SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of biocompatibility of polyethylene glycol derivatives and preparation of solid dispersion containing ketoprofen

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1. Introduction

Polyethylene glycols (PEGs) are widely used polymer that have a variety of applications in many fields, including biomedical, chemical, and industrial processes. It has many applications in various industries, including pharmaceuticals, cosmetics, and food. These compounds are important group of excipients due to their widespread use in different pharmaceutical formulations, especially is a favorable choice to reach the optimal drug delivery system. Polyethylene glycols (PEGs) possess a range of physicochemical properties that make it an invaluable polymer in pharmaceutical sciences. Its solubility, viscosity, molecular weight, thermal behavior, and interactions with biological systems play pivotal roles in drug delivery system, formulation design, and therapeutic applications. PEGs have a favorable safety profile. They are ideal excipients due to their excellent features such as inert, non-immunogenicity, hydrophilicity, high biocompatibility making them ideal for pharmaceutical formulations. Its ability to increase solubility and stability of drugs makes it a valuable tool in pharmaceutical and biomedical research. For PEG to be used to theirs full potential in the creation of novel drug delivery systems, a thorough understanding of these properties is essential.

In recent years, the quest for biocompatible materials has become paramount in biomedical research and applications. Every stage of the drug formulation process must take the drug safety profile into consideration. In order to ensure the safe and efficient interaction of biomedical materials with living systems, *biocompatibility is a crucial component* in their development. The aim of biocompatibility testing is to make sure that a substance or device will offer the patient the greatest benefit with the least amount of risk**.** The evaluation of a biomaterial's biocompatibility at the cellular and tissue levels is one of the primary requirements for its suitability for clinical use. Cytotoxicity testing, which illustrates how cultured cells are exposed directly to the test material or to its extract, is the fundamental and required component of biocompatibility evaluation at early stages of drug developmen**t.** The cytotoxicity assays are a low-cost and quick in vitro techniques that assess the impact of drugs on cultured cells. This assay is applied to evaluate and characterize the potentially harmful/toxic of the tested compound to the cells. Based on the concept that living cells have quantifiable intrinsic metabolic activities, cytotoxicity assays are designed to measure the toxicity of a sample. The reduction in cell viability due to drug exposure can be measured through various endpoints, including cell proliferation, membrane integrity, enzymatic activity, and mitochondrial function.

Ketoprofen, which has chemical name that is 2-(3-benzoylphenyl)-propionic acid, belongs to a propionic acid group of a non-steroidal anti-inflammatory drug (NSAID). By blocking the production of prostaglandins, ketoprofen helps to reduce pain, swelling, fever and inflammation. This medication is commonly used to relieve symptoms associated with various conditions, including rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout, menstrual cramps, and musculoskeletal injuries such as sprains and strains. It is available in various formulations, including oral tablets, capsules, extended-release capsules, and topical gels. The solubility of ketoprofen is an important characteristic that affects its dissolution rate, absorption, and bioavailability. The solubility of ketoprofen in water is 51 mg/L at 22^{o}C which means that ketoprofen is practically insoluble in water. This solubility profile is critical for formulation development, as it affects the choice of appropriate solvents, co-solvents, and surfactants to enhance drug solubility and bioavailability.

As solubility and permeability play a critical role in determining the bioavailability of the drug, The Biopharmaceutics Drug Classification System (BCS) is a system that categorizes a drug (API) based on aqueous solubility and permeability across intestinal membrane properties which is classified in to four classes which are class I: high solubility (S), high permeability (P), class II: low S, high P, Class II: high S, low P, class IV: low S, low P. More than 70% of medications are reported to be poorly soluble and belong to BCS classes II and IV. It is estimated that most compounds undergoing development at the present time are subjected to poor bioavailability of these compounds. The solubility is an important factor for drug release which is an essential and limiting step for oral drug bioavailability. The poor solubility and low dissolution rate of poorly water-soluble drugs in the gastro intestinal fluid often lead to an insufficient efficacy. The solubilization behavior of the drug is the key determinant for the oral bioavailability determination The low solubility of the drugs which leads to low dissolution rate is the biggest obstacle in the performance of poorly water-soluble (PWS) drugs. . The creation of efficient drug formulations presents many difficulties in the field of pharmaceutical sciences, especially when dealing with poorly soluble drugs. Poor solubility and limited permeability significantly hinder the successful delivery of therapeutic compounds. Therefore, improving the solubility and permeability of poorly soluble/permeable drugs is necessary to increase the drug bioavailability, thereby enhancing therapeutic efficacy, and optimizing the pharmacokinetic parameter of pharmaceutical products for better patient outcomes. To solve this problem, various strategies for solubilization enhancement have been developed such as nanoparticle, inclusion complex, lipid formulation, salt formation and solid dispersion. Among

these strategies, solid dispersion (SD) is a well-known method for improvement in the solubility, dissolution rate and bioavailability of poorly water-soluble drugs. Solid dispersion is defined as dispersion of one or more active ingredients (hydrophobic) in an inert carrier matrix (hydrophilic) at in the solid state**.** The characteristics of the API, the carriers or the excipient should be evaluated carefully before selecting appropriate method to prepare the SD because each method have its own advantages and disadvantages. Solid dispersion shows many mechanisms to become one of the promising techniques for the low solubility drugs. The underlying mechanisms for improvement of the bioavailability of poorly water soluble (PWS) drug by SD technique are particle size reduction, particles with high porosity, wettability and dispersibility improvement, drug in amorphous state. Based on the points mentioned above, solid dispersion techniques have the potential to significantly increase the bioavailability and dissolution rate of drugs with poor water solubility, such as NSAID.

2. Aims

General objective of my thesis was to investigate various PEGs derivatives and their characteristics and followed by the preparation of ketoprofen-containing solid dispersion using selected PEG derivatives.As most studies involved only a limited number of derivatives and I wished to better understand the cytotoxicity of these compounds, eleven substances of various molecular weights on a much wider scale: PEG 200, PEG 300, PEG 400, PEG 600, PEG 1000, PEG 1500, PEG 4000, PEG 8000, PEG 10000, PEG 12000, PEG 20000 were examined. Also, I would like to check how the change of PEGs' molecular weight affects on the properties of PEGs and their SDs.

In the first part of my thesis is to examine the toxicity of various PEG derivatives based on cellular effects (cytotoxicity and autophagy) and *in vivo* toxicity which is a necessary step before investigating the solid dispersion containing PEG derivatives and poorly soluble API.

Because PEGs are a common excipient used to increase the solubility of active pharmaceutical ingredients, I looked for an API that is not well soluble. The solubility of ketoprofen was improved by solid dispersion preparation using PEG derivatives that I had previously studied for the first section of my thesis.

In the second part of the thesis, the aim is to formulate binary ketoprofen - PEG hot melt homogenization solid dispersions with low molecular weight polymers which were PEG 1000, 1500 and 2000. The physicochemical characteristics and the dissolution profiles of theses solid dispersions were examined. The relationship between low molecular weight PEG derivates and ketoprofen-SD characterization were also investigated regarding the effects of molecular weight of PEGs.

3. Materials and methods

3.1. Materials

A total of 12 polyethylene glycols derivatives were chosen to be investigated based on their average molecular weight. Range of molecular weight provided by the manufacturer is also indicated. All of PEGs are listed below:

- PEG 200 (190-210 MW), PEG 300 (290-305 MW) and PEG 600 (550-650 MW) were obtained from TCI (Zwijndrecht, Belgium).
- PEG 1000 (950-1050 MW), PEG 8000 (7000-9000 MW), PEG 10000 (9000-11250 MW), PEG 12000 (11000-13000 MW) and PEG 20000 (16000-24000 MW) were purchased from Alfa Aesar (Karlsruhe, Germany)
- PEG 2000 (1900-2200 MW) were originated from Merck Lifesciences Budapest (Hungary) and
- PEG 400 (380-420 MW), PEG 1500 (1400-1600 MW), PEG 4000 (3500-4500 MW) and sorbitol were obtained from Molar Chemicals (Halásztelek, Hungary).

Ketoprofen to formulate solid dispersion with 3 chosen PEGs was purchased from TCI (Zwijndrecht, Belgium).

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium chloride, Dulbecco's Modified Eagle's Medium with high glucose and L-glutamin (DMEM), phosphate-buffered saline (PBS), trypsin from porcine, EDTA, heat-inactivated foetal bovine serum (FBS) and propidium iodide solution were purchased from Sigma-Aldrich (Budapest, Hungary). Non-essential amino acids solution and penicillin-streptomycin mix were obtained from Lonza (Basel, Switzerland). GlutaMax™ supplement and Annexin V Alexa Fluor™ 647 conjugate were purchased from Thermo Fisher (Budapest, Hungary). Neutral Red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) was obtained from Alfa Aesar (Karlsruhe, Germany). CYTO-ID Autophagy Detection Kit was purchased from Enzo Life Sciences (Farmingdale, NY, USA).

3.2. Methods

3.2.1. Cell culture

The Caco-2 cell line was received from the European Collection of Cell Cultures ((ECACC), Salisbury, United Kingdom (catalogue No. 8601020). Cells were grown in plastic Nunc™ EasyFlask™ cell culture flasks (Thermo Fisher, Darmstadt, Germany) in Dulbecco's Modified Eagle's Medium, supplemented with 3.7 g/L $NaHCO_3$, 10% ($^{v}/_{v}$) FBS, 1% ($^{v}/_{v}$) non-essential amino acids solution, 0.584 g/L L-glutamine, 4.5 g/L D-glucose, 100 IU/mL penicillin, and 100 µg/mL streptomycin at 37 °C in an atmosphere of 5% CO_2. Passaging was routinely done for cell maintenance and glutamine was supplemented by GlutaMax™. For the experiments, cells were between passage numbers 25 and 40.

3.2.2. Cytotoxicity tests:

The cytotoxic effects of all PEG and sorbitol solutions were evaluated by using the MTT and Neutral Red (NR) methods. Caco-2 cells with media were seeded on 96-wells plates at a density of 10.000 cells per well. After 7 days, the medium was removed, and the cells were treated with 100 µl of the test solutions for 30 minutes at 37°C. Concentrations of the test solutions were 30 $^{w}/_{v}$% for all the PEG solutions and 7-10-12.5-35 $^{w}/_{v}$% for the sorbitol solutions. During the preliminary studies, lower concentrations of PEG solutions were tested also, but cytotoxicity did not exceed 20%. In case of MTT assay, the test solution was taken out and a 0.5 mg/mL MTT solution (MTT dissolved in PBS) was added to each well. The plate was again incubated for 3 hours at 37°C. In case of Neutral Red assay, a 16.6 mg/mL NR solution (NR dissolved in cell culture medium) was added to each well after removing all test solutions. The plate was incubated for 2 hours in this case. After incubation time, all dyes were completely removed and 0.1 mL of an isopropanol – 1 M hydrochloride acid (25:1) solution was added to each well to dissolve the cells and solubilize the formazan crystals and the incorporated Neutral Red. The absorbance of the wells was measured at 565 nm for MTT assay and 540 nm for NR assay. We used empty wells of the plate as a reference and all the measurements were carried out with a Thermo-Fisher Multiskan Go (Thermo-Fisher, USA) microplate reader. Cell viability was expressed as a percent of the cell viability of the untreated control cells, which were incubated with PBS for 30 minutes.

3.2.3. Osmolality measurement

The OSMOMAT 070 vapor pressure osmometer (Gonotec GmbH, Berlin, Germany) is suitable for directly determining the total osmolality of aqueous solutions. The measurement temperature was 45°C and the sampling time was 5 minutes. The solvent chosen was ultrapure (Type 1) water obtained from a Millipore Direct-Q 5 UV system (Millipore SAS, Molsheim, France). Before each experiment, the baseline was determined by ultrapure water. After the baseline was stable, the calibration was carried out with a 1 $^{w/}_{v}$% sodium chloride solution. After the cell constant was calculated and the system calibrated with it, the PEG and sorbitol samples with increasing concentration were measured sequentially. All liquids were dropped upon the sensors twice and the second drop was used for measurement. The concentrations of the test solutions were 30 $^{w/}_{v}$% for all the PEG solutions and 7-10-12.5-35 $^{w/}_{v}$% for the sorbitol solutions. The osmolality of the samples was expressed in mOsmol/kg, as an average of 4 individual measurements.

3.2.4. Flow cytometry analysis

For the flow cytometry measurements, a Guava® easyCyte™ 5HT (Austin, TX, USA) flow cytometer was used. 3 × 6.5 million Caco-2 cells were harvested from cell culture flasks with trypsin-EDTA solution, and 1-1 million cells were treated with 1-1 ml of PEG test solutions dissolved in PBS. The concentration of the test solutions was 30 $^{w/}_{v}$% for all the PEG solutions. After 30 min, the cells were centrifuged, the test solutions were removed, and the cells were gently washed with cold PBS and centrifuged again. Supernatant was removed and with annexin-binding puffer a 1 million cells/mL cell suspension was created. 100 µl of this suspension was treated with 5 µl of Alexa Fluor™ 647 and 1 µl of 100 µg/mL propidium iodide solution. The cell suspension was stained for 15 min on ice then immediately analyzed with the flow cytometer. The propidium iodide was excited with a 488 nm laser and detected at the 525/30 nm channel (green parameter). The Alexa Fluor™ 647 was excited with the same laser line and detected at the 695/50 nm channel (red parameter). On the FSC-SSC scatterplot the non-cellular events were excluded. On the FSC-A-FSC-W scatterplot the duplets were excluded. The remaining events (8000–10.000) were analyzed on a propidium iodide-Alexa Fluor 647 scatterplot, the quadrant gates were determined on non-labelled samples. The double positive cells are regarded as necrotic/late apoptotic cells. The annexin V positive population was regarded as early apoptotic, the double negative population regarded as viable cells.

3.2.5. Autophagy Assay

For the quantitative analysis of autophagy, we used the CYTO-ID Autophagy Detection Kit (Enzo Life Sciences, Farmingdale, NY, USA) which is based on the staining autophagosomes. Caco-2 cells were seeded into black 96-well plates at the density of 10.000 cells per well. After four days, when cells reached the appropriate confluence, they were incubated for 30 minutes with 100 µl of the PEG and sorbitol test solutions at 37 °C. Concentrations of the test solutions were 30 $^{w/}_{v}$% for all the PEG solutions and 7-10-12.5-35 $^{w/}_{v}$% for the sorbitol solutions. After this, cells were washed once with PBS and were treated according to the Kit specification. Cells were incubated with a solution which contains 1 µL CYTO-ID® Green Detection Reagent and 1 µL Hoechst 33,342 Nuclear Stain in 1 mL buffer for 30 min at 37 °C. After incubation time, cells were washed once with PBS. Green fluorescence intensities of the samples were measured with FLUOstar Optima microplate reader (BMG Labtech, Offenburg, Germany) at 485 nm excitation and 520 nm emission wavelengths. Hoechst 33,342 Nuclear Stain was measured at 365 nm excitation and 445 emission wavelengths. According to the instructions of the kit, green fluorescence values were normalized to the blue fluorescence values.

3.2.6. G. mellonella larvae survivability tests

Larvae of the sixth developmental stage of *G. mellonella* were obtained from Bugs World Inc. (Budapest, Hungary). Larvae were stored at 10 °C and in a dark environment prior to use. Larvae size was between 2 and 3 cm and they showed no sign of melanisation. For each treatment, 10 healthy larvae were placed in sterile vented Petri dishes. The test solutions were injected into the *G. mellonella* haemocoel through the last pro-leg using a 29 G needle in the volume of 20 µl. Concentration of the test solutions was 30 $^{w/}_{v}$% for all the PEG solutions. The injected larvae were incubated at 30 °C for 48 h in dark environment. For the assessment of larval viability, larvae were gently probed with a blunt-ended needle and if no response was observed, the larvae were considered to be dead. Viability was observed at 19 h, 24 h, 43 h, and 48 h.

3.2.7. Statistical analysis

All data were analysed using GraphPad Prism (version 8; GraphPad Software, San Diego, California, USA). All data were presented as means ± SEM. In case of MTT assay and NR assay, each cell viability value represents the mean of ten independent, parallel wells. PEGs

and sorbitol treated cells' absorbance values were compared with their given control group as they were measured on multiple microplates. All data groups were analysed with Shapiro–Wilk test for Gaussian distribution and Bartlett's test for equal variances. If the data set passed both tests, a one-way ANOVA was calculated, if Bartlett's test failed, a Welsch's ANOVA was calculated and if Gaussian distribution was not proved, then Kruskal–Wallis test was carried out. As a post test, Dunnett's test was used to compare the treated cells' results to the controls. In each case we used significance level $p < 0.05$. Significance is labelled as ns = $p \geq 0.05$; * = $p < 0.05$; ** =$p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$. *In vivo* survival curves of *G. mellonella* larvae were plotted according to the Kaplan-Meier analysis, the survival curves were compared with Mantel-Cox log-rank test and Gehan–Breslow–Wilcoxon test. Osmolality, autophagy and flow cytometry results were not analysed because of low number of parallel experiments (n=4, n=4, n=3). Due to this, we used Spearman correlation to calculate the relationship between molecular weight and other measured data ($p < 0.05$, two-tailed).

3.2.8. Phase solubility

The phase-solubility test was performed by adding a fixed excess amount of ketoprofen powder to 1 mL solutions containing the different molecular weight PEGs at increasing concentrations (1 – 20 $^{w}/_{v}$ %). The solvent was hydrochloric acid media, set to pH 1.2 without pepsin (simulated gastric fluid without enzyme). The vials were vortexed for 30 seconds to achieve well-mixed dispersions. They were rotated for 24 hours at room temperature at 50 rpm while being protected from light. Then each vial was centrifuged at 4500 rpm for 20 min. The samples were taken from the clear supernatant, and after filtering, the ketoprofen content of the samples was analyzed by a UV spectrophotometer (Shimadzu UV-1900) at 258 nm. The phase solubility profiles were plotted as the solubility of ketoprofen versus the w/w% concentration of the given PEG by GraphPad Prism (version 9; GrapPad Software, San Diego, California, USA).

The Gibbs free energy of transfer (ΔG_{tr}^{0}) of ketoprofen from pure water to the aqueous solutions of the PEGs was calculated as the following:

$$\Delta G_{tr}^{0} = -2.303*R*T*\log (Ss/S_0)$$

where Ss/S_0 is the ratio of molar solubility of ketoprofen in water (0.41 μg/mL according to our measurements) to that in the aqueous solutions of the PEGs. The apparent stability constant (*Ka*) was determined as:

$$Ka = Slope/(Intercept*(1-Slope))$$

where slope and intercept were obtained from the plotted curve.

3.2.9. Preparation of solid dispersions and physical mixtures

The PEG and the ketoprofen were measured in a ratio of 9:1. We chose this ratio according to literature results, because in this concentration, the properties of the polymer are the dominant factors of dissolution, not that of the API.

3.2.10. Fourier-transform infrared spectroscopy (FTIR)

The infrared spectra of the pure PEGs, physical mixtures, and solid dispersions were obtained by using a JASCO FT/IR-4600 type (ABL&E-JASCO, Hungary) apparatus coupled with a Zn/Se ATR PRO ONE Single-Reflection ATR accessory. Each sample was directly placed on the cleaned crystal of the equipment; the scanning was running in the wavelength range of 500–4000 cm^{-1} at the resolution of 1 cm^{-1} 24 times to obtain smooth spectrum. Corrections of environmental CO_2 and H_2O used the software's built-in method. Spectra were evaluated with SpectraGryph 1.2.16d software (Software for optical spectroscopy by Dr. Friedrich Menges, Oberstdorf, Germany, http://spectroscopy.ninja (accessed on 15 January 2021))

3.2.11. Powder X-ray Diffraction (PXRD)

The finely powdered samples were fixed onto a Mitegen MicroMeshes sample holder (MiTeGen Co., Ithaca, NY, USA) with a minimal amount of oil. Powder diffraction data of the samples with Debye Scherer geometry were collected using a Bruker-D8 Venture (Bruker AXS. GmbH, Karlsruhe, Germany) diffractometer equipped with INCOATEC IμS 3.0 dual (Cu and Mo) sealed tube micro sources (50 kV, 1.4 mA). A Photon 200 Charge-integrating Pixel Array detector and CuKα (λ = 1.54178 Å) radiation was applied. Several frames were collected with various detector-sample distances in phi scan mode. Data collection and integration was performed using the APEX3 and DiffracEva software (Bruker AXS Inc., Madison, WI, USA, Version 4.2.2.3), respectively.

3.2.12. Scanning Electron Microscopy (SEM)

Surface area exploration used a Hitachi Tabletop microscope (TM3030 Plus, Hitachi High-Technologies Corporation, Tokyo, Japan) in high-resolution mode. The samples were attached to a fixture with a double-sided adhesive tape containing graphite. Before SEM

examination gold-sputtered coating was not deposited on the surface of the samples, as the instrument is suitable for the direct investigation of the specimens without any surface pre-treatments. The measurement requires a vacuum and low, 5kV accelerating voltage. The magnifications were 1000×.

3.2.13. In Vitro Dissolution Test

During the experiment, three parallel measurements were performed with size 0 capsules filled with 100 mg of the samples, meaning 90 mg of the given PEG and 10 mg of ketoprofen in form of either a solid dispersion or a physical mixture. The dissolution media was 450 mL of hydrochloric acid media (same as the media of the phase solubility study). The volume was set as the half of the maximum recommended USP volume to stay within range of the limit of UV spectrophotometric detection of the ketoprofen. USP 2 rotating paddle method was with the rotation speed of 75 rpm and at 37 °C in an Erweka DT 128 light dissolution tester (Erweka GmbH, Langen, Germany). Samples of 1 mL were withdrawn after 5, 10, 15, 20, 30, 45 and 60 minutes and filtered through a 0.2 µm polyethersulfone membrane filter and measured as in case of the phase solubility study. The graphs were plotted by GraphPad Prism 9, all calculations were executed in Microsoft Excel.

In order to compare the dissolution profiles of the different formulations similarity (f1) and difference factors (f2) were calculated, as a model independent approach. [148] The exact calculations were the following:

$$\mathrm{f1} = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100$$

where f1 is the difference factor (relative errors between the two curves), n is the sampling number, R_j and T_j are the percent of the dissolved API from the two compared preparations at time point j.

$$f2 = 50 \times \log\left\{\left[1 + (1/n)\sum_{j=1}^{n} w_j |R_j - T_j|^2\right]^{-0,5} \times 100\right\}$$

where f2 is the similarity factor (the logarithmic reciprocal square root transformation of the sum of squared error is a similarity factor which provides percentage dissolution between the two curves) and w_j is an optional weight factor which was 1 our experiment.

For the fitting of dissolution profiles on different dissolution models, the following calculations of zero-order, first-order, Kormeyer-Peppas model were used

4. Results I.

In this section, I conducted experiments to explore the impact of PEGs on both Caco-2 cells and G. mellonella larvae. The objective was to establish correlations between these effects and the molecular weight and osmolality of the PEGs. Eleven PEGs with varying molecular weights (PEG 200, PEG 300, PEG 400, PEG 600, PEG 1000, PEG 1500, PEG 4000, PEG 8000, PEG 10,000, PEG 12,000, and PEG 20,000) was employed in these experiments. The examined cellular effects encompassed cytotoxicity, assessed through MTT and Neutral Red assays, as well as flow cytometry utilizing propidium iodide and annexin V, along with autophagy. The osmolality of PEG solutions at various concentrations was measured using the vapor pressure osmometer OSMOMAT 070, and G. mellonella larvae were injected with the PEG solutions, with sorbitol serving as controls to maintain equivalent osmolality.

4.1. Osmolality results

Initially, the osmolality of PEGs was standardized at 30 $^{w/}_{v}$ %. Our preliminary findings indicate minimal cytotoxicity of PEGs, as cell viability consistently exceeded 80% for all derivatives at concentrations below 30 $^{w/}_{v}$%. To comprehensively assess the impact of all derivatives on both cells and in vivo specimens, a further escalation in concentration was deemed necessary. Additionally, we employed sorbitol solutions to differentiate between biological effects linked to osmolality and those unrelated to it. The result illustrates a noteworthy decline in osmolality from PEG 200 to PEG 4000, with marginal increases observed until PEG 10000, 12000, and 20000, which exhibited nearly identical values.

4.2. Cytotoxicity assay results

Sorbitol solutions did not exert a notable influence on the cell viability of treated Caco-2 cells, as evidenced by the MTT assay. Conversely, low molecular weight PEGs, such as PEG 200, 300, and 400, exhibited a significant reduction in the number of surviving cells. The remaining derivatives had a comparatively milder effect on cell viability, with none of them yielding values higher than 85%. It is crucial to highlight the substantial variation in results across different molecular weights.

The results from the Neutral Red assay display similar trends to those observed in the MTT assay. The sorbitol solutions did not exhibit any cytotoxic effects. Low molecular weight PEGs had a more pronounced impact on cell viability, with a narrower difference between high

and low molecular weight derivatives. Nevertheless, notable variation was evident in the results for high molecular weight derivatives.

4.3. Autophagy assay results

The results indicate that nearly all treatments led to an increase in the number of autophagosomes. Notably, there was a decreasing trend in fluorescence intensity from PEG 200 to PEG 1500, which then changed and started to increase again. The sorbitol solutions produced similar results to their PEG counterparts, except for 12.5 $^{w}/_{v}$%, , which yielded higher results than PEG 1000. It is worth mentioning that PEG 200 exhibited significantly higher intensity than any other treatment, while the remaining chemicals produced results in the range of approximately 180% to 100%.

4.4. Flow cytometry results

The sorbitol solutions were excluded from further experiments due to their minimal impact on cells. In the flow cytometry analysis, Caco-2 cells were stained with propidium iodide and annexin V labeling to distinguish between necrotic and early apoptotic cells. The result depicts the distribution of gated cells, revealing that the proportion of unstained, living cells (PI- AV-) increases with molecular weight. Remarkably, for low molecular weight PEGs, necrotic cells (PI+ AV+) predominate among the deceased cells. However, with increasing molecular weight, the ratio of early apoptotic cells (PI- AV+) also rises.

4.5. In vivo toxicity test

Over the course of the two-day experiment, G. mellonella larvae received 20 µl injections of PEG solutions, and their viability was assessed four times. Each group consisted of 10 healthy larvae. The analysis presented unveiled notable mortality rates for low molecular weight PEGs 200 and 300, as well as medium molecular weight PEGs 4000 and 6000. Importantly, no other PEG exhibited a substantial impact on larval survival.

4.6. Correlation of results with molecular weight

We employed Spearman correlation analysis to establish the statistical relationship between the measured data and the average molecular weight of PEGs. Interestingly, as the molecular weight of PEGs increased, we observed a significant decrease in osmolality, the proportion of necrotic cells (PI+ AV+ cells according to flow cytometry), and total larvae

mortality. Furthermore, the ratio of living cells, as determined by both cytotoxicity assays and flow cytometry, increased with the average molecular weight of PEGs. However, we found no statistical relationship between autophagy and the proportion of apoptotic cells with the chemical structure of PEGs

The correlation matrix was calculated to analyze the relationships between the different data sets. It was observed that autophagy showed no statistical relationship overall. However, the proportion of early apoptotic cells exhibited a negative correlation with larvae mortality and a positive correlation with the NR assay. Additionally, the various cell viability measurements, including MTT, NR assays, and the proportion of necrotic and living cells according to flow cytometry, displayed significant correlations with each other and with osmolality. The last data set, the total larvae mortality was only linked to the flow cytometry results. Notably, the most significant correlation was found between the distribution of necrotic and living cells in the flow cytometry analysis.

5. Results II.

In the second segment of my thesis, I developed solid dispersions using low molecular weight PEGs (PEG 1000, PEG 1500, PEG 2000) and the active pharmaceutical ingredient (API) ketoprofen through the hot melting method. A phase solubility study was conducted in hydrochloric acid media, and the physicochemical properties of the solid dispersions prepared with hot melt homogenization, along with their respective physical mixtures, were examined using Fourier transform infrared spectroscopy, powder X-ray diffraction, and scanning electron microscopy techniques. The primary goal was to investigate the relationship between selected PEGs and ketoprofen, with a specific focus on the impact of molecular weight.

5.1 Phase solubility

Our research has shown that the solubility of ketoprofen in simulated gastric juice was 41 μg/mL. 1 $^{w/}_{v}$%, PEG had minimal effect on the solubility of the API. However, as the concentration increased, a linear correlation was observed up to 10 $^{w/}_{v}$%, with a significant increase at 20 $^{w/}_{v}$%, concentration. No significant differences were observed among different molecular weight PEGs regarding their ability to increase solubility. The apparent stability constants (Ka) of ketoprofen for PEG 1000, PEG 1500, and PEG 2000 were calculated to be 3.317*10-5, 3.346*10-5, and 3.569*10-5, respectively.

5.2. Fourier-transform infrared spectroscopy (FT-IR)

Ketoprofen was prepared for further experimentation in two different forms: solid dispersion by hot melt homogenization (SD) and gently mixed powders (physical mixture, PM) of ketoprofen and PEGs. FTIR spectroscopy was employed to scrutinize potential interactions between ketoprofen and PEGs in these formulations. Remarkably, in the 1650-1700 cm^{-1} range, ketoprofen exhibited characteristic peaks absent in the PEGs. For PEG 1000, the spectra of the solid dispersion and physical mixture were nearly identical. In contrast, for PEG 1500 and 2000, the characteristic ketoprofen peaks were discernible in the physical mixture within the same range as the pure API. However, in the solid dispersions, the formation of a new hydrogen bond caused a shift in the ketoprofen bands from 1693 cm^{-1} to 1732 cm^{-1}.

5.3. Powder X-ray Diffraction (PXRD)

The PXRD results confirm that for PEG 1500 and 2000, the physical mixture contains ketoprofen in a crystalline form. In contrast, in the case of solid dispersions, there are no indicative signals of a crystalline structure. This suggests that in the solid dispersions, the active ingredient primarily exists in an amorphous form, indicating that the API was dissolved in the PEG. In comparison to the FT-IR results, both the PEG 1000 PM and the PEG 1000 SD exhibit nearly identical characteristics.

5.4. Scanning Electron Microscopy (SEM)

The typical crystalline form of ketoprofen is visible with particle size below ~100 μm. In the case of the polymers, there is not any detectable crystalline form, they are amorphous particles. Upon magnification at 500x, the electron microscope images confirm that the crystals of the active ingredient can be found between the larger particles of the PEG, whereas, in the case of solid dispersion (SD), a predominantly homogeneous, molten substance is observed. In physical mixtures, the crystals of ketoprofen are detectable, except PEG 1000, which again shows similarities to its respective solid dispersions. The lack of ketoprofen crystals indicates the successful formation of a solid dispersion. In the case of PEG 1000, the solid dispersion is achieved due to its low melting point, allowing ketoprofen to dissolved during the gentle homogenization process in the experiment.

5.5. In Vitro Dissolution Test

The dissolution test results reveal that the highest amount of dissolved drug was observed for PEG 1000, with the other two PEGs exhibiting mostly similar profiles. The difference and similarity factors for the solid dispersions is caculated. The compared curves are statistically similar when f1 values fall between 0 and 15 and f2 values exceed 50. The graph illustrates the similarity between PEG 1500 SD and 2000 SD. It also must be noted, in all cases, the concentration of the dissolved ketoprofen was multiple times the amount calculated from the phase solubility study results, indicating the formation of a supersaturated solution.

The majority of samples were best described by the Korsmeyer-Peppas equation, commonly used to characterize release from polymeric systems. Though, in specific instances, the zero-order model was the most suitable, particularly for matrix tablets containing low solubility API.

In various articles, multiple PEGs have been studied to understand how changes in their molecular weight affect specific characteristics. The relationship between molecular weight and these characteristics can be either proportional or inversely proportional, but some studies have failed to find any correlation. The upcoming chapters will detail my findings on the relationship between molecular weight and properties related to PEG (cellular effect and solid dispersion formulation).

6. Discussion I.

Polyethylene glycols, referred to as Macrogols in the European Pharmacopoeia, play a pivotal role as excipients in both oral and parenteral dosage forms. Their primary applications include constipation treatment and colonoscopy preparation. These polymers exhibit noteworthy characteristics, including chemical neutrality, hydrophilicity, and high to moderate solubility in aqueous and organic solvents. Such attributes make them widely employed for enhancing the pharmacological and biological properties of diverse pharmaceutical formulations. Additionally, PEGs are recognized for their contribution to the development of stealth coatings on nanoparticle surfaces, leveraging the interaction between opsonins and the PEGylated surface. While the physical properties and cellular effects of PEGs hold significance, most literature tends to focus on individual PEGs, with limited studies involving multiple derivatives. Our study aimed to delve into the cellular effects of PEGs spanning a broad range of molecular weights, seeking to establish correlations among different measured properties. Prior research has indicated that fundamental biological effects, such as the cytotoxicity of PEGs, are influenced by osmolality and can be predicted based on the molecular weight.

Our study sought to conduct a comprehensive exploration of the biological effects of PEGs. Initially, we measured osmolality and subsequently delved into cytotoxicity, autophagy, and the distribution of necrotic/apoptotic/living cells, along with in vivo toxicity assessment. To ascertain the osmolality of our solutions, we employed the OSMOMAT 070 osmometer, given its suitability for assessing osmolality or molecular weight through the vapor pressure method. In our in vitro cytotoxicity experiments, we utilized Caco-2 human colorectal adenocarcinoma cells. These cells were chosen for their role as a general model cell line and their ability to reflect the susceptibility of the gastrointestinal tract, given their morphological resemblance to the intestinal epithelium. Cytotoxicity evaluation was conducted through the commonly employed MTT and Neutral Red assays, both of which complement each other. The MTT assay detects cell viability through enzymatic conversion, while the Neutral Red assay measures cellular uptake and the incorporation of neutral red. The combination of these assays is often utilized in various studies to explore the cytotoxicity of different chemicals, benefiting from their distinct mechanisms of action. To delve deeper into the cytotoxic characteristics of PEGs, we utilized labeled annexin V and propidium iodide in conjunction with flow cytometry. This approach allowed us to distinguish between necrotic and early apoptotic cells. Additionally, we measured autophagosome formation through cellular organelle staining as a

marker of autophagy, an inducible mechanism of cytotoxicity. We complemented our cytotoxic experiments by employing the Galleria mellonella injection method, an emerging in vivo model organism that serves to assess the toxicity of different xenobiotics. This method has shown a robust correlation with cellular and other in vivo techniques in studies focusing on acute toxicity.

In the field of cytotoxicity, there has been limited research on the cytotoxic properties of PEGs across a wide range of molecular weights. Liu et al. conducted a study on a variety of PEG derivatives using the MTT assay, focusing on HeLa (human cervical cancer cells) and the L929 cell line (fibroblasts derived from mice). The PEG derivatives involved in the study included TEG (triethylene glycol), PEG oligomers (PEG 400, PEG 1000, PEG 2000, PEG 4000), and PEG-based monomers such as PEG methyl ether acrylate (mPEGA) and PEG methyl ether methacrylate (mPEGMA-500, mPEGMA-950). Previous research suggested that PEG 400 and PEG 2000 exhibited minimal cytotoxicity at a concentration of 5 mg/mL, while PEG 1000 and PEG 4000 displayed higher toxicity, particularly towards the L929 cell line. This implies that molecular weight and osmolality might not be directly correlated with cytotoxicity. However, our study contradicts this report, as we found that the cytotoxicity of PEG 400, PEG 1000, and PEG 4000 increased based on the MTT assay. Discrepancies between the studies may arise from differences in the MTT assay protocols. In our approach, cells were exposed to a higher concentration of PEGs for a shorter duration, emphasizing acute effects, whereas Lie et al. incubated cells for 24 hours, emphasizing the impact of PEGs on cell proliferation. Furthermore, Hodaei et al. reported that after 24 hours of incubation, PEG 4000, PEG 6000, PEG 10000, and PEG 35000 at 4 w/v% had no cytotoxic effect on Caco-2 cells. In contrast, our study revealed significant toxicity for PEG 400 and PEG 15000 at 4 w/v%, indicating a distinct pattern with acute, high-concentration treatment compared to long-term incubation. Postic et al. explored the impact of PEG 200, 2000, 20000, and poly(vinyl pyrrolidone) 8000 on various cellular parameters in metastatic melanoma A375, mouse fibroblast 3T3, and human corneal epithelial cells over a 72-hour incubation period. Their results indicated that higher concentrations of low molecular weight PEGs significantly altered the number and morphology of A375 cells compared to PVP 8000. The Resazurin assay revealed that PEG 200 was more cytotoxic to A375 and 3T3 cells than other PEGs, while PVP 8000 exhibited a unique dose-dependent killing action against the cells. The effects on human corneal epithelial cells varied among all three PEGs. Caspase 3/7 activation displayed a time-dependent pattern, showing no

correlation with molecular weight. In summary, the diverse cellular effects of PEGs are strongly influenced by incubation time and the specific cell types under investigation.

Collectively, the Spearman correlation analysis, unveiled significant positive correlations between the average molecular weight of the investigated PEGs and cell viability, as indicated by both MTT ($p=0.0055$) and NR assays ($p=0.0444$), along with the percentage of living cells according to flow cytometry ($p= 0.0018$). In contrast, a negative correlation was noted between molecular weight and osmolality ($p=0.0039$), as well as the proportion of necrotic cells ($p= 0.0001$). These findings suggest that lower molecular weight PEGs exhibit heightened cytotoxic activity, with this effect diminishing as the average PEG chain length increases. Additionally, the correlation matrix revealed associations between osmolality and MTT ($p<0.0001$), NR ($p=0.0018$), the proportion of living cells ($p=0.0182$), and necrotic cells ($p=0.0098$). These results reinforce the concept that the cytotoxicity of PEGs may be attributed to the severe osmotic shock induced during cell exposure to 30 $^{w}/_{v}$ % PEG solutions over a 30-minute incubation period. However, sorbitol solutions did not manifest cytotoxic effects in both the MTT and NR assays, despite comparable osmolality to PEGs. This suggests that osmolality alone might not be solely responsible for the observed decrease in cell viability. There may be an unexplored factor or cellular effect related to molecular weight contributing to the cytotoxicity of PEGs.

Wang et al. discovered that the cellular uptake mechanism of PEGs depended on their molecular weight. They found that low molecular weight PEGs (750-2000) were exclusively taken up through passive diffusion, while longer derivatives also utilized endocytosis as a significant mechanism. Additionally, the study highlighted that cellular uptake was influenced by factors such as incubation time and temperature. Notably, mesenchymal stem cells exhibited varying tolerance levels to sodium chloride, sorbitol, and PEG 3000, despite being applied at the same osmolality. Additionally, the type of osmolyte had a significant impact on various factors related to chondrogenesis, regardless of osmolality. This suggests that only low molecular weight PEGs could penetrate the cells within the brief incubation period, possibly explaining our results. Furthermore, the specific sensitivity of Caco-2 cells to PEGs and their tolerance to sorbitol could also contribute to these findings.

Unfortunately, there is a dearth of prior research on the influence of non-modified PEGs in G. mellonella larvae and their role in autophagosome formation. This absence of pre-existing studies poses a challenge in comparing our findings with previous literature. Nevertheless, the

well-established correlation between osmotic stress and the induction of autophagy has been extensively explored, revealing a substantial impact. Autophagosome formation did not show any correlation with molecular weight, suggesting that autophagy is generally a late-phase response to osmotic stress. The significant increase in autophagosome formation observed in this investigation can be attributed to the facile penetration of low molecular weight PEGs into the cells. Past research has demonstrated that a mere 30 minutes of hyperosmotic incubation can initiate the formation of proteasomes. It is essential to conduct additional research to identify the specific property of PEGs that exerts the most substantial influence on the process. Notably, unexpected cellular effects of PEGs have been documented, including the capacity of PEG 35 to influence the uptake of exosomes and diminish the levels of IL-1β. Unmodified PEGs had not been previously injected into G. mellonella for toxicity evaluation. Our findings showed that only molecular weight ($p=0.0403$) demonstrated a notable correlation with overall larvae mortality. Macromolecules are infrequently examined in this novel model organism; nevertheless, at elevated concentrations, they display toxicity that is dependent on concentration. Moreover, labeled annexin V served as an indicator for early-stage apoptosis, and our results indicated no correlation between the proportion of stained cells and osmolality. Literature suggests that the conjugation of PEG 12000 to interferon-α2b did not lead to a noteworthy increase in apoptotic activity, in contrast to observations with PEG 5000-coated silver nanoparticles compared to citrate-coated counterparts. Additional flow cytometry experiments are required to clarify the division of cells between necrotic and early apoptotic populations after PEG treatment. We hypothesize it is significantly influenced by the PEG concentration and the duration of incubation.

In summary, the analysis indicates a significant correlation between osmolality, cell viability (measured by various methods), and the molecular weight of PEGs. However, more intricate phenomena such as early-stage apoptosis, autophagosome formation, and in vivo toxicity (larvae survival) do not display a direct association with molecular weight.

When anticipating the biological effects of a previously untested PEG, only straightforward cellular interactions, such as (cyto)toxicity through necrosis, can be approximated based on prior experiments with PEGs of varying molecular weights. Further investigation is essential to unveil which properties of PEGs influence more complex mechanisms like autophagosome formation or apoptosis. Through thorough testing, the contributing factors can be identified, and statistical correlations can be established to elucidate the connection between biological effects and chemical or physical attributes.

Until additional research is conducted, it is prudent to approach the estimation of cellular actions of PEGs cautiously, particularly when comparing new derivatives to established ones based solely on molecular characteristics.

7. Discussion II.

Ketoprofen, a widely utilized NSAID with diverse therapeutic applications, exhibits remarkably low water solubility. Enhancing its solubility is imperative for improved bioavailability, and various methods can be employed to achieve this. Solid dispersions have become increasingly popular due to their favorable characteristics. In our study, we aimed to investigate the binary dispersions of ketoprofen with three polymers of distinct molecular weights: PEG 1000, 1500, and 2000. Our objective was to delineate the variations among the solid dispersions produced by these three molecules and their dissolution properties.

Our phase solubility study reveals a significant increase in PEG solubility. Specifically, we observed an augmentation in ketoprofen solubility from 41 μg/mL to 300 μg/mL. Importantly, there was no statistically significant difference among the three polymers. This observation aligns with the findings of Khattab et al., who demonstrated minimal differences in the solubility-enhancing properties of PEG 4000, 10000, and 20000 for gliclazide. Our results, within the 1-10 $^{w/}_{v}$% range, also correspond well with the previous research by Mura et al., indicating a proportional increase in the solubility of ketoprofen when using PEGs within this range. The negative free energy transfer across all concentrations and polymers indicates the spontaneity of the drug solubilization process. This process is even more advantageous at higher concentrations of the polymers, as evident from the decreasing free energy for the solutions with higher concentrations. Furthermore, based on the Ka values, it is observed an increase in the binding affinity between ketoprofen and the PEGs as the molecular weight of the PEGs increases.

When examining the six samples, outcomes from both the FT-IR and the PXRD analyses suggest the formation, albeit partial, of a solid dispersion even at room temperature, particularly in the case of the physical mixture involving PEG 1000. Despite the initial mixing being conducted with liquid and subsequent cooled storage before investigation, the infrequently used PEG 1000 for preparing hot melt solid dispersions is attributed to its low melting range. The shifts observed in the characteristic IR bands are ascribed to hydrogen bond formation between ketoprofen and the polymer, a phenomenon previously reported in different

ketoprofen solid dispersions. Overall, the noted alterations in the spectra indicate the formation of a solid dispersion, distinguishing it from a mere physical mixture. The scanning electron microscopy images affirm the homogeneous distribution of the API, but the presence of ketoprofen crystals in the solid dispersion texture suggests incomplete conversion into the amorphous phase.

The primary merit of a solid dispersion lies in its ability to generate supersaturated solutions of the given API. In an ideal system, the API exists 100% in an amorphous state. Consequently, the dissolution of the matrix polymer results in a supersaturated colloidal solution, where the kinetic solubility exceeds the "normal" thermodynamic solubility determined by the physicochemical properties of the crystalline form. The difference between the two solubility values can be observed when comparing the results . In the phase solubility studies, a 24-hour interval between initial dissolution and ketoprofen measurement initiated amorphous API particle crystallization, which could be separated by centrifugation, leading to a significant decrease in the dissolved ketoprofen amount. During the dissolution experiment, we observed a sufficiently short time window to detect the formation of a supersaturated solution. This supersaturation phenomenon can be attributed to the prevention of crystallization by the polymer matrix. Consequently, enhanced bioavailability can be assumed.

There is a compilation of diverse studies investigating solid dispersions using different PEGs as the polymer matrix. It is evident from the data that establishing a universal correlation between molecular weight and dissolution rate is challenging, as this relationship is significantly influenced by the specific APIs employed. Duong and Van den Mooter propose a potential explanation for this phenomenon in their review. According to them, fenofibrate, which does not form hydrogen bonds with PEG, and flurbiprofen, which does, show that the dissolution of the solid dispersion is unaffected by molecular weight as PEG remains neutral. However, this explanation does not exclusively account for the observed outcomes. For example, in the case of other potential hydrogen-bond-forming molecules like naproxen, the dissolution profiles of solid dispersions were unaffected by molecular weight. Unfortunately, our findings fall under the "None" classification, with PEG 1000 exhibiting a higher percentage of released drug compared to PEG 1500 and PEG 2000, despite the nearly identical characteristics of the latter two. Despite the confirmed formation of hydrogen bonds in the interaction between ketoprofen and PEG 1000, 1500, and 2000, our study did not reveal a clear correlation between molecular weight and dissolution properties.

Our findings indicate that both the physical mixtures and solid dispersions of PEG 1500 and 2000 yielded similar. It has been noted that the dissolution rate of the polymer may decrease when it is part of a solid dispersion rather than being freely present as in a physical mixture. However, the low melting ranges of PEG 1500 and 2000 may have led to the in situ formation of a solid dispersion from the physical mixtures while inside the capsule. Even if this phase transition occurred only partially, the physical mixture exhibited the potential to elevate the percentage of released drug to the level observed in the original solid dispersion. The difference between PEG 1000 SD and PM could be explained by the lowest Ka values, indicating a lower binding affinity of ketoprofen toward the carrier. We believe that the unique combination of ketoprofen-PEG interactions altering the dissolution rate of the polymer and the in situ phase transition of physical mixtures accounts for our results.

Our observation of the kinetics profile for the samples is consistent with existing literature, when SDs commonly exhibit a good fit for both Korsmeyer-Peppas and zero-order models.

Overall, our investigation supports the conclusion that the molecular weight of PEGs does not have a straightforward impact on the dissolution profile of ketoprofen. Surprisingly, the physical mixtures of PEG 1500 and 2000 were nearly identical to their respective solid dispersions, suggesting a potential method for easily and simply enhancing the solubility of ketoprofen. Further research confirming the in situ solid dispersion formation could lead to the development of simple powder-loaded capsules that enhance the bioavailability of the API compared to conventional dosage forms.

8. Summary

To summarize, our study investigated numerous cellular effects, in vivo toxicity, as well as osmolality of eleven different polyethylene glycols on Caco-2 cells. Data analysis with different statistical method revealed a notable correlation among cytotoxicity, osmolality, and the molecular weight of PEGs. In general, lower molecular weight PEGs demonstrated a substantial decrease in cell viability, elevated osmolality, and elevated the mortality of larvae, while PEGs with higher molecular weights generally exhibited minimal impact on cells and lowered osmolality. Further scientific inquiries are necessary to gain a deeper understanding of the impact of PEGs on autophagosome formation and early apoptosis. However, it can be affirmed that specific biological effects of PEGs cannot be accurately evaluated solely from their molecular weight.

Overall, it can be concluded, that no rule of thumb can be applied for the molecular weight of PEGs as polymer matrices of solid dispersions, if the question is their ability to modify dissolution curve of a drug molecule. Each PEG-API interaction must be investigated on its own and trial-error methods must be used to test out what is the optimal molecular weight, drug:polymer ratio for each situation. Also, the comparison of physical mixtures and solid dispersions must be carried out especially in case of carriers where the melting point/range is in the physiological range of the gastro-intestinal tract.

We describe the formulation of ketoprofen and PEG physical mixtures as well as solid dispersions in a ratio of 1:9. It was observed that at room temperature, the physical mixture PEG 1000 began to melt, leading to the creation of a partial solid dispersion. On the contrary, the formulations of PEG 1500 and 2000 exhibited distinct PXRD and FT-IR spectra, and their physical characteristics varied as observed in scanning electron microscopic images. The dissolution study carried out in hydrochloric acid media indicated no difference between the formulations with PEG 1500 and 2000, while demonstrating a notably higher quantity of dissolved API in the PEG 1000 solid dispersion. However, in each case, a supersaturated solution was identified, as the quantity of dissolved ketoprofen exceeded the theoretical maximum suggested by the phase solubility study.

In summary, our findings demonstrate that, in the case of low molecular weight PEGs, polymer's length does no influence dissolution profile of ketoprofen solid dispersions directly. Unfortunately, our results can only be moderately compared to other research articles, as the

investigation of binary solid dispersions is currently outside the scope of researchers, and most publications do not focus on the impact of molecular weight but on other aspects of solid dispersion formulation. Further investigation is required to study the dissolution of different formulations to explain the interesting results of the dissolution study. Only the PEG 1000-ketoprofen solid dispersion surpassed its physical mixture counterpart, with PEG 1500 and 2000 exhibiting considerable similarity to each other. Additionally, the physical mixture of PEG 1000 showed a relatively high amount of dissolved API. These findings indicate that, for low molecular weight PEGs, forming a solid dispersion may not be essential to achieve a supersaturated solution in the stomach, thereby significantly enhancing the bioavailability of the given API. Even a simple physical mixture can improve drug delivery due to the combination of the melting point and dissolution speed of PEGs, along with the unique interaction between ketoprofen and the polymers. Further investigation is necessary to delve into the potential utilization of this characteristic of low molecular weight polyethylene glycol derivatives.

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