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Release kinetics of [Lidocainium][Ibuprofenate] as Active Pharmaceutical Ingredient-Ionic Liquid from a plasticized zein matrix in simulated digestion

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Abstract

An *in vitro* approach is proposed to study the release of an Active Pharmaceutical Ingredient-Ionic Liquid (API-IL) from a natural biopolymer matrix based on zein, a maize storage protein. Zein can be processed in the molten state with 20 w% [Lidocainium][Ibuprofenate] added as API-IL also acting as plasticizer and potentially co-plasticized by glycerol. The thermal stability of the matrix is checked, as well as the *in vivo* biological activity of the API-IL confirming anesthetic and anti-inflammatory activities. Model tablets are thermomolded at 130 °C (ø20 mm, 0.2 mm thick) and submitted to simulated digestion based on the INFOGEST static protocol of gastrointestinal food digestion at 37 °C (2h under gastric conditions followed by 2h under intestinal ones). The release of the API-IL is evaluated by HPLC-UV to dissociate lidocainium, that shows a progressive release (35% after 2h and 60% after 4h digestion), from ibuprofenate, that is mainly released under intestinal conditions due to low solubility in acidic conditions. The monitoring of the tablets reveals release

mechanisms based on diffusion without noticeable erosion of the matrix. These results demonstrate the interest of this thermoplastic material to provide a relevant drug delivery system.

Keywords: Biopolymer, Controlled release, Diffusion, *in vivo* activity, *in vitro* digestion, Thermal stability

1. Introduction

Thermomechanical processing is widely studied in the pharmaceutical manufacturing domain, especially using biopolymers as edible and biodegradable alternative to synthetic polymers. Indeed, biopolymers are of particular interest to form matrices in order to assure protection and transport of the drug when administered. Zein, a prolamin extracted from maize corn, is a protein with hydrophobic behavior due to numerous apolar amino acids in its structure. It has been largely studied to propose a sustainable material as excipient for pharmaceutical application (Berardi et al., 2018; Corradini et al., 2014; Jayan et al., 2019). It is approved as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA, 2008) for food and pharmaceutical applications. Thus, various studies reported its potential for controlled drug delivery, as coating material, for example (Vattanagijyong et al., 2022), or as nanoparticle material for carrying poorly soluble molecules (Tran et al., 2019), or even as matrices obtained by hot melt extrusion (Bisharat et al., 2020; Bouman et al., 2016). Indeed, zein possesses thermoplastic properties, i.e. it can be molten, allowing its thermomechanical processing, along with ability to interact with many compounds (such as plasticizers) due to both hydrophobic and hydrophilic domains contained in its structure (Shukla and Cheryan, 2001). Glycerol, for instance, is a well-known biocompatible plasticizer often used in pharmaceuticals able to modify zein mechanical properties (Chaunier et al., 2020; Corradini et al., 2006).

Considering the great proportion of Active Pharmaceutical Ingredients (APIs) that show a low solubility in water, many strategies have been developed to allow their administration and improve their bioavailability. The use of ionic liquids (ILs), for example, gains more and more interest in order to enhance solubility. ILs are salts, made of two ionized components, that can have the particularity to be in liquid state at room temperature, and many of them have been studied in the biomedical and pharmaceutical field (Curreri et al., 2021). While some ILs are used as solvents to solubilize the API or as nanocarriers, others are made of active molecules themselves, as part of their components, and are used as liquid forms of API (Shamshina et al., 2013). These are called Active Pharmaceutical Ingredients – Ionic Liquids (API-ILs). API-ILs offer numerous advantages such as a better chemical and thermal stability than crystalline APIs, an increased solubility in water due to their ionized state and less polymorphism issues, since not under a crystalline form for most cases, allowing a better bioavailability compared to the crystalline form of the APIs. For instance, Balk et al., in 2015 worked on turning active molecules of poor water solubility into room temperature ionic liquids. Among them, ibuprofen forming an ionic liquid with Tetrabutylphosphonium showed a greater solubility than the free acid and even the sodium salt. Pharmacological activity enhancing was also reported in several examples with APIs turned into ionic liquids (Klebeko et al., 2022; Pedro et al., 2020). Moreover, special interest is given to ionic liquids in the processing of biopolymer materials (Chen et al., 2018), our group has reported the ability of the API-IL [Lidocainium][Ibuprofenate] to plasticize zein, in order to obtain zein-based drug delivery systems by hot melt extrusion (Chaunier et al., 2020). This API-IL has been selected among other ionic liquids for various reasons. Firstly, it has shown some promising features in drug delivery systems, to enhance the permeability through skin layers for example (Berton et al., 2017; Zhang et al., 2020). Secondly, it possesses the ability to interact and form drug delivery systems with biocompatible polymers such as PLA (Jouannin et al., 2014) and

thirdly, its dual activity due to both anesthetic and anti-inflammatory properties of respectively lidocaine and ibuprofen make it a good candidate as API model in the studied systems (Pedro et al., 2020). In this study, thin films of zein plasticized by [Lid][Ibu] are used as a model geometry representing the hot-melt molded material.

In this work we investigate the release of [Lid][Ibu] from zein-based matrices, specifically under simulated digestive conditions using an original method implemented. It is based on the harmonized INFOGEST protocol which is an *in vitro* digestion method proposed to implement a unique optimized protocol mimicking relevant physiological gastrointestinal conditions (Brodkorb et al., 2019; Minekus et al., 2014). Moreover, while former study of zein matrices containing [Lid][Ibu] only measured its release in phosphate buffer by UV spectroscopy, we use an analytical HPLC-UV dosage, giving access to the concentrations of each component of the ionic liquid. Additionally, the effect of heating on [Lid][Ibu] therapeutic activity is tested with *in vivo* experiments to ensure no damaging of the ionic liquid occurs during processing. Furthermore, the impact of the digestion on the macroscopic aspect of matrices is investigated through evaluation of their swelling, water uptake and mechanical properties. Zein materials containing glycerol as a co-plasticizer are also characterized in order to evaluate the impact of plasticization upon the release of [Lid][Ibu] and optimize thermochemically processed systems suitable for controlled drug delivery.

2. Materials and methods

2.1. Materials

All reagents used were analytical grade. Zein-Z3625, glycerol-G7893, pepsin-P7012, pancreatin-P7545, bile-B3883, trifluoroacetic acid (TFA), and carrageenan-22048 were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Ibuprofen-B20989 was from Thermo Fisher Scientific (Kandel, Germany). All reagents for gel electrophoresis were

from Bio-Rad Life Science, France. Acetonitrile were from Carlo Erba reagents S.A.S. (Val de Reuil Cedex, France). Veterinary lidocaine was used as LAOCAÏNE® specialty.

[Lid][Ibu] was synthesized according to Jouannin et al. (2014).

2.2. Formulation

Physical mixtures were prepared preliminary to matrix thermomolding by mixing the powdery raw zein with [Lid][Ibu] and/or glycerol (Table 1).

Table 1. Formulations of the different materials based on zein, [Lid][Ibu] and/or glycerol.

Moisture Content (MC) and glass transition temperature (T_g) were determined after reaching equilibrium under an intermediate humidity of 59 %RH in a closed vessel at 20 °C, except for the raw commercial zein used as obtained from the supplier (with MC corresponding to commercial specification, < 8%).

| Formulation ID | Zein [g] | Glycerol [g] | [Lid][Ibu] [g] | MC [%] | T_g [°C] |
|----------------|----------|--------------|----------------|--------|------------|
| Z | 100 | 0 | 0 | 6 | 85 |
| [Lid][Ibu] | 0 | 0 | 100 | ≈0 | -30 |
| Z20LI | 100 | 0 | 20 | 7 | 60 |
| Z10GLY10LI | 100 | 10 | 10 | 9 | 37 |

2.3. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out on a TGA-2050, TA Instruments-Waters SAS (Guyancourt, France) under a nitrogen gas flow. Samples of physical mixtures were weighted (about 10 mg) and heated from 20 °C to 130 °C with a heating rate of 10 °C/min. Then, the temperature was kept constant for 30 min when reached 130°C and then subsequently increased to 500 °C at 10 °C/min.

2.4. Differential scanning calorimetry (DSC)

Thermal properties were studied using a TA Instruments (Guyancourt, France) Q100 DSC, with a nitrogen gas flow rate 50 mL/min. Samples (about 10 mg) were placed in aluminum hermetic pans and heated in the DSC from -60 °C to 140 °C at a rate of 3 °C/min. Two successive cycles for heating and cooling were carried out, to cancel the physical aging of the material.

2.5. Thermomolding

Thin films were obtained as disks by thermomolding physical powdery mixtures in order to study zein as an homogeneous thermoplastic matrix. Approximately 0.2 g were weighted for each disk in a 0.2 mm thick steel mold composed of 20 mm diameter holes. The mold was then compressed between two Teflon sheets and hot plates of a hydraulic press VS35 Pinette® (Chalon-sur-Saône, France) at 150 bar and 130 °C during 3 min. Plates were then cooled down to 90 °C before opening and taking zein-based disks out of the mold. They were placed at constant humidity level (59 %RH, 20 °C) during one week for subsequent analysis.

2.6. Dynamic mechanical thermal analysis (DMTA)

Dynamic mechanical analysis was performed on thermomolded zein materials using a dynamic mechanical thermal analyzer (DMTA; model MK-IVA, Rheometric Scientific Inc., USA). Samples were thermomolded as rectangular bands of 3x20 mm in the same way as disks with the hydraulic press and then covered with a thin film of silicone-based hydrophobic grease to avoid dehydration during characterization. Samples were placed between two 10 mm away grips and submitted to a 0.1 % strain oscillating at 1 Hz frequency while temperature varied from -60 °C to 140 °C increasing at a rate of 3 °C/min.

2.7. *In vitro* study

The release of the API-IL from the matrices was studied using an optimized method based on the INFOGEST standardized protocol (Brodkorb et al., 2019). Films were placed in 1 mL of simulated gastric fluid (SGF, pH=3) and incubated under gentle shaking at 37.4 °C during 2 hours, then pH was increased to 7 with NaOH 1M. Then, 1 mL of simulated intestinal fluid (SIF, pH=7) was added to switch to intestinal conditions for 2 more hours. SGF and SIF were prepared following the recommendations of the INFOGEST protocol, respectively enriched with pepsin from porcine gastric mucosa (2000 UI/mL) and pancreatin from porcine pancreas (100 U trypsin activity/mL). Incubating media were removed at 7 time points (0, 15, 30, 60, 120, 180 and 240 minutes) for [Lid][Ibu] dosage, one sample was used for each time point. The amount of [Lid][Ibu] released was then quantified by Reverse-Phase HPLC (RP-HPLC) with UV detection. Prior to HPLC-UV analysis, media were centrifuged 10 min at 10000g. All experiments were performed in duplicate.

2.7.1. HPLC-UV analysis

Quantification of API-IL was performed by RP-HPLC system (Dionex Ultimate 3000, Thermo Fisher Scientific, Courtaboeuf Cedex, France). The system consisted of a pump system (Dionex, LPG34005D), autosampler (Dionex, WPS 3000 TSL), and rapid separation variable wavelength detector (Dionex, VND3400R5) equipped with a Symmetry C18 RP column 3.5 μm , 4.6 mm x 100 mm (Waters, Milford, MA, USA). Elution was carried out with a solvent gradient water/acetonitrile at a flow rate of 1 mL/min. The column effluent was monitored by UV detection ($\lambda=225$ nm). The column was equilibrated with solvent A water/acetonitrile 98/2 v/v with 0.05 % TFA 2 min, then a gradient was applied from 0-30 % of solvent B acetonitrile/water 98/2 v/v with 0.04 % TFA over 22 min and finally 100 % of solvent B until 25 min. Lidocainium was detected after around 12 min and ibuprofen after 23

min as retention time. Calibration curve was obtained using specific amount of [Lid][Ibu] dissolved in MilliQ water.

2.7.2. Gel electrophoresis (SDS-PAGE)

The samples were collected from each digestion time. Protein analyses was performed by SDS-PAGE with Mini-Protean TGX Precast gels gradient 8-16 % using Mini-Protean Tetra Cell system (Bio-Rad Life Science, France). All samples were diluted with 2x Laemmli sample buffer in reducing conditions with 2-mercaptoethanol 5 %. The migration buffer contained 25 mM Tris, 192 mM glycine, 0.1 % SDS according to Laemmli (1970) protocol. The samples were heated at 100°C for 5 min. Each sample was then loaded on a sample well of gradient gel 8 – 16 % polyacrylamide gel electrophoresis. Molecular weight (M_w) protein from M_w 14.4 to 116 kDa (unstained Molecular weight marker, Euromedex, Souffelweyersheim, France) were used for M_w calibration. The migration was carried out under standard conditions at 200 V for 45 min. The gel was stained with Coomassie Brilliant Blue G-250 staining solution for 2h with gentle agitation on platform rocker according to Lawrence and Besir (2009). The gel was rinsed with distilled water before scanning on a flatbed scanner (Image Scanner iii, GE Healthcare Europe, Velizy-Villacoublay, France).

2.7.3. Swelling

After medium removal, images of the film were taken at each time point of the *in vitro* digestion using an SVS-Vistek Camera exo265CU3. Those images were treated via MatLab® software (The MathWorks Inc., Natick, MA, USA) to be converted into binary images where films were represented in white on a black background. Surface of the films were measured by white pixels counting followed by a conversion into meters with a scaled image (resolution $\approx 20 \mu\text{m}/\text{pixel}$). The thickness is evaluated on each film after image acquisition, thanks to a

digital micrometer. Then the films volume can be determined by combining surface measurements *via* image analysis and thickness measurements. Since all the films produced had approximately the dimensions of molds used ($\varnothing 20$ mm, 0.2 mm thick), their initial volume was constant, about 60 mm³.

2.7.4. Moisture content

Moisture content (MC) of films that underwent digestion was determined by measuring their mass before (M1) and after (M2) dehydration at 103 °C overnight in oven (Chopin Technologies, F-92 Villeneuve-la-Garenne). Moisture content reported on total weight (zein+plasticizer+water) was calculated as follow:

$$\text{Moisture Content (\%)} = [(M1-M2) / M1] \times 100 \quad \text{Eq. 1}$$

2.7.5. Mechanical testing

At each time point of the digestion, films were taken out and gently drained on a filter paper, in order to remove the medium in excess. Then films were placed in the compression cell of a universal testing machine to perform mechanical testing (MTS Systems DY 34B, Eden Prairie, MN, US). A 10 mm-diameter cylinder was used to perform the compression at 5 mm/min speed rate. Force was acquired at 20 Hz and corresponding stresses were recorded. Mechanical compression tests gave curves representing stress versus strain applied on the films (Figure 1) and mean elastic modulus was obtained calculating the slope of the curve for the different steps of the digestion.

2.8. *In vivo* testing

2.8.1. Animal model

Six-week-old female BALB/cJRj mice (Janvier Labs, F-53940 Le Genest-Saint-Isle) were housed in a ventilated cage system and in specific pathogen-free conditions in the animal facility (INRAE-BIA, F-44300 Nantes) and used for analgesic and acute inflammation study. All experiments respect EU Directive 2010/63/EU for animal experiments and the protocol was approved by the ethics committee on Animals Experimentation Pays de Loire (accreditation number 34619). Experimental mice were divided into the following groups each having twenty animals. Lidocaine and Ibuprofen as sole chemical compounds were given through intraperitoneal (I.P.) route for standard group and through oral gavage for [Lid][Ibu] groups: Control group (PBS), Standard group Ibuprofen/Lidocaine (20 mg/kg, ratio 1:1), LI and LI^{°C} (20 mg/kg of each molecule). LI received [Lid][Ibu] dissolved in water whereas LI^{°C} received [Lid][Ibu] that has been maintained at 130 °C during 3 min before dissolution in water such as in thermomolding conditions during material processing.

2.8.2. Hot plate analgesia

The analgesic activity of test drug was assessed using the hot plate method of Eddy and Leimbach, 1953. One hour after dosing group specific drugs, mice were placed on the hot plate. The temperature of the hot plate was maintained at 55 ± 0.20 °C. Time until either licking or jumping (indication of pain) was recorded by a stopwatch. Time interval between placing the animal on hot plate and either licking or jumping was considered as reaction time (latency period).

2.8.3. Carrageenan-induced mice ear edema

The anti-inflammatory properties of the drug were determined by injecting carrageenan in the ear of each mouse (Winter et al., 1962). A few minutes after treatment administration, an acute inflammation was produced by injection of carrageenan (0.01 ml of 1 % w/v

suspension). The ear edema was determined using a digital micrometer (Mitutoyo France, F-95 Roissy CDG) before and 60 min after carrageenan injection. Subcutaneous injection of carrageenan into the ear produces inflammation resulting from plasma extravasations, increased tissue water, and plasma protein exudation, along with neutrophil extravasation due to the metabolism of arachidonic acid.

2.8.4. Statistics

Data were analyzed using GraphPad Prism 6.0 (La Jolla, CA, USA). Values are expressed as the mean \pm SEM (Standard Error of Mean) and were compared using nonparametric ANOVA with a Kruskal-Wallis test. A p-value of less than 0.05 was considered significant.

3. Results and discussion

3.1. Physical mixtures characterization and therapeutic effect of API-IL

3.1.1. Thermogravimetric analysis

Prior to any thermal treatment, thermal stability was studied for the different materials. Raw zein lost 5-10 % of its mass with water evaporation at 100 °C, then its mass remained unaltered at 130 °C for 30 min. As seen in Chaunier et al. (2020), pure [Lid][Ibu] does not show mass loss below 130 °C (Figure 2A) which is also the case for pure lidocaine and ibuprofen (Figure 2B). During the 30 min at 130 °C, these 3 species progressively decomposed but at different rate. [Lid][Ibu] weight seemed to decrease slower compared to its pure constituents which indicates the protective effect of the ionic liquid form against heat for a better thermal stability (Panić et al., 2020). Formulations of zein mixed with the plasticizers showed also few alterations, with a loss of 5-10 % of their mass before reaching 130 °C that can be attributed to loss of water initially contained in zein. Total weight loss then stays between 15-20 % after 30 min for the two formulations. Above 130 °C, [Lid][Ibu], lidocaine,

ibuprofen and glycerol were degraded, resulting in a thermal step occurring for the blends. Then degradation of zein and its plasticized mixtures occurred around 270 °C. These results confirmed that plasticized zein can undergo a process at 130 °C such as thermomolding or hot melt extrusion up to 15 min with moderated degradation (Federici et al., 2020; Selling, 2010; Selling and Utt, 2013).

3.1.2. [Lidocainium][Ibuprofenate] *in vivo* activity

For the evaluation of [Lid][Ibu] activity, *in vivo* tests were carried out in mice. The objective was to verify that both lidocainium and ibuprofenate kept their biological activity under the form of ionic liquid and to check if the thermal treatment applied during the processing of the molten zein had an impact on such activity. One group of mice was then treated with [Lid][Ibu] (LI), another one with heated [Lid][Ibu] (LI°C) and results were compared with control groups receiving either a placebo (CTL) or commercial active molecule (Ibuprofen or Lidocaine).

Figure 3A represents the ear thickness of mice induced by the injection of carrageenan for different groups of mice for anti-inflammatory activity evaluation. Non-treated group (CTL) showed the highest ear inflammation with an increase of around 100 µm of thickness. As it could be expected, the treatment with ibuprofen led to a much less inflammation with a mean thickness increase of less than 20 µm. Furthermore, mice treated with [Lid][Ibu] and even [Lid][Ibu] that has been heated (LI°C group), also showed a significative lower inflammation (respectively around 25 and 50 µm Δ ear thickness) compared to the non-treated group that showed a thickness increase around 100 µm. This confirms the anti-inflammatory activity of ionic liquid [Lid][Ibu] and, more importantly, shows that this API-IL can be submitted to a thermal treatment without altering its activity. Moreover, this experiment also shows that the ionic liquid form does not hinder the absorption of the molecules. Indeed, [Lid][Ibu] was

administered orally and successfully absorbed through gastrointestinal mucosa to the blood and site of action (ears here).

For the anesthetic activity, hot plate assays were carried out to measure the time reaction of mice to thermal stimulation. Longer reaction times mean a lower sensibility threshold that can be induced by an anesthetic for example. As for the anti-inflammatory activity, [Lid][Ibu] anesthetic activity was also confirmed with a reaction time increased from under 25 seconds to around 50 seconds compared to non-treated mice (Figure 3B). This effect occurred with mice treated with either pharmaceutical lidocaine (Lido), [Lid][Ibu] (LI) or heated [Lid][Ibu] (LI^{°C}), confirming the ability of the ionic liquid to undergo thermal processing without activity loss.

3.2. Plasticizing effect of API-IL and co-plasticization by glycerol

Plasticizing effect was studied via Differential Scanning Calorimetry. While the sole zein and the raw [Lid][Ibu] showed, respectively, a glass transition at around 85 °C and -30 °C, the one for blend of zein with 20 w% [Lid][Ibu] was found to be about 60 °C (Supplementary Figure A.1 and Table 1). As this latter transition happened over a relatively wide temperature range, it hampers the accurate evaluation of T_g but allows checking its order of magnitude. Addition of glycerol in the formulation (Z10GLY10LI) led to a quite similar transition occurring between 10 and 70 °C with a T_g evaluated around 37 °C. This demonstrated that a good miscibility was maintained with glycerol and showed its greater plasticizing effect that would facilitate the processing of zein-based materials in the molten state if used as co-plasticizer.

Mechanical properties of the thermomolded materials are represented as storage (E') and loss (E'') moduli, as well as damping factor $\tan(\delta)$ for the different zein formulations against temperature (Supplementary Figure A.2). Both materials are vitreous at room temperature

with moduli around 2 GPa for E' and 0.1 GPa for E'' . For Z10GLY10LI, a peak in the damping factor curve indicated a main mechanical relaxation occurring at $T_{\alpha} \approx 80$ °C whereas Z20LI showed a much higher $T_{\alpha} \approx 120$ °C consistent with a higher glass transition temperature determined by DSC (see discussion above). These results are in accordance with those found on extrudates (Chaunier et al., 2020). Moreover, two events could be seen in the loss modulus curve for Z10GLY10LI at around 60 °C and 70 °C that could be attributed to each plasticizer interacting with different sites of zein according to its polarity. These results also confirm the ductility of materials at 130 °C, allowing their processability by hot melt molding, or extrusion.

3.3. Simulated digestion

Release study was performed on thermomolded films obtained from the zein materials. The protocol implemented deliberately skipped the oral phase and focused on the GI tract conditions to mimic a drug delivery system that would be directly swallowed.

In this study, Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were used in order to mimic gastric and intestinal fluid properties. As indicated in 2.7., media contained digestive enzymes but another series of experiments were also carried out using media without enzymes in order to study the effect of diffusion and pH. These latest will be further indicated “NE” (No Enzyme) whereas simulated digestion will be noted “E” (including Enzymes).

3.3.1. Release kinetics

Advantages that represented an HPLC-UV method dosage was to be able to evaluate separately the concentration of lidocainium and ibuprofen within the same sample in one measurement. Whereas UV spectroscopy only enables the evaluation of the concentration of

[Lid][Ibu] as the maximum absorbance of both species is around 225 nm (Chaunier et al., 2020). Figure 4 typically shows a chromatogram of a sample containing [Lid][Ibu]. Two distinct major peaks can be seen, one representing lidocainium (12 min retention) and the other one ibuprofen (23 min). Area under curve increased with incubation time during simulated digestion as the quantity of species released increased.

Figure 5A shows the rate of lidocainium released from the original amount contained in the film during the simulated digestion and Figure 5B the release rate of ibuprofenate. The change of medium is represented by the grey filled background. The release of lidocainium is quite progressive with more than 30 % released after the 120 first minutes (gastric phase), followed by 50-70 % after 120 more minutes in the intestinal phase. Medium changing seemed to be of small impact on the release kinetics. Conversely, the release of ibuprofenate mainly occurred in the last 120 minutes of the simulated digestion, corresponding to the intestinal phase. It is triggered when pH is increased to neutral values. This is due to the low solubility of ibuprofen in acidic solutions (with around 5 % released at the end of the gastric phase). As presented in Figure 6, whereas lidocaine remains positively charged at both gastric and intestinal pH, ibuprofen is under its ionic form at intestinal pH, which explains its release triggered at medium change from the gastric to the intestinal one. This means each sample along the simulated digestion contains lidocaine and ibuprofen in their ionic form since intestinal samples have a neutral pH and gastric samples are neutralized following take out. We can also note that control of simulated fluids pH is of particular importance in order to stay in the range of enzymes activity.

The Ritger and Peppas power law (Ritger and Peppas, 1987) was applied to characterize the kinetics such as:

$$M_t/M_{inf} = k.t^n \quad \text{Eq.2}$$

Where M_t/M_{inf} is the released rate at a time t (min), k is a constant depending on the system structure and n is the exponent characterizing the transport mechanisms.

Considering the progressive release of lidocainium, curves from the entire digestion could be fitted by equations presented in Table 2. For a plane geometry, the exponent n being between 0.5 and 1, the release can be associated to an anomalous transport combining release by diffusion and by swelling (case II) (Ritger and Peppas, 1987).

Table 2. Power law fitted for release kinetics of lidocainium during whole simulated digestions (gastric+intestinal conditions) with (E) and without enzymes (NE) for Z20LI and Z10GLY10LI films

| Formulation | NE | E |
|-------------|--------------------|--------------------|
| Z20LI | $y = 0.76x^{0.80}$ | $y = 3.60x^{0.50}$ |
| Z10GLY10LI | $y = 1.91x^{0.65}$ | $y = 2.77x^{0.63}$ |

Moreover, k and n coefficients are assessed respectively in the gastric and the intestinal media in order to assess the effect of pH change (Table 3). Exponent n values stay between 0.5 and 1 for lidocainium either in the gastric or intestinal conditions, likewise for ibuprofenate but only in intestinal medium. This means that release mechanisms of the two species are the same but triggered at different time during the simulated digestion. However, values found for ibuprofenate in gastric medium are due to very weak release occurring in this phase.

Table 3. Coefficients k (min^{-1}) and exponents n from fitting the release curves of lidocainium and ibuprofenate with a power law from thermomolded films of Z20LI and Z10GLY10LI in separated gastric or intestinal media with (E) or without enzymes (NE)

| | Z20LI | | | | Z10GLY10LI | | | |
|-----------------|-------------|------|--------------|------|-------------|------|--------------|------|
| | lidocainium | | ibuprofenate | | lidocainium | | ibuprofenate | |
| | k | n | k | n | k | n | k | n |
| gastric (NE) | 0.7 | 0.82 | 6E-10 | 4.93 | 2.56 | 0.56 | 8E-6 | 2.23 |
| gastric (E) | 3.81 | 0.49 | 1E-7 | 3.88 | 2.54 | 0.65 | 2E-6 | 2.65 |
| intestinal (NE) | 0.99 | 0.67 | 0.99 | 0.79 | 1.02 | 0.75 | 1.01 | 0.8 |
| intestinal (E) | 0.98 | 0.66 | 1 | 0.78 | 1.03 | 0.7 | 1.03 | 0.91 |

The only kinetics that exhibited a significant higher release was from Z10GLY10LI films when incubated with digestive enzymes. In this case, 55 % lidocainium was released after 120 min versus 30 % for other cases that only reached 55 % after 240 min when Z10GLY10LI (E) reached 80 %. A maximum of 70 % of ibuprofenate was released for Z10GLY10LI (E) versus 45-50 % for other cases. This means that digestive enzymes have a positive effect on the release of both species when films contain glycerol as seen in Figure 5. The presence of glycerol in this material, compared to Z20LI films, facilitates protein mobility in the matrix and might explain this increase in the release of [Lid][Ibu]. Plus, considering the hydrophilicity of glycerol and incubating media, its fast liberation may occur at the beginning of the incubation (Paolicelli et al., 2018). We can hypothesize that it creates more space and degree of freedom for API-IL to diffuse out of the matrix and for enzymes to diffuse inside the matrix. Thus, glycerol combined to API-IL in the formulation eases the release of [Lid][Ibu] from zein matrices.

3.3.2. Swelling

As films were incubated in aqueous media, matrices went through different changes implying water uptake, plasticizer leaking and decrease in the stiffness. Macroscopically, the evolution of samples volume due to swelling was noticeable during the simulated digestion. Figure 7 shows that few differences were observed between the different formulations with no effect of the presence of enzymes. Volume of the films increased to around 200 mm³, reaching the maximum swelling in the first hour of immersion and then remaining stable for the rest of the simulated digestion. This represents more than three times their original size. This swelling seemed isotropic, then X-ray diffraction measurements (presented in Supplementary Figure A.3) confirmed that no structural orientation influenced the direction of swelling (2θ measurements) and that no crystallization of compounds constitutive of the API-IL occurred in the materials. Indeed, peaks seen at 4.5Å and 9.5Å correspond to characteristic peaks of structural organization of the protein zein (Matsushima et al., 1997). These data also showed a good consistency of the materials that can undergo 4 hours digestion without any significant effect of erosion by the enzymes (Tran et al., 2019). Indeed, no solubilized zein was found in the media after migration on electrophoresis gel (SDS-PAGE) accounting for a resistance of the materials to erosion (Supplementary Figure A.4). Films integrity looked barely affected in the 4 hours digestion compared to water uptake and swelling evolution. Moreover, as volume evaluation is not based on weight evaluation that can be potentially disturbed by chemical species migration to and from the matrix (Paolicelli et al., 2018), swelling results obtained with this method are not impacted by the composition of the materials and can be easily used to compare various formulations only according to dimensional criteria.

3.3.3. Water uptake

Figure 8 shows that moisture content of films followed the same pattern as the expansion of the volume. Films absorbed most of their moisture content during the first hour of digestion reaching an amount of 60-70 % and then stayed at this equilibrium for the rest of the digestion. Bouman et al., (2015) effectively reported a favored incorporation of water in zein matrix in acidic media due to de-amination of glutamine and asparagine. Moisture content of Z10GLY10LI films tended to be slightly higher than those without glycerol which can be explained by the hydrophilicity of glycerol that rapidly leaks out of the films favoring water entry and [Lid][Ibu] release, as mentioned in section 3.3.1.. If we calculate the ratio hydrated weight/dry zein weight for Z10GLY10LI_NE, as proposed by Bouman and co-workers, we find values increasing from 1.3 before immersion to 4.2 for 70 % MC after 4 hours digestion. This factor is of the same order of magnitude as that of the increase in volume seen in 3.3.2. (from 60 mm³ to about 240 mm³ for Z10GLY10LI_NE, for example).

3.3.4. Mechanical properties

Mean elastic modulus decreased from above 10 MPa to less than 5 MPa with water absorption at the beginning of the digestion, then remained quite constant around 2 MPa for the rest of the incubation after 1 hour (Table 4). This witnesses a rather good stability in the film consistency during the whole simulated digestion, which confirms zein resistance to degradation at gastric pH and its good potential for gastroresistant application (Nguyen et al., 2017).

Table 4. Mean elastic modulus of Z20LI and Z10GLY10LI films during simulated digestion, with (E) or without enzymes (NE). Results are given as mean \pm SD

| | Elastic modulus (MPa) | | | | | |
|-----------------|-----------------------|----------|-----------|----------|-----------|-----------|
| | 0 min | 15 min | 60 min | 120 min | 180 min | 240 min |
| Z20LI (NE) | 12.2 | 5.5 ±2.6 | 1.9 ±0.01 | 1.1 ±0.5 | 0.7 ±0.1 | 0.9 ±0.3 |
| Z20LI (E) | 12.2 | 3.7 ±0.2 | 1.3 ±0.2 | 2.4 ±1.0 | 1.6 ±0.03 | 1.3 ±0.03 |
| Z10GLY10LI (NE) | 10.6 | 3.6 ±0.7 | 2.5 ±0.5 | 2.2 ±0.6 | 2.0 ±1.1 | 1.8 ±0.5 |
| Z10GLY10LI (E) | 10.6 | 3.1 ±0.5 | 1.5 ±0.02 | 2.8 ±1.3 | 1.8 ±0.2 | 1.5 ±0.2 |

4. Conclusions

This study allowed confirming the processability of zein at 130 °C in the presence of plasticizer for which further characterization was carried out. Work was done on representative materials formulating zein with an API-IL plasticizer and thermomolded in the molten state. Plus, a method was optimized in order to study the release of [Lid][Ibu] from zein matrices under gastrointestinal conditions. HPLC-UV measurements gave access to lidocainium and ibuprofenate release separately: lidocainium release happened progressively following the immersion, whereas ibuprofenate release was triggered in intestinal medium due to low solubility in acidic conditions. Glycerol, as a co-plasticizer, also permitted to modulate the release with an increased release rate when added to the formulation. Zein matrices integrity was quite stable under immersion, considering the stability of their mechanical properties during the whole simulated digestion, showing a low degradation of the materials. This accounted for release mechanisms explained by water uptake, swelling and diffusion out of the material rather than matrix degradation. Moreover, conservation of [Lid][Ibu] activity was verified *in vivo* in mice that showed equivalent pharmacological response to API-IL that underwent high temperature and to each drug taken separately. Further investigations could include histological study, or evaluation of markers of

inflammation, to highlight these findings. All these data confirmed that zein has promising features for further investigations in thermomechanical processing, such as extrusion or additive manufacturing, to target pharmaceutical matrices including API-IL plasticizers. Furthermore, they were collected for non-porous systems allowing the study of [Lid][Ibu] release without the effect of any macroscopic porosity. Then, the robust method that was set up in this work to study such complex drug release, combining two compounds with various release kinetics, could be applied to more complex objects presenting varying geometries and porous structures designed to target specific release profiles.

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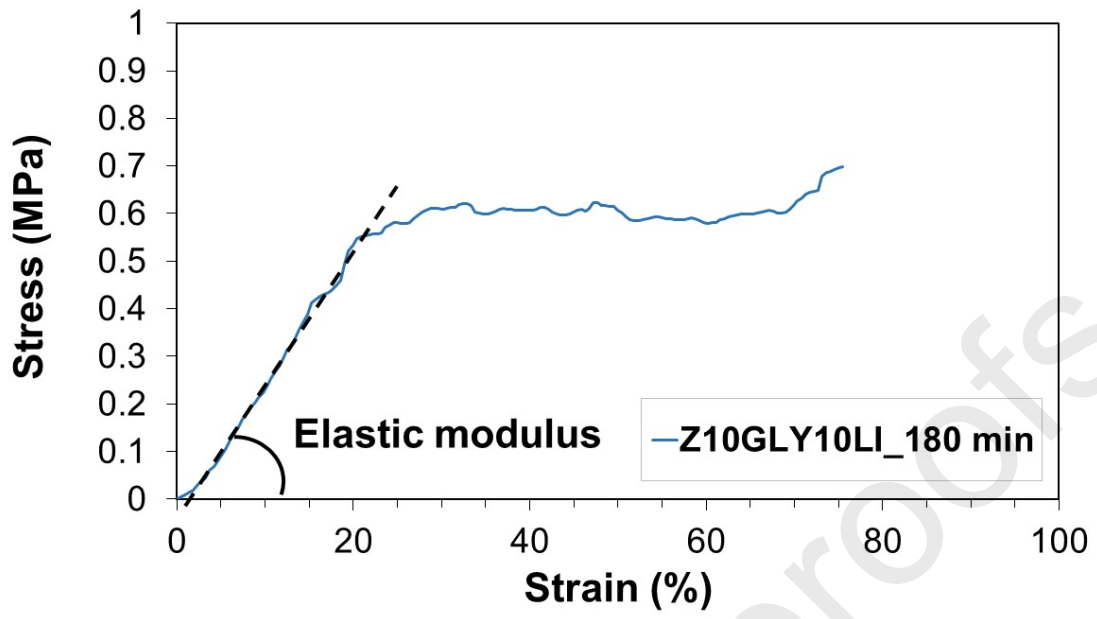
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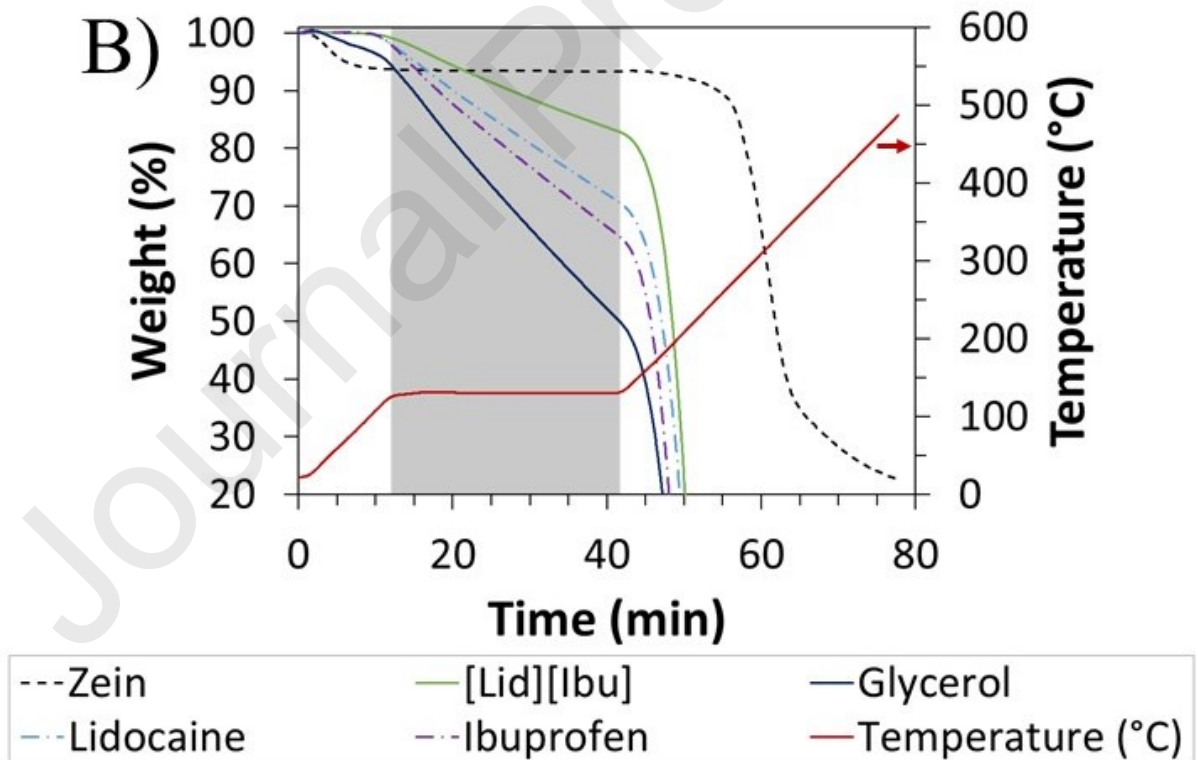
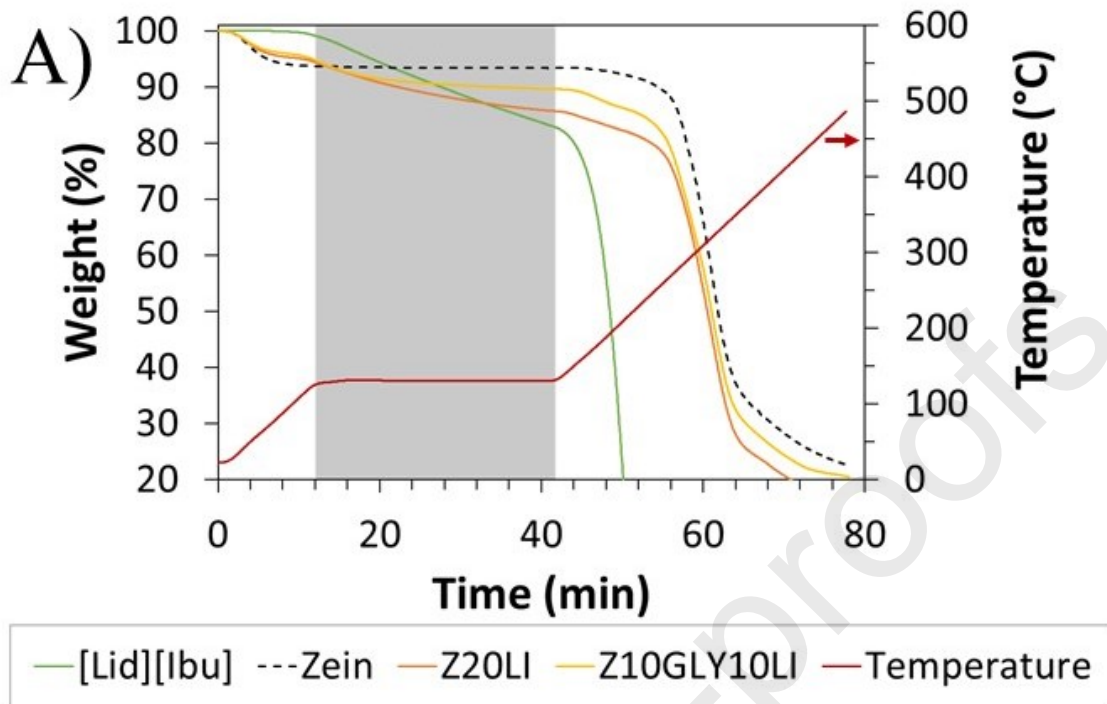
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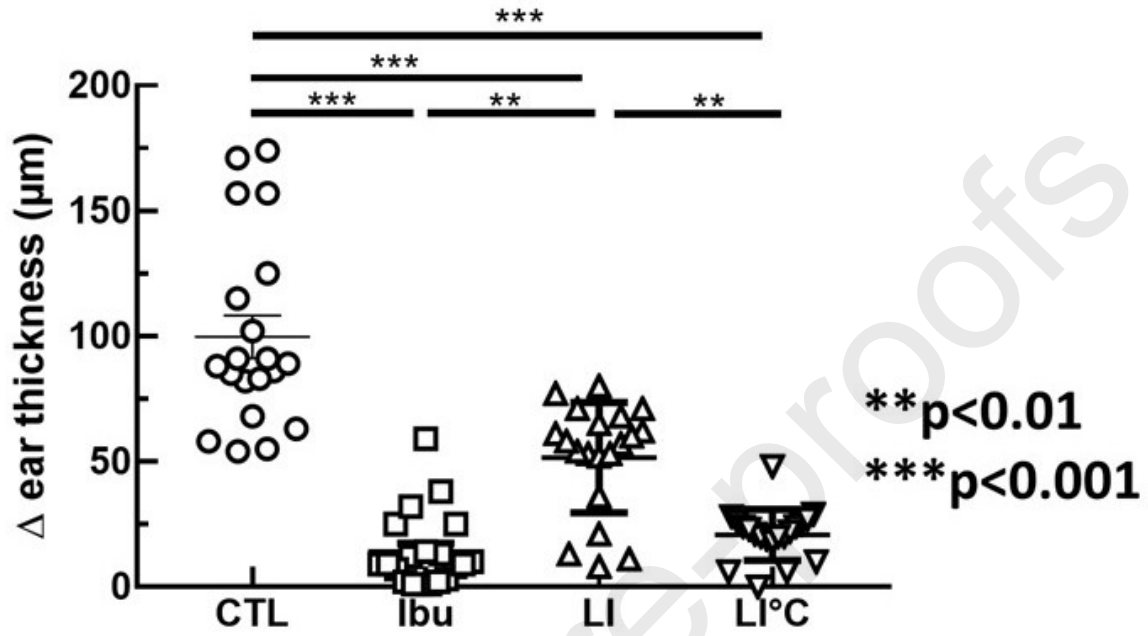
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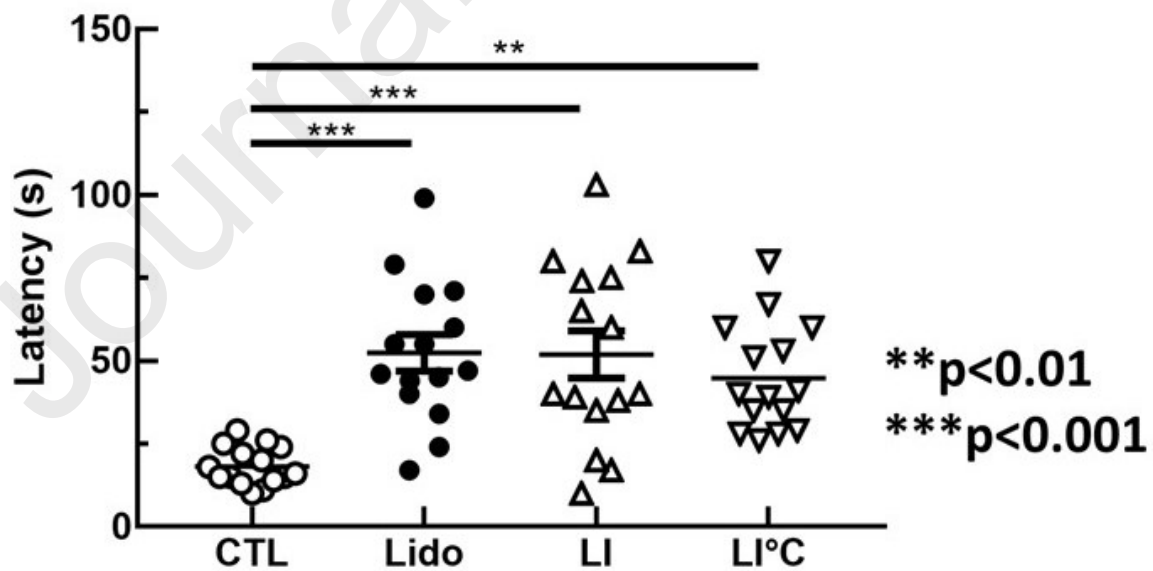


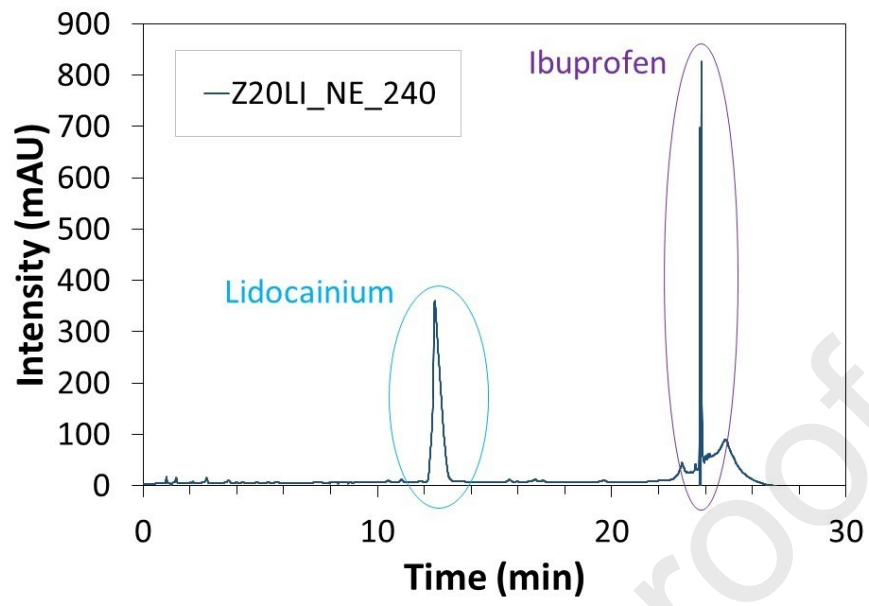


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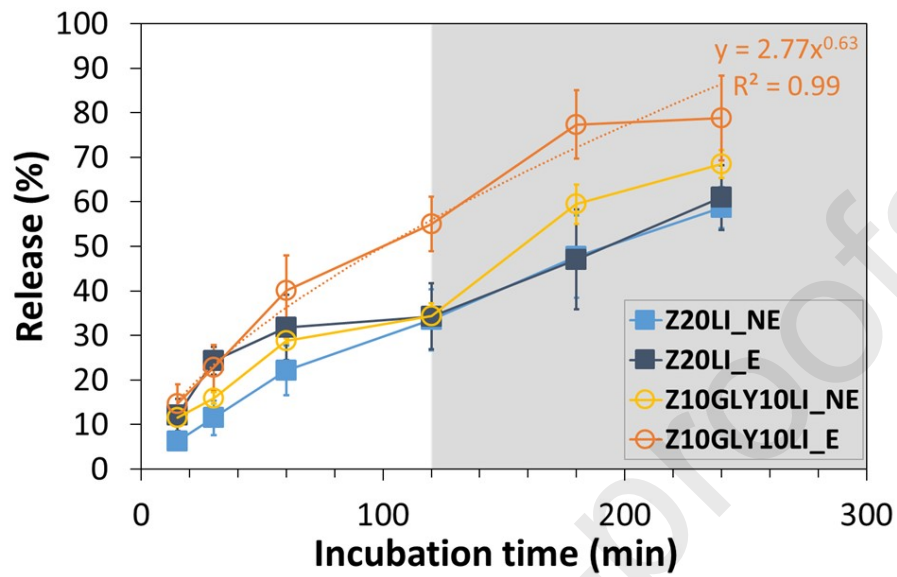


B)

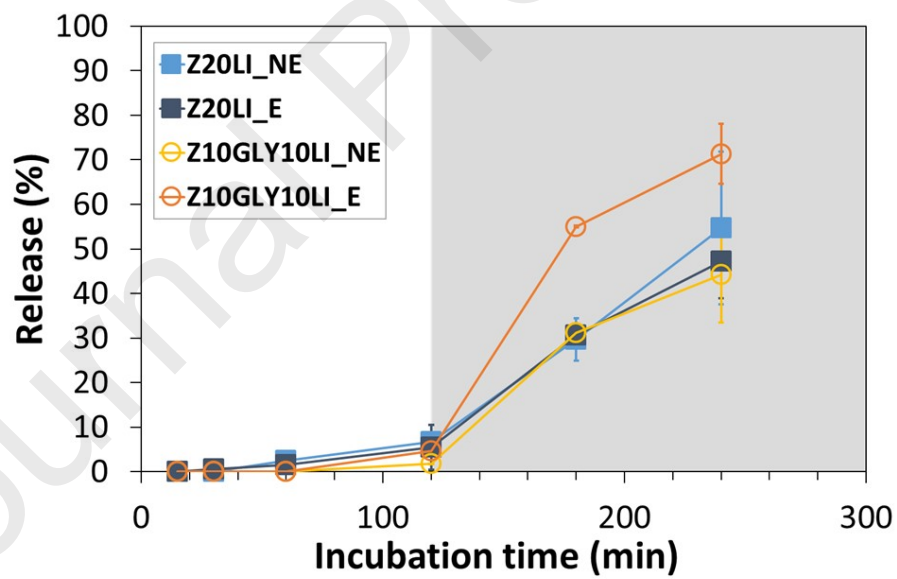


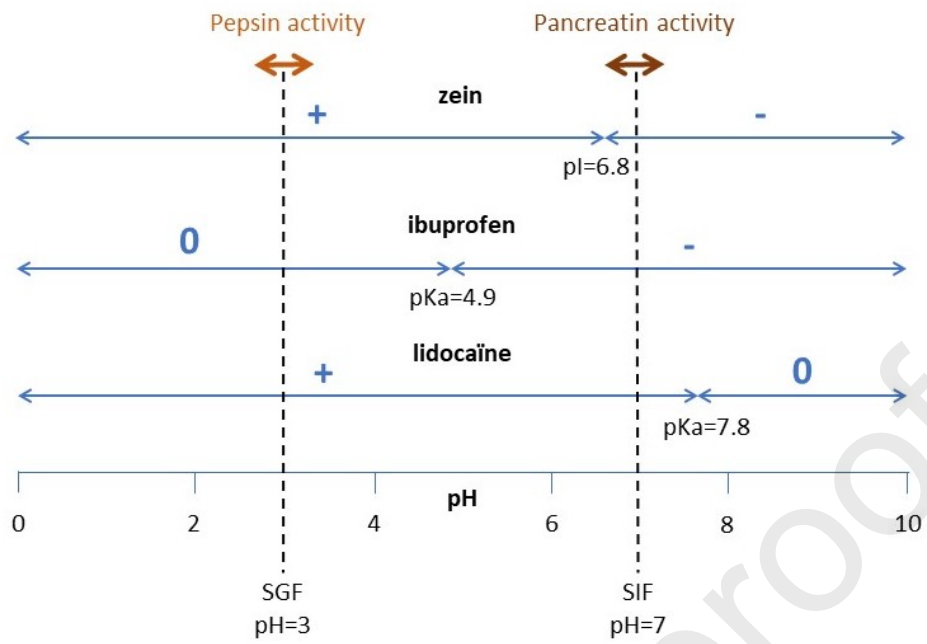


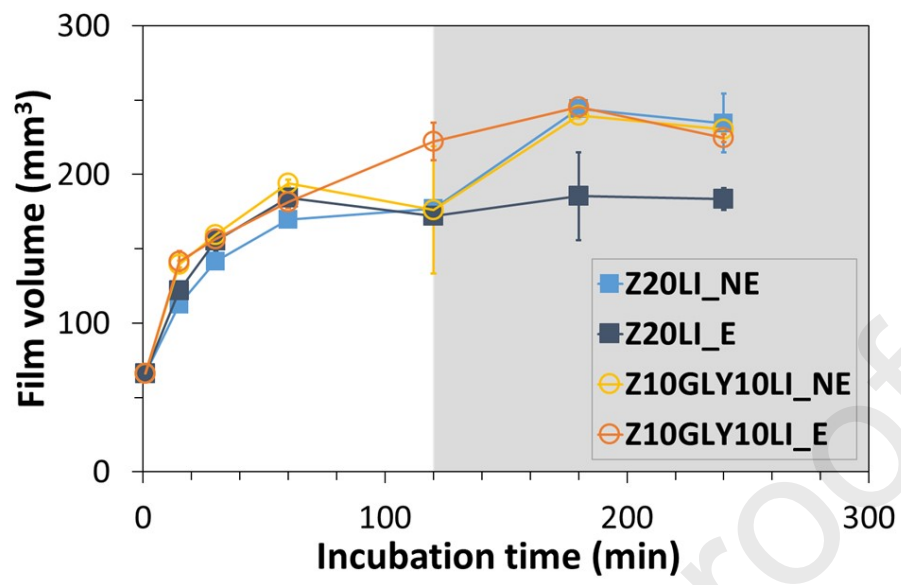
A) Lidocainium

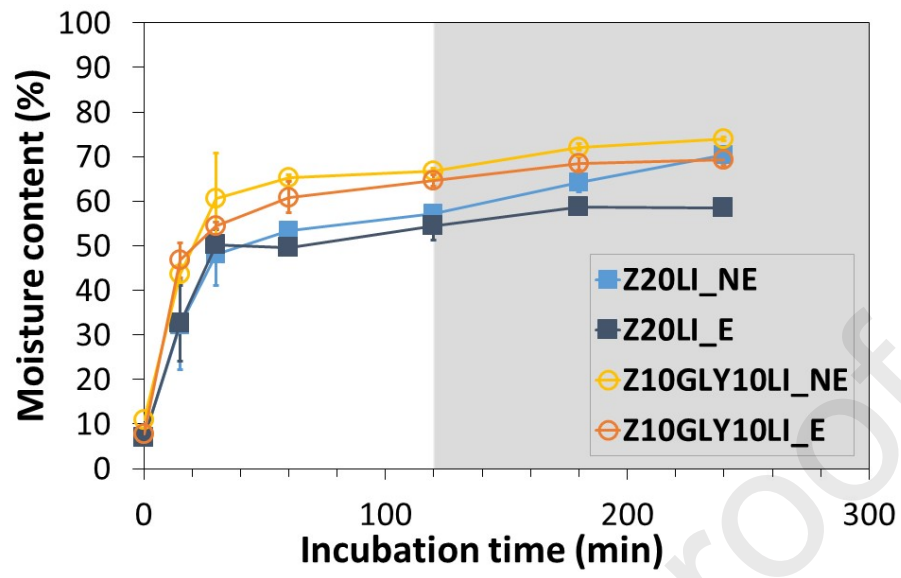


B) Ibuprofenate









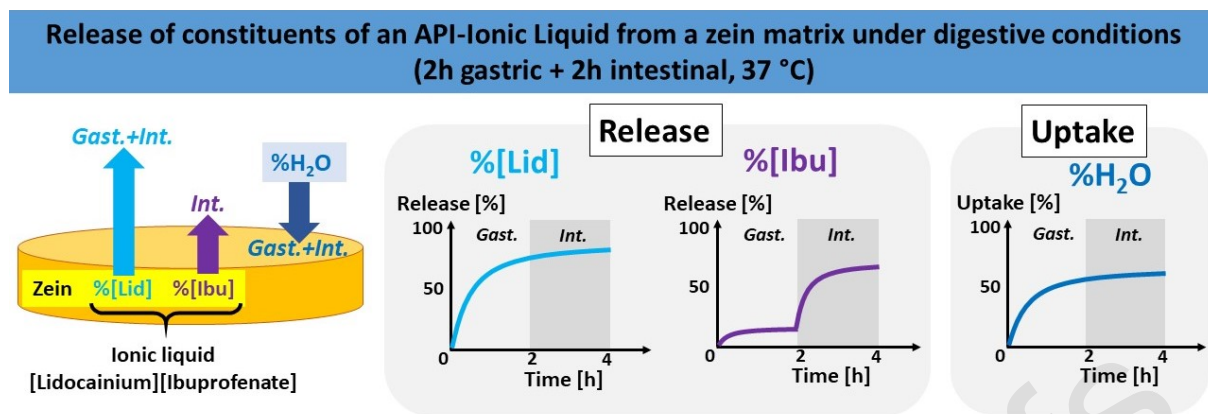


Figure 1. Compression curve representing stress vs strain and determination of the mean elastic modulus (example for sample Z10GLY10LI, after 180 min of simulated digestion).

Figure 2. TGA analysis of API-IL [Lid][Ibu], raw powdery zein, formulations Z20LI and Z10GLY10LI (A), (B) shows glycerol, crystalline lidocaine and ibuprofen. The grey band indicates the 30 min during which the temperature was kept constant at 130 °C.

Figure 3. (A) Difference of ear thickness (μm) before and after induced inflammation for different tested groups of mice. (CTL: control, Ibu: ibuprofene, LI: [Lid][Ibu], LI°C: pre-heated [Lid][Ibu]) - (B) Time (s) before the mouse leaves or reacts to the hot plates for different tested groups. (CTL: control, Lido: lidocaine, LI: [Lid][Ibu], LI°C: pre-heated [Lid][Ibu])

Figure 4. Chromatogram from HPLC-UV measurement on digestive media after 240 min of Z20LI films simulated digestion without enzymes.

Figure 5. Release profile of lidocainium (A) and ibuprofenate (B) in the digestive media during the simulated digestion of thermomolded films of Z20LI and Z10GLY10LI, with

enzymes (E) or without enzyme (NE). Gastric phase is represented in white and intestinal phase in grey. Results are given as mean \pm Standard Deviations.

Figure 6. Schematic representation of zein, ibuprofen and lidocaine charge at different pH with their pKa/pI values (adapted from Bouman et al., 2016 and Brodkorb et al., 2019))

Figure 7. Volume of Z20LI and Z10GLY10LI films during the simulated digestion with enzymes (E) or without enzyme (NE). Size at 0 min is the volume of a 20 mm diameter film that was not yet immersed. Gastric phase is represented in white and intestinal phase in grey. Results are given as mean \pm SD.

Figure 8. Evolution of moisture content of Z20LI and Z10GLY10LI films during the simulated digestion, with enzymes (E) or without enzyme (NE). Gastric phase is represented in white and intestinal phase in grey. Results are given as mean \pm SD.

- Zein-based matrices are formed with plasticizing ionic liquid and glycerol
- The active ingredients keep their biological activity after thermal treatment
- The release of [Lidocainium] and [Ibuprofenate] is studied under digestive conditions
- Zein matrices undergo gastric and intestinal phases without noticeable erosion
- Release kinetics are controlled by diffusion and swelling mechanisms

Credit Author Statement

Mélanie Thadasack: Conceptualization, Data acquisition and analysis, Investigation, Methodology, Writing the original draft and validation of the manuscript. **Laurent Chaunier:** Conceptualization, Investigation, Methodology, Writing - Review and editing, Validation of the manuscript. **Hanitra Rabesona:** Data acquisition and analysis, Methodology, Validation of the manuscript. **Lydie Viau:** Methodology, Writing - Review and editing, Validation of the manuscript. **Marion De-Carvalho:** Data acquisition, Methodology, Validation of the manuscript. **Grégory Bouchaud:** Data acquisition and analysis, Methodology, Writing – Review, Validation of the manuscript. **Denis Lourdin:** Conceptualization, Methodology, Writing - Review and editing, Validation of the manuscript.

Declaration of interests

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: