer permeability assay. The Caco-2 cell permeability assay is considered the gold standard *in vitro* assay for oral drug absorption and is widely used in predicting *in vivo* human intestinal drug absorption. The methoxyflavones in PEG400 were found to exhibit an apparent permeability coefficient (P_{app}) of 24.07 × 10^{-6}, 22.59 × 10^{-6} and 19.63 × 10^{-6} cm s^{-1} for DMF, TMF and PMF, respectively (Fig. 7a). Besides solubility, permeability is the other vital parameter utilized by biopharmaceutics classification system (BCS) for predicting the intestinal absorption of drugs administered *via* the oral route (47). In terms of permeability across intestinal barriers, the BCS framework recognizes orally administered drugs as either high or low permeability. Drugs with high permeability feature a human intestinal absorption (F_a) of > 90 % of the oral dose, while those with low intestinal absorption exhibit a F_a value of < 90 %. A strong correlation is known to exist between the F_a value and the experimental permeability (P_{app}) values across

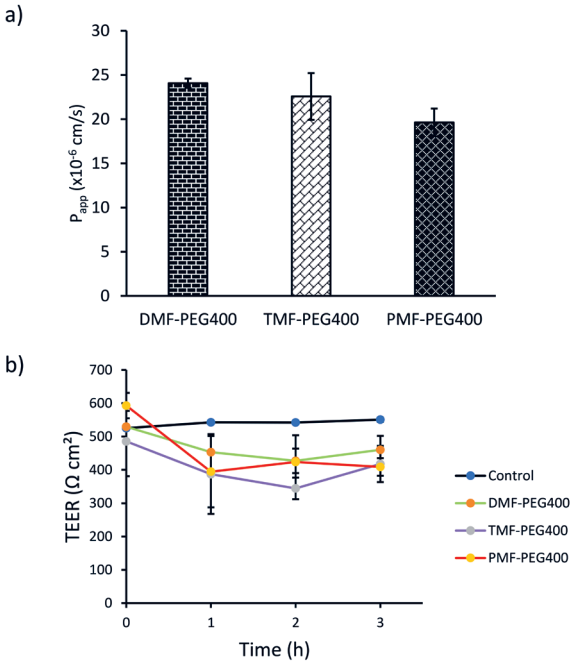

Fig. 7. a) Permeability of methoxyflavone-PEG400 solutions across Caco-2 cell monolayers; b) TEER values of Caco-2 cell monolayers before and after exposure to methoxyflavone-PEG400 solution reflecting the impact on cells tight junctions.

Caco-2 cell monolayers. In fact, Volpe *et al*. found that for oral drugs with passive transport, experimental P_{app} values of > 14.0×10^{-6} cm s^{-1} correlated well with drugs having more than 90 % *in vivo* human intestinal absorption (*i.e*., highly permeable) and *vice versa* (48). As such, the high P_{app} values obtained for KAPE methoxyflavones in PEG400 are suggestive of their high permeability across the cell monolayer and suggestive of their high *in vivo* intestinal absorption.

Improvement of intestinal absorption of drugs may be underpinned by actions that influence factors associated with an intestinal barrier, for instance, improvement in membrane fluidity, inhibition of efflux system action, or opening of tight junctions of epithelial cells (49). Herein, we looked at the impact of KAPE methoxyflavone in PEG400 on the tight junction of Caco-2 cell monolayers. Considering that a strong correlation exists between epithelial resistance and tight junction barrier integrity, TEER values of the Caco-2 cell monolayers were measured before and after the introduction of the methoxyflavones (Fig. 7b). For the negative control, we found that the TEER value of Caco-2 cell monolayers was slightly increased with time, however, the values at 0 and 3 h were not significantly different ($p < 0.05$). The Caco-2 cell monolayers exposed to the methoxyflavone-PEG400 mixtures exhibited lower TEER values at 2 h compared to 0 h, but the TEER values apparently increased thereafter. It was found that but for the PMF-PEG400 mixture, the TEER values of the methoxyflavone-PEG400 exposed cell monolayers at the start (0 h) and completion

(3 h) were not significantly different ($p < 0.05$). These results seem to suggest that the methoxyflavone-PEG400 mixtures opened the tight junctions of the cell monolayers, and the effects were apparently reversible at the concentrations of methoxyflavones tested. This indicated that no damage was done to the integrity of the cell monolayers. In addition, the increase in permeability of the methoxyflavones could partly be due to the impact of the mixture on the tight junctions of the epithelial cell monolayers. Now, the effect of the mixtures on the tight junctions did not mirror the trend in the apparent permeability of the individual compounds. This is indicative that the observed increase in their apparent permeability might be the result of multiple actions by the mixtures on Caco-2 cell monolayers. The impact of different concentrations of the methoxyflavones on TEER or P_{app} values was not considered in this study. Given that beyond a certain concentration, hydrophobic compounds dissolved in PEG400 tend to precipitate upon contact with water, it is strongly suggested that the impact of these parameters be delineated in future investigations.

CONCLUSIONS

We have revealed here that methoxyflavones purified from *K. parviflora* were compatible with PEG400 and PG, two pharmaceutical excipients with limited toxicity. Cell viability assays confirm that, at pharmacological concentrations, glycol-solvated methoxyflavones had minimal cytotoxicity on Caco-2 cells. Furthermore, the methoxyflavones solubilized in PEG400 were found to exhibit high permeability across Caco-2 cell monolayers, indicative of enhanced absorption across intestinal barriers. Therefore, it is reasonable to suggest that these glycols, especially PEG400 will be effective for enhancing the solubility and oral bioavailable of KAPE methoxyflavones and worthy of further consideration towards the development of oral liquid dosage forms.

Acknowledements. – The authors are thankful to Assoc. Prof. Dr. Nantakan Muensit, Division of Physical Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110 for assistance with dielectric measurements.

Funding. – This work was supported by the Drug Delivery Systems Excellence Center, Prince of Songkla University, Hat Yai.

Conflicts of interest. – The authors have no competing interests to declare that are relevant to the content of this article.

Authors contributions. – Conceptualization, T.S.; methodology, F.N.E., C.J. and T.S.; analysis, F.N.E., C.J. and T.S.; investigation, F.N.E., C.J. and T.S.; writing, original draft preparation, F.N.E. and C.J.; writing, review and editing, F.N.E., C.J. and T.S. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. D. Chen, H. Li, W. Li, S. Feng and D. Deng, *Kaempferia parviflora* and its methoxyflavones: Chemistry and biological activities, *Evid. Based Complement Alternat. Med.* **2018** (2018) Article ID 4057456 (16 pages); https://doi.org/10.1155/2018/4057456
2. S. Yoshino, R. Awa, Y. Miyake, I. Fukuhara, H. Sato, T. Ashino, S. Tomita and H. Kuwahara, Daily intake of *Kaempferia parviflora* extract decreases abdominal fat in overweight and pre-obese subjects: a randomized, double-blind, placebo-controlled clinical study, *DMSO* **11** (2018) 447–458; https://doi.org/10.2147/DMSO.S169925

3. C. Mekjaruskul, Y.-T. Yang, M. G. D. Leed, M. P. Sadgrove, M. Jay and B. Sripanidkulchai, Novel formulation strategies for enhancing oral delivery of methoxyflavones in *Kaempferia parviflora* by SMEDDS or complexation with 2-hydroxypropyl-β-cyclodextrin, *Int. J. Pharm.* **445**(1-2) (2013) 1–11; https://doi.org/10.1016/j.ijpharm.2013.01.052
4. P. Chairuk, S. Tubtimsri, C. Jansakul, P. Sriamornsak and Y. Weerapol, Enhancing oral absorption of poorly water-soluble herb (*Kaempferia parviflora*) extract using self-nanoemulsifying formulation, *Pharm. Dev. Technol.* **25**(3) (2020) 340–350; https://doi.org/10.1080/10837450.2019.1703134
5. C. Mekjaruskul and B. Sripanidkulchai, *Kaempferia parviflora* nanosuspension formulation for scalability and improvement of dissolution profiles and intestinal absorption, *AAPS PharmSciTech.* **21** (2020) Article ID 52; https://doi.org/10.1208/s12249-019-1588-4
6. Y. Weerapol, S. Tubtimsri, C. Jansakul and P. Sriamornsak, Improved dissolution of *Kaempferia parviflora* extract for oral administration by preparing solid dispersion via solvent evaporation, *Asian J. Pharm. Sci.* **12**(2) (2017) 124–133; https://doi.org/10.1016/j.ajps.2016.09.005
7. K. Sutthanut, B. Sripanidkulchai, C. Yenjai and M. Jay, Simultaneous identification and quantitation of 11 flavonoid constituents in *Kaempferia parviflora* by gas chromatography, *J. Chrom. A* **1143**(1-2) (2007) 227–233; https://doi.org/10.1016/j.chroma.2007.01.033
8. N. Koirala, N. H. Thuan, G. P. Ghimire, D. V. Thang and J. K. Sohng, Methylation of flavonoids: Chemical structures, bioactivities, progress and perspectives for biotechnological production, *Enzyme Microbial. Tech.* **86** (2016) 103–116; https://doi.org/10.1016/j.enzmictec.2016.02.003
9. L. Zhang, H. Luan, W. Lu and H. Wang, Preformulation studies and enabling formulation selection for an insoluble compound at preclinical stage – from in vitro, in silico to in vivo, *J. Pharm. Sci.* **109** (2020) 950–958; https://doi.org/10.1016/j.xphs.2019.10.023
10. A. Hussain, M. A. Altamimi, O. Afzal, A. S. A. Altamimi, A. Ali, A. Ali, F. Martinez, M. U. Mohd Siddique, W. E. Acree and A. Jouyban, Preferential solvation study of the synthesized aldose reductase inhibitor (SE415) in the {PEG 400 (1) + water (2)} cosolvent mixture and gastroplus-based prediction, *ACS Omega* **7**(1) (2022) 1197–1210; https://doi.org/10.1021/acsomega.1c05788
11. S. Kalepu and V. Nekkanti, Insoluble drug delivery strategies: review of recent advances and business prospects, *Acta Pharm. Sin. B* **5**(5) (2015) 442–453; https://doi.org/10.1016/j.apsb.2015.07.003
12. A. A. D'souza and R. Shegokar, Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications, *Expert Opin. Drug Deliv.* **13**(9) (2016) 1257–1275; https://doi.org/10.1080/17425247.2016.1182485
13. R. P. Gullapalli and C. L. Mazzitelli, Polyethylene glycols in oral and parenteral formulations – A critical review, *Int. J. Pharm.* **496**(2) (2015) 219–239; https://doi.org/10.1016/j.ijpharm.2015.11.015
14. C. Jansakul, K. Tachanaparuksa, M. J. Mulvany and Y. Sukpondma, Relaxant mechanisms of 3,5,7,3′,4′-pentamethoxyflavone on isolated human cavernosum, *Eur. J. Pharmacol.* **691**(1-3) (2012) 235–244; https://doi.org/10.1016/j.ejphar.2012.07.019
15. S. Yorsin, K. Kanokwiroon, N. Radenahmad and C. Jansakul, Increased vascular eNOS and cystathionine-γ-lyase protein after 6 weeks oral administration of 3,5,7,3′,4′-pentamethoxyflavone to middle-aged male rats, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **389** (2016) 1183–1194; https://doi.org/10.1007/s00210-016-1280-0
16. S. Sawatdee, H. Phetmung and T. Srichana, Sildenafil citrate monohydrate-cyclodextrin nanosuspension complexes for use in metered-dose inhalers, *Int. J. Pharm.* **455**(1-2) (2013) 248–258; https://doi.org/10.1016/j.ijpharm.2013.07.023
17. P. B. Rathi, M. Kale, J. Soleymani and A. Jouyban, Solubility of etoricoxib in aqueous solutions of glycerin, methanol, polyethylene glycols 200, 400, 600, and propylene glycol at 298.2 K, *J. Chem. Eng. Data* **63**(2) (2018) 321–330; https://doi.org/10.1021/acs.jced.7b00709
18. B.-L. Ma, Y. Yang, Y. Dai, Q. Li, G. Lin and Y.-M. Ma, Polyethylene glycol 400 (PEG400) affects the systemic exposure of oral drugs based on multiple mechanisms: taking berberine as an example, *RSC Adv.* **7** (2017) 2435–2442; https://doi.org/10.1039/C6RA26284H

19. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1)*, Current Step 4 version, November 2005; https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf last acess date May 16, 2023.
20. F. N. Eze and O. F. Nwabor, Valorization of *Pichia* spent medium via one-pot synthesis of biocompatible silver nanoparticles with potent antioxidant, antimicrobial, tyrosinase inhibitory and reusable catalytic activities, *Mater. Sci. Eng. C* **115** (2020) Article ID 111104 (14 pages); https://doi.org/10.1016/j.msec.2020.111104
21. Y. Kamiya, S. Otsuka, T. Miura, H. Takaku, R. Yamada, M. Nakazato, H. Nakamura, S. Mizuno, F. Shono, K. Funatsu and H. Yamazaki, Plasma and hepatic concentrations of chemicals after virtual oral administrations extrapolated using rat plasma data and simple physiologically based pharmacokinetic models, *Chem. Res. Toxicol.* **32**(1) (2019) 211–218; https://doi.org/10.1021/acs.chemrestox.8b00307
22. R. C. R. Beck, A. R. Pohlmann, C. Hoffmeister, M. R. Gallas, E. Collnot, U. F. Schaefer, S. S. Guterres and C. M. Lehr, Dexamethasone-loaded nanoparticle-coated microparticles: Correlation between in vitro drug release and drug transport across Caco-2 cell monolayers, *Eur. J. Pharm. Biopharm.* **67**(1) (2007) 18–30; https://doi.org/10.1016/j.ejpb.2007.01.007
23. G. Kigen and G. Edwards, Drug-transporter mediated interactions between anthelminthic and antiretroviral drugs across the Caco-2 cell monolayers, *BMC Pharmacol. Toxicol.* **18** (2017) Article ID 20 (18 pages); https://doi.org/10.1186/s40360-017-0129-6
24. S. Songngam, M. Sukwattanasinitt, K. Siralertmukul and P. Sawasdee, A 5,7-dimethoxyflavone/hydroxypropyl-β-cyclodextrin inclusion complex with anti-butyrylcholinesterase activity, *AAPS PharmSciTech.* **15** (2014) 1189–1196; https://doi.org/10.1208/s12249-014-0157-0
25. R. Paberit, E. Rilby, J. Göhl, J. Swenson, Z. Refaa, P. Johansson and H. Jansson, Cycling stability of poly(ethylene glycol) of six molecular weights: Influence of thermal conditions for energy applications, *ACS Appl. Energy Mater.* **3** (2020) 10578–10589; https://doi.org/10.1021/acsaem.0c01621
26. T. Uemura, N. Yanai, S. Watanabe, H. Tanaka, R. Numaguchi, M. T. Miyahara, Y. Ohta, M. Nagaoka and S. Kitagawa, Unveiling thermal transitions of polymers in subnanometre pores, *Nat. Commun.* **1** (2010) Article ID 83 (8 pages); https://doi.org/10.1038/ncomms1091
27. C. Belingheri, E. Curti, A. Ferrillo and E. Vittadini, Evaluation of porous starch as a flavour carrier, *Food Funct.* **3** (2012) 255–261; https://doi.org/10.1039/C1FO10184F
28. H. J. Oh, B. D. Freeman, J. E. McGrath, C. H. Lee and D. R. Paul, Thermal analysis of disulfonated poly(arylene ether sulfone) plasticized with poly(ethylene glycol) for membrane formation, *Polymer*, Special issue: *Porous Polymers* **55**(1) (2014) 235–247; https://doi.org/10.1016/j.polymer.2013.11.041
29. J. A. Manthey, Fourier transform infrared spectroscopic analysis of the polymethoxylated flavone content of orange oil residues, *J. Agric. Food Chem.* **54**(9) (2006) 3215–3218; https://doi.org/10.1021/jf053134a
30. I. M. Deygen and E. V. Kudryashova, New versatile approach for analysis of PEG content in conjugates and complexes with biomacromolecules based on FTIR spectroscopy, *Colloids Surfaces B: Biointerfaces* **141** (2016) 36–43; https://doi.org/10.1016/j.colsurfb.2016.01.030
31. K. S. Suganthi, N. Anusha and K. S. Rajan, Low viscous ZnO-propylene glycol nanofluid: a potential coolant candidate, *J. Nanopart. Res.* **15** (2013) Article ID 1986; https://doi.org/10.1007/s11051-013-1986-6
32. K. Adhikari, W. Buatong, E. Thawithong, T. Suwandecha and T. Srichana, Factors affecting enhanced permeation of amphotericin B across cell membranes and safety of formulation, *AAPS PharmSciTech.* **17** (2016) 820–828; https://doi.org/10.1208/s12249-015-0406-x
33. M. Z. Jora, M. V. C. Cardoso and E. Sabadini, Dynamical aspects of water-poly(ethylene glycol) solutions studied by ^{1}H NMR, *J. Mol. Liquids* **222** (2016) 94–100; https://doi.org/10.1016/j.molliq.2016.06.101

34. L. L. Tshweu, M. A. Shemis, A. Abdelghany, A. Gouda, L. A. Pilcher, N. R. S. Sibuyi, M. Meyer, A. Dube and M. O. Balogun, Synthesis, physicochemical characterization, toxicity and efficacy of a PEG conjugate and a hybrid PEG conjugate nanoparticle formulation of the antibiotic moxifloxacin, *RSC Adv.* **34** (2020) 19770–19780; https://doi.org/10.1039/C9RA10872F
35. J. Fattori, F. H. S. Rodrigues, J. G. M. Pontes, A. Paula Espíndola and L. Tasic, *Chapter 6 – Monitoring Intermolecular and Intramolecular Interactions by NMR Spectroscopy*, in *Applications of NMR Spectroscopy* (Eds. A. ur-Rahman and M. I. Choudhary), Vol. 3, Bentham Science Publishers, Karachi 2015, pp. 180–266; https://doi.org/10.1016/B978-1-68108-063-5.50006-0
36. G. Akerlof, Dielectric constants of some organic solvent-water mixtures at various temperatures, *J. Am. Chem. Soc.* **54**(11) (1932) 4125–4139; https://doi.org/10.1021/ja01350a001
37. M.-J. Ko, H.-H. Nam and M.-S. Chung, Subcritical water extraction of bioactive compounds from Orostachys japonicus A. Berger (Crassulaceae), *Sci. Rep.* **10** (2020) Article ID 10890 (10 pages); https://doi.org/10.1038/s41598-020-67508-2
38. M.-J. Ko, C.-I. Cheigh and M.-S. Chung, Relationship analysis between flavonoids structure and subcritical water extraction (SWE), *Food Chem.* **143** (2014) 147–155; https://doi.org/10.1016/j.foodchem.2013.07.104
39. Z. Zhou, Y. Qu, J. Wang, S. Wang, J. Liu and M. Wu, Measurement and correlation of solubilities of (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid in different pure solvents and binary mixtures of water + (ethanol, methanol, or glycol), *J. Chem. Eng. Data* **56**(4) (2011) 1622–1628; https://doi.org/10.1021/je101351w
40. C. MacDonald, W. Lyzenga, D. Shao and R. U. Agu, Water-soluble organic solubilizers for in vitro drug delivery studies with respiratory epithelial cells: selection based on various toxicity indicators, *Drug Deliv.* **17** (2010) 434–442; https://doi.org/10.3109/10717541003777548
41. L. Le Hegarat, S. Huet, E. Pasquier and S. Charles, Impact of solvents on the in vitro genotoxicity of TMPTA in human HepG2 cells, *Toxicol. In Vitro* **69** (2020) Article ID 105003; https://doi.org/10.1016/j.tiv.2020.105003
42. H. Li, X. Zhang and W. Wang, Antiancer activity of 5,7-dimethoxyflavone against liver cancer cell line HEPG2 involves apoptosis, ROS generation and cell cycle arrest, *Afr. J. Tradit. Complement Altern. Med.* **14**(4) (2017) 213–220; https://doi.org/10.21010/ajtcam.v14i4.24
43. Y. Miyata, J. Tatsuzaki, J. Yang and H. Kosano, Potential therapeutic agents, polymethoxylated flavones isolated from *Kaempferia parviflora* for cataract prevention through inhibition of matrix metalloproteinase-9 in lens epithelial cells, *Biol. Pharm. Bull.* **42**(10) (2019) 1658–1664; https://doi.org/10.1248/bpb.b19-00244
44. P. Tep-areenan, P. Sawasdee and M. Randall, Possible mechanisms of vasorelaxation for 5,7-dimethoxyflavone from *Kaempferia parviflora* in the rat aorta, *Phytother. Res.* **24**(10) (2010) 1520–1525; https://doi.org/https://doi.org/10.1002/ptr.3164
45. H. F. Smyth, C. P. Carpenter and C. S. Weil, The chronic oral toxicology of the polyethylene glycols, *J. Am. Pharm. Assoc.* **44**(1) (1955) 27–30; https://doi.org/10.1002/jps.3030440111
46. J. D. R. Schulze, W. A. Waddington, P. J. Eli, G. E. Parsons, M. D. Coffin and A. W. Basit, Concentration-dependent effects of polyethylene glycol 400 on gastrointestinal transit and drug absorption, *Pharm. Res.* **20** (2003) 1984–1988; https://doi.org/10.1023/b:pham.0000008046.64409.bd
47. G. L. Amidon, H. Lennernäs, V. P. Shah and J. R. Crison, A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability, *Pharm. Res.* **12** (1995) 413–420; https://doi.org/10.1023/A:1016212804288
48. D. A. Volpe, P. J. Faustino, A. B. Ciavarella, E. B. Asafu-Adjaye, C. D. Ellison, L. X. Yu and A. S. Hussain, Classification of drug permeability with a Caco-2 cell monolayer assay, *Clin. Res. Regul. Affairs* **24**(1) (2007) 39–47; https://doi.org/10.1080/10601330701273669
49. X.-L. Liang, J. Zhang, G.-W. Zhao, Z. Li, Y. Luo, Z.-G. Liao and D.-M. Yan, Mechanisms of improvement of intestinal transport of baicalin and puerarin by extracts of Radix *Angelicae dahuricae*, *Phytother. Res.* **29**(2) (2015) 220–227; https://doi.org/10.1002/ptr.5242