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β-galactosidase orodispersible dosage forms for the treatment of lactose intolerance

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ABSTRACT

Lactose intolerance is associated with the insurgence of mild to severe gastrointestinal symptoms. The administration of β-galactosidase (β-gal) tablets or capsules, which are unsuitable for the dysphagic population, is the main symptomatic treatment. This work aimed to demonstrate the feasibility of β-gal orodispersible films (ODF) prepared by solvent casting technique. Since the preparation involves thermal and mechanical stresses, which can compromise the enzyme stability, *in vitro* performances of ODF were compared to those of oral lyophilizates (OL).

ODF were made of maltodextrin DE6, glycerol and Capryol®90. OL made of different grades of maltodextrins, and sorbitol or trehalose were prepared in aluminium blisters. ODF and OL were assayed for disintegration time and β-gal activity. The hydrolysis rate of lactose was determined using: a) a placebo capsule (500 mg lactose) disintegrated in a glass of water; b) 150 mL milk in biorelevant media. ODF (6 cm^2) and OL (made of maltodextrin DE19 and trehalose). ODF and OL loaded with about 4000 UI of β-gal were stable over 3 months of storage at 25 °C/60% RH. Both of them allowed the hydrolysis of lactose in water within 15 min. The complex composition of milk affected the hydrolysis rate (K) of lactose: the reaction was faster in fasted-state media ($K \approx -0.07$ min^{-1}) than in those simulating the fed state of subjects with physiologically appreciable residual gastric fluid in the stomach (grade 1 antrum: 20 mL, $K \approx -0.005$ min^{-1}; grade 2 antrum: 180 mL, $K \approx -0.01$ min^{-1}). No significant differences were noticed between ODF and OL. Overall, ODF can be proposed to hydrolyse lactose contained in immediate release dosage forms, improving the patient's adherence to therapy. Moreover, the dependence of the lactose degradation kinetic not only on the fed or fasted conditions, but also on the antrum phenotype may allow the development of ODF in a more patient-centric perspective.

1. Introduction

Lactose intolerance is related to the manifestation of abdominal pain, bloating and diarrhoea after the food ingestion which are distressing to patients. It can be due to congenital lactase deficiency, that is a rare paediatric disease, or an abnormal reduction of the lactase activity, which peaks at the time of birth and progressively decreases till adulthood [1]. In the most serious cases, lactose malabsorption not only limits the food consumption due to the manifestation of symptoms, but also it affects the bioavailability of drugs administered by oral dosage forms containing lactose as an excipient. As an example, the presence of lactose in levothyroxine tablets requires a significant increase of the dose in patients affected by lactose intolerance [2]. Beside reducing or eliminating the consumption of dairy products, possible treatments also include the use of food supplements containing probiotic strains such as *Lactobacilli* spp., *Bifidobacteria* encoding the glycoside hydrolase β-galactosidase (β-gal), or, more frequently, β-gal itself [3,4]. Exogenically supplemented β-gal formulations are usually available as capsules or tablets, which are not adequate for patients with dysphagia, travellers, and people with fear of chocking. Orodispersible dosage forms (ODx) are among the first choices to solve these issues since they rapidly dissolve/disintegrate in saliva, producing a fine suspension or solution of the drug, without requiring fluid intake or chewing. The disintegration occurs within 3 min, depending on the excipients selected and adopted production strategy [5]. The first developed ODx were oral lyophilizates (OL) which disintegrate within a few seconds, thanks the high solubility of excipients and the porosity of matrices [6]. Orodispersible films (ODF) are plasticized polymeric sheets [7], which

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combine the prompt release of the payload (i.e. small molecules, nanocrystals or microparticles) with the elimination of the fear of chocking [5].

Although they represent a valid alternative to OL, ODF have not yet been exploited to administer biomolecules because of the relatively high temperature required in the main production techniques (i.e., solvent-casting, hot-melt extrusion and 3D printing). Moreover, the compatibility between proteins and film forming polymers and the limited formulation space are among the common challenges to address to avoid protein denaturation [8]. Among polysaccharides generally used to design ODF, maltodextrins (MDX) are an amorphous film-forming material obtained by the depolymerization of starch, which was demonstrated suitable to microencapsulate sensitive biomolecules, especially probiotics [9].

Based on these considerations, the main purpose of this study was to develop ODF based on MDX for the release of β-gal. The importance of this investigation lies in offering a novel formulation able to address current challenges associated with lactose intolerance. First, ODF meet patient's special needs, keeping in mind not only the paediatric, but also the increased age of population and the occurrence of psychiatric and neurodegenerative diseases. In addition, β-gal loaded ODF can be proposed to hydrolyse lactose released from immediate-release dosage forms before intake.

The enzymatic activity of different amounts of β-gal loaded into ODF was investigated after preparation by solvent-casting evaporation and subsequent storage. In particular, the best formulations were selected to hydrolyse lactose released from a placebo capsule in a glass of commercially-available spring water, or contained in a complex matrix (i.e., milk). All results were compared to the performance of β-gal loaded in an OL formulated with a similar quali-quantitative composition to better evaluate the possible impact of stirring and heating on the stability of the protein and more in general the ODF performances.

2. Materials and methods

2.1. Materials

β-galactosidase from *Aspergillus* oryzae (β-gal, ACEF, I); maltodextrin DE6 (MDX 6), DE12 (MDX 12), DE19 (MDX 19) and DE38 (MDX 38; Roquette, F); trehalose, (VWR International, I); glycerol and sorbitol (Farmalabor, I); propylene glycol monocaprylate type II (CAPRYOL®90, Gattefosse, F).

All the solvents were of analytic grade, unless otherwise specified.

2.2. Orodispersible film (ODF)

ODF with a β-gal content ranging from 50 to 650 UI/cm^2 were prepared by the solvent-casting technique using a Mathis LTE apparatus [10]. The formula containing MDX 6 plasticized by glycerol; Capryol®90 was added to allow the wetting of a siliconized foil upon spreading of the slurry (Table 1). The impact of process parameters (e.g., drying temperature and time) on enzyme activity was also preliminarily evaluated and set at 70 °C, 20 min and 1500 rpm (Table 2).

The technological features of ODF were assessed by determining the loss on drying (LOD) (thermobalance, Gilbertini, I), film thickness (MI 1000 μm, ChemInstruments, USA), and stickiness following the same experimental protocols already described by Musazzi and coworkers [11]. In particular, ODF stickiness was measured by the thumb tack test and expressed by the following score system: A (not sticky), B (sticky), and C (very sticky).

2.3. Oral lyophilizate (OL) preparation

2.3.1. Thermal characterization of the MDX slurries

To tailor the process and formulation parameters, the glass transition temperature of a maximally cryo-concentrated solution (T_g') of MDX 12, MDX 19, MDX 38, or MDX/cryoprotectant (i.e., trehalose and sorbitol) solutions with or without β-gal (Table 1) were investigated using a DSC Star System (Mettler Toledo, CH). Aliquots of each solution of about 20 mg were accurately weighed and transferred to an aluminium pan, then closed with crucible lid and sealed with a press. Samples were cooled from 25 °C to −40 °C at a rate of 1.5 °C min^{-1} and maintained for 10 min, then thawed from −40 °C to 20 °C at a rate of 5 °C/min under a nitrogen gas flow of 80 mL/min.

2.3.2. Freeze-drying process

For the preparation of OL, blisters composed by an aluminium sheet comprising 80 cavities with a maximum volume of 1.5 mL each were used. One sheet was cut into pieces with 10 cavities each to allow the placement procedure on the central plate of a Martin Christ freeze-drier Epsilon 2–6 (Martin Christ, D). Slurries made of MDX 19, β-gal and with/without cryoprotectants (i.e., trehalose and sorbitol) were frozen at the rate of 1 K min^{-1} to −40 °C and held for 5 h.

Then, the chamber pressure was set to 0.180 mBar to initiate the main drying at −10 °C for 23 h. In the secondary drying, the shelf temperature was increased to 30 °C at the rate of 0.1 K/min and held for 5 h.

After freeze-drying, samples were removed for the blisters and transferred into vials. To protect them from the environmental moisture, the vials were stoppered under vacuum. OL were stored at 25 ± 1 °C until use and characterized in terms of aspects, uniformity of mass, enzymatic content, and residual activity.

2.4. Disintegration time

The disintegration test on OL and ODF (6 cm^2) was carried out in purified water using apparatus and specifications described in the Ph. Eur. monograph on "Disintegration of tablets and capsules" for orodispersible tablets.

Table 1
Composition of the slurries used for the preparation of ODx (%, w/w).

Form.	Theoretical β-gal UI/ODx	Slurry composition (%, w/w)							
		β-gal	MDX 6	MDX 19	Glycerol	Capriol90	Trehalose	Sorbitol	Water
ODF-1	300	2.23	56.20	–	15.53	2.22	–	–	23.77
ODF-2	800	6.17	53.96	–	14.91	2.13	–	–	22.83
ODF-3	2400	11.78	50.74	–	14.02	2.00	–	–	21.46
ODF-4	3900	24.49	43.43	–	12.00	1.71	–	–	18.37
OL-1	1200	1.20	–	40.00	–	–	–	–	58.80
OL-2	2400	2.40	–	40.00	–	–	–	–	57.60
OL-3	3600	3.60	–	40.00	–	–	–	–	56.40
OL-4	1800	1.80	–	20.00	–	–	–	10.00	69.10
OL-5	1800	1.80	–	20.00	–	–	10.00	–	68.20
OL-6	2700	2.70	–	20.00	–	–	10.00	–	67.30
OL-7	4000	4.00	–	20.00	–	–	10.00	–	66.00

Table 2
Set up of process parameters to obtain homogeneous and not-sticky laminates for ODF. The experiments were carried out using the formulation ODF-2, containing 8% w/w of β-gal. ODF stickiness was expressed by the following score system: A (not sticky), B (sticky), and C (very sticky).

Run	Thickness (μm)	Air		Drying time (min)	Stickiness	LOD (%, w/w)	β-gal	
		Temperature (°C)	Speed (rpm)				mg/ODF	UI/ODF (%)
1	300	60	1200	30	C	n.d.[a]	n.d.[a]	n.d.[a]
2	300	70	1200	20	C	n.d.[a]	n.d.[a]	n.d.[a]
3	300	70	1200	25	B	7.8 ± 2.3	8.09 ± 0.85	743 ± 73 (90.52)
4	300	70	1500	20	A	6.0 ± 1.2	8.23 ± 1.45	821 ± 73 (99.71)
5	300	70	1500	45	A	5.2 ± 0.8	8.14 ± 2.57	623 ± 42 (75.80)

[a] Not determined due to the stickiness of the ODF.

2.5. β-gal content

The quantification of β-gal in the ODx was carried out by using a bicinchoninic acid protein assay (BCA) under manufacturer's instructions (Thermo Scientific, USA). Briefly, ODx were dissolved in citrate buffer at pH = 5 so that all samples had the same excipients concentration. Then, 25 μL of samples were mixed with 200 μL of working solution in 96-well polystyrene microtiter plates and incubated for 30 min at 37 ± 1 °C. Subsequently, the absorbance was measured at 562 nm using a Tecan Spark microplate reader (Tecan, CH) and values were correlated with protein concentration by referring to a calibration curve of unformulated β-gal solution (0.25–1.00 mg/mL).

2.6. Enzymatic activity assay

The β-gal activity was determined by a spectrophotometric assay as follows: 96-well microtiter plates were filled with a mixture of i) various concentrations of β-gal from ODF and OL and ii) ortho-nitrophenyl β-D-galactopyranoside (ONP-G) substrate (1.5 mg/mL) in 50 mM citrate buffer at pH = 5 (total volume, 0.1 mL). The mixtures were subsequently incubated for 10 min at room temperature and, then, the reaction was stopped by adding 0.1 mL of 1 M Na_2CO_3 to reach pH = 9. The substrate cleavage was correlated with enzyme activity by measuring the absorbance of ortho-nitrophenol (ONP) at 410 nm after 0 and 10 min of incubation, as well as after Na_2CO_3 addition using Tecan Spark microplate reader (Tecan, CH). The enzyme units in each sample were calculated by referring to those of the unformulated β-gal used as standard (theoretical specific activity of β-gal: 100 UI/mg).

2.7. In vitro lactose hydrolysis

2.7.1. Hydrolysis of lactose contained in placebo capsules

To simulate the ability of β-gal released from ODF1-4 or OL-7 to hydrolyse lactose used as excipient in a medicinal product, a hard-capsule containing 500 mg lactose was compounded and put in a glass containing 50 mL of commercially available spring water (measured pH = 6.40; fixed residue = 14 mg/L) and mixed with a teaspoon until disintegration. After 10 min, an OL or an ODF with a surface area of 6 cm^2 was added. At predetermined times (i.e., 5 and 10 min), aliquots of 200 μL were withdrawn and mixed with 100 μL of 0.1 M NaOH to stop the hydrolysis. The experiment was performed in triplicate for each formulation.

2.7.2. Simulated digestion of lactose contained in milk

To simulate the performance of β-gal contained in ODx after the ingestion of 150 mL of UHT-milk, a set of experiments was carried out using the simulated gastric fluid media (Table 3).

Briefly, about 6 cm^2 ODF-4 or OL-7 were dispersed in 180 mL (simulating the condition in which patients with grade 1 antrum who took 150 mL of UHT-milk; Fe–V1) or 300 mL (simulating the condition of patients with grade 2 antrum who took 150 mL of UHT-milk, Fe–V2) of the digestion medium. Independently of the vessel volume, each vessel contained approximately 6.5 g of lactose. A series of experiments was performed dissolving 6.5 g of lactose in Fa-V0 to control for the effect of milk composition on β-gal hydrolysis activity. For both experimental series, the parameters of USP dissolution apparatus were set at 37 ± 1 °C under paddle stirring at 50 rpm. At predetermined times, an aliquot of 2 mL was withdrawn, and the enzymatic reaction was stopped by adding 0.1 M NaOH.

Table 3
Composition and physicochemical properties of fed gastric medium simulating two phenotypes (Fe–V1 and Fe–V2) and fasted gastric medium Fa-V0 [12].

Component	Composition		
	Fe–V1	Fe–V2	Fa-V0
Acetic acid (mM)	–	17.12	–
Sodium acetate (mM)	–	29.75	–
Ortho-phosphoric acid (mM)	–	–	–
Sodium dihydrogen phosphate (mM)	–	–	–
Milk/buffer	1:0	1:1	–
Hydrochloric acid/sodium hydroxide	qs pH = 6.4	qs pH = 5	–
Sodium taurocholate (μM)	–	–	80
Lecithin (μM)	–	–	20
Pepsin (mg/mL)	–	–	0.1
Sodium chloride (mM)	148	237.02	34.2
Hydrochloric acid	–	–	qs pH = 1.6
Deionized water	–	–	qs ad 1 L
pH	6.4	5.0	1.6
Osmolality (mOsm kg^{-1})	559	400	120.7
Buffer capacity (mmol L^{-1} ΔpH^{-1})	21.33	21.33	–

2.7.3. HPLC determination of undigested lactose

The remaining lactose was quantified using an isocratic HPLC (HP1100 series, Agilent, UK), equipped with a quaternary pump, an auto-sampler, a thermostated column compartment at 35.0 ± 0.1 °C, and a RI detector. An aliquot of 10 μL was eluted through a HILIC column (Luna® Omega SUGAR, 3 μm, 100 Å 150 × 4.6 mm, Phenomenex, I) using a mixture of Milli Q® water/acetonitrile (25:75, % v/v) at the flow rate of 1 mL/min. Calibration curve was in the 1–25 mg/mL range ($R^2 > 0.99$).

2.8. Stability assay

Both ODF-4 and OL-7 were stored for 3 months at 25 °C/60% RH to evaluate the enzyme stability. At the end of study, formulation specimens were tested in terms of disintegration time, β-gal content, and enzymatic activity. Experiments were performed in triplicate for each formulation.

3. Results and discussion

3.1. Orodispersible films (ODF) preparation

Preliminarily, the drying variables were optimized to avoid stickiness of ODF (Table 2): air temperature and speed were the main parameters worthy of consideration as potentially affecting handling and the enzymatic activity. The solvent evaporation carried out at 70 °C for

20 min allowed to obtain ODF homogeneously opaque in appearance, easy to handle and able to disintegrated within 30 s. Independently of the enzyme content (3%–30% w/w), the drying parameters did not significantly affect either the protein content, or the enzymatic activity (Table 4). ODF with enzymatic activity ranging between 300 and 4000 UI were easily obtained considering an ODF size lower than 6 cm^2. It is worth noting that a good recovery of β-gal activity was obtained despite the drying step, which is a possible cause of protein denaturation. Indeed, the possible loss of the protein hydration shells upon drying can lead to a change in the three-dimensional structures. In ODF, 7% moisture content, generally present after drying to allow the proper handling of films, and glycerol may be responsible of the hydrogen bonds between the protein and hydroxyl groups of water and/or plasticizer, conserving the protein's three-dimensional structure. Moreover, it can be assumed that, in agreement with the vitrification theory, the molecular mobility of a protein is strongly reduced when incorporated the amorphous matrix made of MDX.

Furthermore, no significant deviations from expected values of β-gal content and activity were observed either at preparation or after 3 months of storage at room temperature (Table 4).

3.2. Oral lyophilizates (OL) preparation

A preliminary study was carried out to tune up the composition of slurries to be freeze-dried. Since T_g' of MDX was strongly dependent on the molecular weight, MDX 38 solutions presenting a T_g' value of −23 °C was discarded. Despite the similar values of T_g' between MDX 12 and MDX 19 (T_g' MDX 12 = −10.7 °C and T_g' MDX 19 = −13.3 °C), MDX 19 was preferred due to the faster disintegration time.

At the highest amount of β-gal loaded, lyophilized cakes presented bubbles and an inhomogeneous structure (formulations OL-1-3, Table 1). This result may be due to the significant decrease of T_g' values in presence of increasing amount of β-gal (Fig. 1). Hence, the concentration of MDX 19 was decreased from 40% to 20% and sugar, i.e. trehalose or sorbitol, was added (formulations OL4-6, Table 1). In all cases, the visual appearance improved without compromising the enzymatic activity. However, only the composition MDX 19/trehalose in the ratio 2:1 w/w allowed to preserve about 97% of β-gal activity after 3 months of storage at 25 °C; while in presence of MDX or MDX 19/sorbitol, a 50% reduction of activity was measured. The combination of MDX 19/trehalose was loaded up with 12% β-gal and the final OL, weighting about 340 mg, presented a final enzyme content of about 4000 UI/OL (formulation OL-7, Table 1).

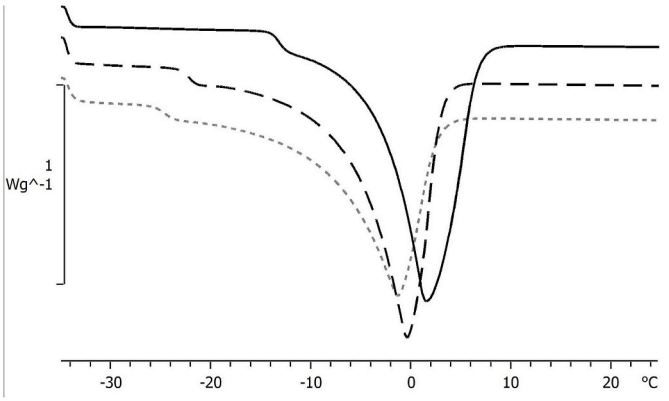

Fig. 1. Thermograms of 20% MDX 19 solution (solid line) containing 1.5 % (dashed line) and 9% (dotted line) β-gal.

3.3. Hydrolysis of lactose contained in a medicinal product in water

The experiment was conducted to evaluate the performances of ODF and OL-7 when used to hydrolyse lactose contained in an oral immediate release dosage form. The idea is that a capsule or tablet would disintegrate in a glass of water. Subsequently, an ODx would be added, allowing the patient to consume a dispersion or solution in which the lactose has already been hydrolysed.

Fig. 2 evidenced that lactose hydrolysis occurred very fast for almost all tested formulations. As expected, the efficiency in lactose hydrolysis is concentration and time dependent. In the case of ODF-1, the amount of loaded β-gal was too low for the intended use. ODx at higher content of β-gal hydrolysed more 40% of lactose in the first 5 min, and more than 55% within 10 min. Only in the case of ODF-4, the concentration of non-hydrolysed lactose after 15 min was lower than the LOQ (data not shown). Similarly, to ODF-4, also the OL-7 containing 3900 UI of β-gal, completely degraded the lactose after 15 min. Thus, based on the performance in water, ODF-4 and OL-7 can be proposed to eliminate lactose contained in tablet or capsule before intake. Indeed, considering the lactose amount generally contained in a single tablet or capsule, it is reasonable to suppose that a polytherapy subject would intake maximum 500 mg of this excipient per oral administration. Hence, this approach can be a valuable alternative if lactose-free oral dosage forms are not available on the marker or cannot be compounded in a pharmacy setting.

Table 4
β-gal content and enzymatic activity in the ODx formulations at preparation and after 3 months at 25 °C/60% RH.

Form. ID	At preparation			After 3 months		
	β-gal content, mg/ODx[a]	β-gal activity		*β-gal content, mg/ODx*[a]	β-gal activity	
		UI/ODx	%[b]		UI/ODx	%[b]
ODF-1	5.16 ± 0.14	303 ± 29	86.35	5.22 ± 0.21	298 ± 30	83.95
ODF-2	12.34 ± 1.45	821 ± 73	97.80	11.8 ± 0.16	830 ± 50	103.44
ODF-3	35.43 ± 1.91	2364 ± 545	98.12	34.8 ± 0.60	2360 ± 157	99.73
ODF-4	57.72 ± 3.02	3910 ± 75	99.62	56.82 ± 0.93	4025 ± 108	104.17
OL-7	56.94 ± 2.04	3890 ± 29	100.79	57.03 ± 1.34	3922 ± 62	101.28

[a] ODF having area equal to 6 cm^2.
[b] Compared to a theoretical β-gal activity estimated by considering the specific activity equal to 67.9 ± 1.2 UI/mg.

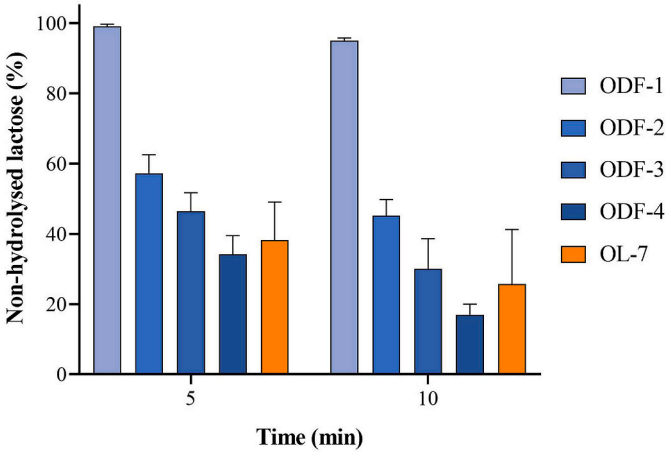

Fig. 2. Remaining percentage of non-hydrolysed lactose 5 and 10 min after the ODx disintegration. A capsule containing 500 mg lactose was previously disintegrated in the same glass of water.

3.4. In vitro lactose hydrolysis to simulate the digestion of milk

The *in vitro* lactose hydrolysis in presence of β-gal released from ODF-4 and OL-7 was evaluated in simulated fed gastric medium, simulating the condition in which 150 mL of UHT-milk was taken by a subject. Considering that it is well-known that volume of pre-existing gastric fluids may vary based on the physiological and anatomical features of patients, two digestion media were prepared: fed state simulated gastric fluids, namely Fe–V1 and Fe–V2. Fe–V1 is referred to a subject with a pre-existing gastric volume of 20 mL (grade 1 antrum) who drinks a glass of milk; Fe–V2 simulates the milk intake in patients with larger gastric volumes (≈150 mL; grade 2 antrum). Experiments using fasted gastric medium (Fa-V0) as control condition were also performed to determine the impact of fed medium on the enzyme performance [12].

The hydrolysis rate constants (K) in the three conditions are summarized in Fig. 3. When ODx were added to Fa-V0, all the K values are maximal (Fig. 3); 3900 UI loaded in ODx seem sufficient to hydrolyse lactose in less than 15 min.

A slightly better performance was observed for OL-7 ($p = 0.017$, Student's T-Test), but this difference is not relevant. When dissolved in biorelevant media simulating the food intake (i.e., Fe–V1 at pH = 6.4 and Fe–V2 at pH = 5.0), the hydrolysis kinetics slowed down, minimizing the differences between ODF-4 and OL-7 in terms of enzyme performances ($p > 0.05$, Student's T-Test). A marked dependence of hydrolysis rate on digestion medium was also observed. After 30 min the amount of hydrolysed lactose was 15% and 22% in the case of Fe–V1 and Fe–V2, respectively. This trend was confirmed over time: at 90 min, almost the whole quantity of lactose present in Fe–V2 was hydrolysed, whereas about half the initial amount of lactose was still present in Fe–V1. These results suggest that both pH and concentration of milk components (e.g., micronutrients) can impact on the enzyme's performance (Fig. 3). These findings were in line with literature data [13], above all the effect related to the pH value. Indeed, it is reported that the optimal pH for β-gal from *A. oryzae* ranges between 5.0 and 6.2 without requiring ionic activators or inhibitors [14]. This range includes the pH values of both media (Fe–V1, pH = 6.4; Fe–V2, pH = 5.0).

Considering that Fe–V1 and Fe–V2 mainly differ in nutrients' composition, a strong dependence of the β-gal activity on concentration of milk components can be foreseen; this confirms the importance of testing ODx performance in media simulating the physiological microenvironment of biomolecules. Although an *in vitro/in vivo* correlation is desirable, these results could be exploited also for a clinical point of view since they provide the evidence that the physiology of the stomach may strongly impact on the β-gal efficiency even if in a fasted state. In general, patients are advised to take β-gal used as dietary supplements between 0 and 30 min before the food consumption [14]. However, these indications do not consider the disintegration time of a dosage form containing β-gal and the physiologic volume of gastric fluids in fasted state. Such parameters are not critical for immediate-release dosage forms (e.g., tablets and capsules), that generally disintegrate between 15 and 30 min, but they can affect the performance of β-gal released from ODx in few minutes. In this context, for light meals (e.g., 150 mL of milk), a different fluid volume in the gastric antrum (patient at grade 0 = 0 ± 2 mL; patients at grade 1 = 16 ± 36 mL; patients at grade 2 = 180 ± 83 mL [15]) may result in a different dilution of bolus after food intake. Based on obtained results on ODx, the lactose hydrolysis rate seemed faster when the buffering effect of bolus was less significant, namely in patients having physiologically appreciable gastric fluid in fasted conditions (corresponding to grade 1 and 2 antrum [15]). On the contrary, the buffer capacity and the fat content of bolus (i.e., milk) have a strong impact in patients with very limited volume of gastric fluid, resulting in a slow degradation of lactose. This implies that patients at grade 0 and 1 would be exposed to a higher risk of side effects due to the remaining amount of not-degraded lactose with respect to patients at grade 2, after administration of the same dose of β-gal. To have similar efficiency of the enzyme in Fe–V1 and Fe–V2 in 30 min, two dosage forms should be taken.

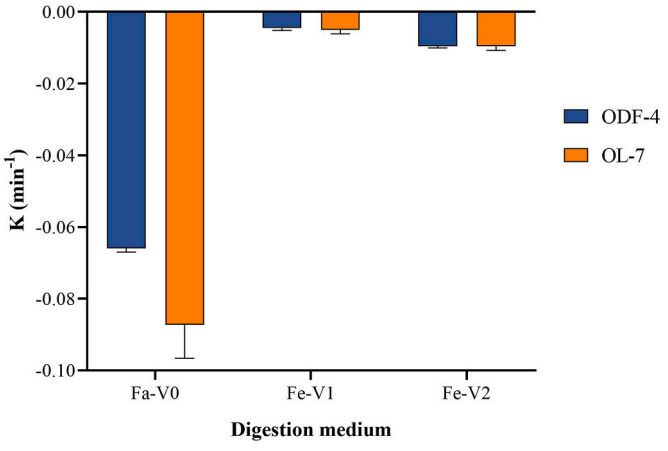

Fig. 3. Hydrolysis rate constants (K) of lactose in presence of ODF-4 and OL-7 in Fa-V0 at pH = 1.2, Fe–V1 at pH = 5.4 and Fe–V2 at pH = 6.8.

4. Conclusion

This proof-of-concept confirms the suitability of ODF to load active substances with different characteristics, including enzymes which can pose some issues in the definition of the production process due to their sensitivity to various stresses (i.e., thermal and shear stress). Based on the preliminary studies, it was possible to design ODF enabling to extend the patient's choice of dosage forms and their applications in the treatment of lactose intolerance. In another words, these dosage forms allow the tune-up of the amount of β-gal as a function of the lactose content in oral dosage forms or foods, and the physiologic features of the patient. The first feature is relevant to fulfil the special needs of patients, when alternatives are not commercially available. Hence, ODF can be proposed to hydrolyse lactose released from an immediate-release dosage form directly in a glass of water, which can help to improve the patient's adherence to treatment, above all in case of chronic diseases or polytherapy. Finally, this study on the ability of β-gal to hydrolyse lactose in different biorelevant conditions reveals the dependence of the degradation kinetic not only on the fed or fasted conditions, but also on phenotype of the subject affected by lactose intolerance. This aspect is important in terms of patient-centric formulations because the proposed ODF can satisfy different phenotypes of subjects.

CRediT authorship contribution statement

Umberto M. Musazzi: Writing – original draft, Supervision, Conceptualization. **Chiara Meazzini:** Formal analysis, Data curation. **Giulia Anderluzzi:** Writing – original draft, Formal analysis, Data curation. **Giorgia Frigerio:** Formal analysis, Data curation. **Francesca Selmin:** Writing – review & editing, Writing – original draft, Conceptualization. **Paola Minghetti:** Conceptualization. **Francesco Cilurzo:** Writing – review & editing, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the Writing process

During the preparation of this work, the Author did not use any AI tool and/or service. The Authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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