MATERIALE PLASTICE
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https://doi.org/10.37358/Mat.Plast.1964

Synthesis and Preliminary Characterization of Polyurethane Matrices Used as a Drug Carrier for Bromelain

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Abstract: *Bromelain, a natural substance sourced from pineapples, was found effective in various colds, in preventing heart attack and respectively in excess weight. On the other hand, it is well-known that pineapples juice increases the gastric acidity and respectively the people with hemophilia, internal bleeding must also be very careful in consuming this fruit. The objectives of this study were to obtain and to characterize a drug delivery system used for the transmembrane transfer of bromelain. The samples based on polyurethane matrices with and respectively without bromelain were synthesized and characterized by measurements of pH, encapsulation efficacy, cumulative drug release in a degradative media and in simulated gastric acid, electron microscopy and by modern techniques such as Zetasizer, cytotoxicity assay, and various non-invasive skin irritation assessment. The results indicate the obtaining of polyurethane matrices with mean sizes between 322-342 nm and neutral pH, that have a medium stability against the clusters formation tendency and a medium drug release rate. The non-irritative potential and the results on their cytotoxicity are important evidences that can be used for the further clinical trials of the polyurethane carriers.*

Keywords*: cell culture, pH, pineapple, release rate, skin tests, UV-Vis, Zetasizer*

1. Introduction

Bromelain ($C_{39}H_{66}N_2O_{29}$, M = 1026.9, Figure 1), a proteolytic enzyme, is often found as a dietary supplement [1]. Literature describes bromelain like an ancient remedy used for its anti-inflammatory and analgesic properties in osteoarthritis [2] and asthma [3], in cardiovascular diseases due to its ability to reduce clot formation [4], in chronic rhinosinusitis [5], colitis [6], burns [7], and cancer [8]. On the other hand, the ingest of bromelain as a dietary supplement must be approved by a physician due to its side effects: vomiting, nausea, diarrhea, and enhanced menstrual bleeding; bromelain is a natural blood thinner and it must be avoided before and after surgery [9]. There are known a few interactions of bromelain with the following drugs: blood thinners (Warfarin, Aspirin, Heparin), antibiotics (Amoxicillin, Tetracyline), and sedatives (Xanax, Valium) [10,11].

The interest to develop a carrier for bromelain can be expressed by the high number of the published studies; thus, there have been reported the obtaining of bromelain capped gold nanoparticles [12] and the development of aquasomes for its oral delivery [13]. A bacterial cellulose membrane containing bromelain was presented in the literature [2]; the authors found that their carrier can improve the antimicrobial activity 9 times than the pure substance.

Drug delivery systems represent a very good solution to protect sensitive pharmaceutical substances against the hostile medium from the gastrointestinal tract, to deliver the loaded active agents to a specific targeted organ with a controlled release, and to modify the properties of drugs (aqueous solubility, bioavailability etc.). The first studies on polyurethane drug carriers were published in the best period of nanotechnology development, the last two decades of the last century, at approx. 50 years after the discovery of the polyurethane foams (O. Bayer, 1937); the drug release from a polyurethane gel [14] and from poly(ethylene oxide)- and poly(tetramethylene oxide)-based segmented polyurethanes [15] was investigated in that period.

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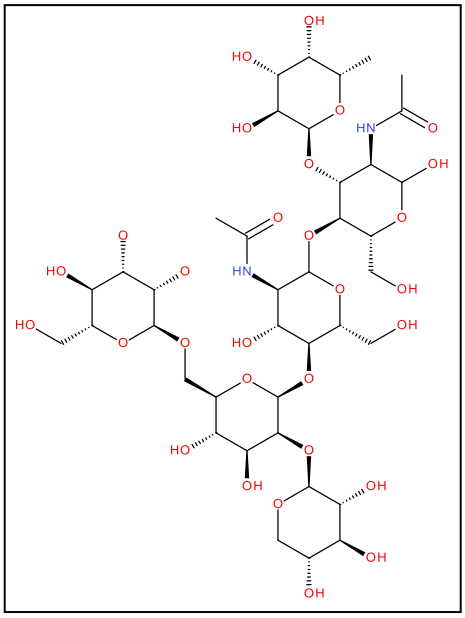

Figure 1. Chemical structure of bromelain

Polyurethane matrices present a high interest in various industrial fields such as the obtaining of foams and automotive appliances due to their simple *in situ* synthesis and to their versatility [16]. Their use was transferred from industrial composites to the drug carriers relatively recent: it has been assessed the drug-release mechanism from polyurethanes matrices [17], while the modulation of the drug release kinetics from polyurethane matrices was also presented in the literature [18].

Our research team has started to develop polyurethane drug carriers almost 10 years ago, when we have reported the first syntheses and *in vivo* evaluations [16] and the optimization of the size and the stability against the tendency to form particles agglomeration [19]. The main aims of the present study were to obtain and to characterize polyurethane matrices that can be used as a drug delivery system for bromelain. The novelty of this research is represented by the use of a small number of raw materials compared to all previous studies when co-polymer matrices were obtained; the elimination of auxiliary reagents is often considered as an effective method of reducing health hazards.

2. Materials and methods

2.1. Chemicals

The following reagents were used in this research: bromelain (from pineapples) 2 U/mg from BioChemica | Hallstar BPC (Arcore, Italy), tris(hydroxymethyl)aminomethane (THAM) as a crosslinker and polyethylene-glycol (PEG, M≈200) from Aldrich (Sofia, Bulgaria), Span® 85 as emulsifier from Sigma (Balcatta, USA), and isophorone-diisocyanate (IPDI) from Merck (Darmstadt, Germany); mono-ethylene glycol (MEG) from Lach-Ner s.r.o. (Neratovice, Czech Rep.) and 1,4-butanediol (BD) from Carl Roth GmbH (Karlsruhe, Germany) as polyurethane chains extenders, while the other chemicals (acetone, acetic anhydride, pyridine, HCl, and inorganic salts) from Chimopar S.A. (Bucharest, Romania). The reagents were used without any previous purification. Double distilled water prepared at in-house facility was used throughout.

2.2. The acetylation of bromelain

The chemical bonds between the hydroxyl groups of bromelain and the -NCO groups of IPDI lead to a very retarded release of the drug from the polyurethane matrices; this is the reason why the esterification of the -OH groups was necessary before the synthesis of the polymer carrier. 5.0 g bromelain was dissolved in a solution containing 20 mL acetic anhydride and anhydrous pyridine (1:1, v/v); the mixture was refluxed using 0.05 g dimethyl-amino-pyridine as catalyst and an excess of acetic

anhydride (10 drops) for 50 min. Finally, the product was concentrated by vacuum filtration at 200 mbar through a G4 Buchner funnel with sintered ware disc and then it was slowly dried at room temperature and atmospheric pressure; 4.1 g dried product was obtained. The efficacy of the esterification process was checked using two samples of bromelain (before and after the esterification); powder of the first sample, respectively of the second sample was mixed with KBr powder (1:20 w/w) and packed into pellets for IR measurement, using a Jasco FTIR spectrometer FT / IR 410. Only the acetylated sample showed a strong vibration displacement at 1743 cm^{-1}, relative to C=O stretching from esters, respectively a strong stretching at 1248 cm^{-1} is corresponding to C–O bonds from esters.

2.3. The synthesis of polyurethane matrices

The next steps to obtain the drug delivery system are based on the formation of macromolecular chains: - a hydroxylic component was prepared by using 2.8 mL MEG, 2.2 mL BD, 3.5 mL PEG, 1.1 g THAM, 1.0 mL Span®85, and 30 mL distilled water; the mixture was homogenized for 15 min. with 350 rpm at room temperature;

- an organic component based on 8.0 mL IPDI in 35 mL acetone was homogenized for 15 min. in the same conditions; it was rapidly injected in the hydroxylic component under magnetic stirring (525 rpm) at 35±2°C. The stirring was continued for 6 h to ensure the complete synthesis of macro-molecular chains.

The synthesized product was purified by repeated washing / centrifugations using a mixture based on water-acetone (1: 1.4, v/v) and then it was dried as thin layers in borosilicate glass Petri dishes at 55°C inside a PolEko SL115 drying oven till no mass change was observed (20±4 h).

The entire experiment was repeated two times (without and with 1.5 g acetylated bromelain inside the hydroxylic component) in order to synthesize different samples for further comparative characterizations: PUM_0 (polyurethane matrices without bromelain), PUM_ 1 (polyurethane matrices with bromelain).

2.4. The preliminary characterization

The *p*H values of the diluted aqueous solutions (2%, w/v) were determined using a portable pH Meter Checker® Hanna Instruments (Woonsocket, USA) that was previously calibrated using commercial buffer solutions with *p*H = 4.01, 7.00, and 10.00 from Mettler-Toledo (Barcelona, Spain).

An UVi Line 9400 SI Analytics Spectrophotometer (Mainz, Germany) was used to investigate the encapsulation efficacy (EE) of bromelain inside the polyurethane matrices and the cumulative drug release (CDR) according to the following procedures that were already published by our team: EE can be estimated by reporting the amount of the free bromelain to the total amount that was used in the synthesis: $EE = [(W_i–W_f)/W_i]\times100$ %, where W_f is the total amount of bromelain from the water-acetone mixture used in the washing / centrifugation process and W_i is the total quantity of bromelain added initially [20], while CDR was calculated after the exposure of sample PUM_1 inside a degradative media consisting in a mixture of $NaHCO_3$, Na_2HPO_4, NaCl, KH_2PO_4, K_2HPO_4, KCl and $MgCl_2$ at similar concentrations and *p*H with the human plasma. CDR = volume of sample withdrawn (mL) / bath volume $\times P_{(t–1)} + P_t$, where P_t is percentage release at time "t" and $P_{(t–1)}$ is percentage release previous to "t" [21]; all spectrometry analyses were based on the difference between the maximum absorption (280 nm for bromelain and around 358 nm for polyurethane matrices), a calibration curve (bromelain absorption vs. its concentration) and the Beer-Lambert law.

The *in vitro* dissolution profile of samples, a critical quality attribute of a pharmaceutical formulation with oral administration, was assessed using a modified Erweka DT instrument (Langen, Germany), at 37 ± 2°C, in 100 mL simulated gastric medium, at 100 rpm; 5 mL samples were collected in each case at 1/ 4, 1/ 2, 1, 2, 4, 6, 8, 10, 18 and 24 h to evaluate the release profile of bromelain; the procedure was already presented in detail in the literature [22].

The average diameter and the surface charge of the polyurethane particles inside the matrices with and without bromelain were assessed using a Vasco Particle Size Analyzer and a Wallis Zeta potential

Analyzer from Cordouan Technology (Pessac, France); the following input parameters have been chosen: evaluation temperature (30±1°C), interval of time (10±3 μs), number of channels (around 420), power of laser (85±5%), acquisition mode (continuous), analysis mode (Pade-Laplace), Wallis resolution (medium), and Smoluchowski model as Henry function.

The morphological aspect of the samples was comparatively investigated using a Quanta 250 FEI scanning electron microscope (Eindhoven, The Netherlands). The accelerating voltages value was set at 10 kV via the "electron column" console and the magnifications to 80x.

DSC curves were obtained by a Mettler-Toledo DSC1 instrument (Greifensee, Switzerland) between 40 and 260°C in an inert atmosphere (100 mL/min Ar) using aluminum crucibles with pierced cap and a 5 degree/min heating rate.

The cytotoxic activity of the synthesized samples was tested on human dermal fibroblasts (Invitrogen, USA); the cells were cultured in Dulbecco's Modified Eagle's Medium containing fetal calf serum (PromoCell, Germany) and penicillin-streptomycin (PromoCell, Germany). Cells were maintained in an atmosphere of 5 % CO_2 at 37°C. The cells viability was assessed by Alamar Blue *in vitro* analysis, using a spectrophotometer and the wavelengths that are specific to oxidized and reduced forms (570 and 600 nm respectively) according to the procedure already presented in the literature [23].

Ten volunteers (4 men and 6 women, mean age 32.7 years) were enrolled to assess the irritation effect of the synthesized samples according to a protocol that was described by our research team [24]. Every volunteer has signed an informed consent about him/her participation in the study and about the publication of the results. All evaluations were done using a professional MPA System from Courage&Khazaka (Koln, Germany) equipped with a Tewameter®TM300 probe for the evaluation of the transepidermal water loss (TEWL) and a Mexameter®MX18 probe to assess the level of erythema; volunteers were maintained approx. 20 min in the lab before any evaluation and all measurements were done by the same operator in triplicate; all values were the subject of statistics evaluation; * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

3. Results and discussions

The *p*H measurements of samples, as diluted aqueous solutions (2%, w/v), indicate the obtaining of almost neutral samples: 6.64±0.17 for sample PUM_0 and 6.32±0.14 for sample PUM_1. All drug delivery systems are very important for the convenient, safe and effective administration of active pharmaceutical substances. The *p*H of these carriers is a very important parameter for the safety of its administration and for its ability to cross biological membranes [25]. The *p*H values of the present samples are proper for a drug carrier that can be included in clinical trials.

A calibration curve between 0.05 and 15.00 μg / mL bromelain was drawn to calculate EE. It was found a very good encapsulation efficacy (EE= 65.7%) based on the estimation of bromelain concentration using the Beer-Lambert law [26, 27]. Figure 2 presents the cumulative drug release from the synthesized drug delivery system.

The main advantage deriving from the use of various degradation media is represented by their ability to mimic real conditions to investigate the degradation of the carrier particles and the release of the loaded drugs to the receptor; the literature presents a simulated body fluid consisting of a mixture of different inorganic salts at similar concentrations and *p*H as human plasma [28], which was used by our team as a degradation medium to study the release profile. The release profile (Figure 2) is specific to a drug delivery system that present a medium degradation rate according to the literature [29]: the profile for the first 120 h can be fitted to a first-order equation (R^2= 0.91), while the last 120 h fitted to the Higuchi equation (R^2= 0.87). It is important to mention that this degradation rate is strongly influenced by the *p*H of the degradative medium and it is very different between an aerobic and an anaerobic medium. Fortunately, the degradation rate of the drug carriers based on polyurethane structures can be easily adjusted by using different ratios of polyether vs. polyesters in their hydroxylic component.

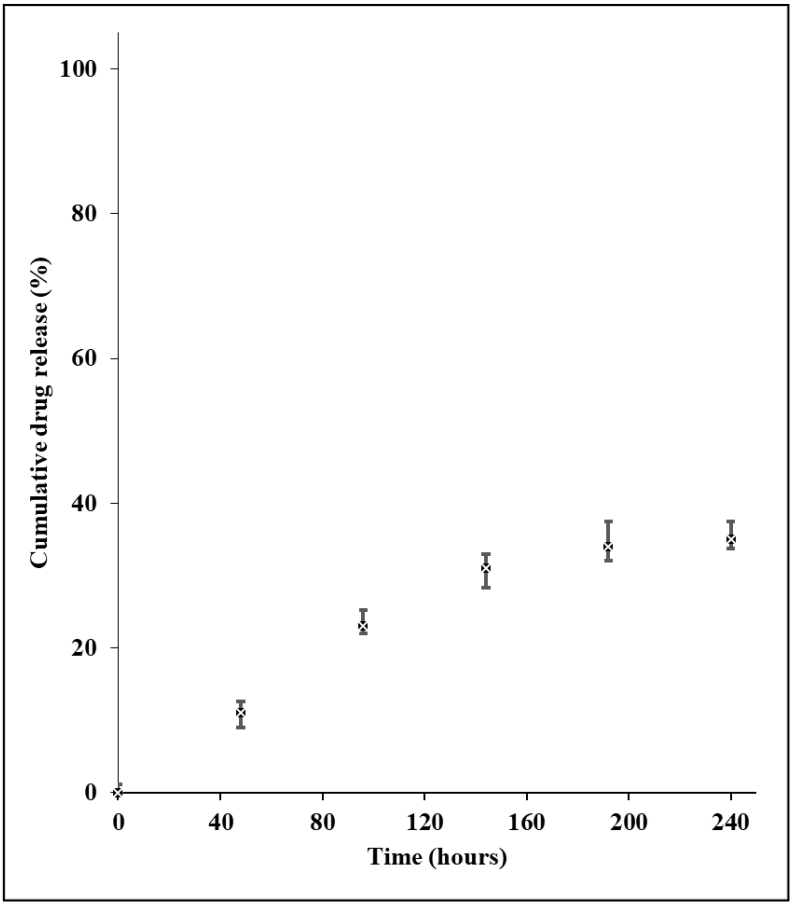

Figure 2. The release profile in T. Kokubo medium

Bromelain is known as a dietary supplement that presents a few side effects such as diarrhea and stomach upset; its oral administration must comply with the prescribed medication. The oral administration of bromelain as 40 mg at every 6 h for 6 days has been studied and presented in the literature [30]. It is well-known that the drug accumulation appears with repeated dosing, and it is necessary a delay in time to eliminate any drug from the body; the encapsulation of the active agents inside polymer carriers represents a solution to this problem. An accelerated degradation of our polyurethane matrices was found in the evaluation of the dissolution profile using a simulated gastric medium (Figure 3) compared to the release of bromelain from polyurethane matrices that were maintained inside a degradative media - almost three quarters of the bromelain amount that was encapsulated was released in the first 24 h. Such a dissolution profile, very similar to the one described in another study [31], is the proof of the obtaining of an oral drug delivery system that ensures a suitable bioavailability.

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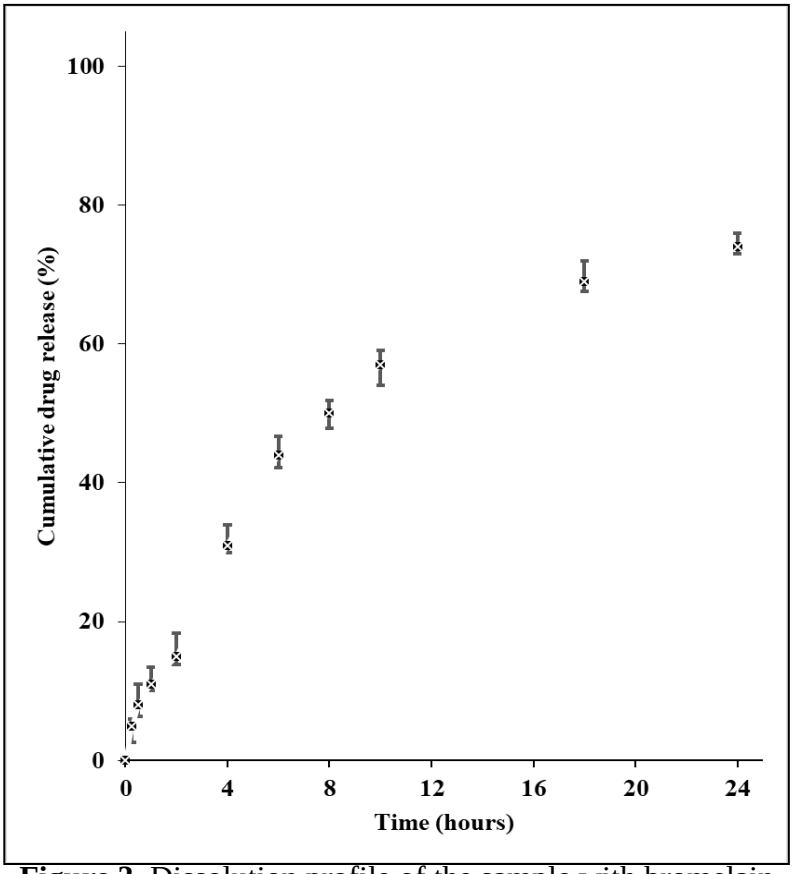


Figure 3. Dissolution profile of the sample with bromelain

A much faster release was observed inside the simulated gastric medium than that observed in the simulated plasma; this aspect is very important for a drug delivery system with oral administration route because the accumulation of synthetic compounds is avoided in this way.

Table 1 presents the Zetasizer characterization of samples PUM_0 and PUM_1. The values of the polydispersity index (PDI) indicate the obtaining of two samples with medium homogeneity and they are specific to colloidal suspensions with multiple populations, while the Zeta potential values show a medium tendency to form particles clusters according to other published data [32].

Table 1. Experimental values of the Zetasizer characterization.

Sample	Size of structures (nm)		Zeta potential (mV)
	Mean ± SD	PDI	
PUM_0	322 ± 18	0.5	+24.17
PUM_1	342 ± 21	0.6	+26.09

A good release of the entrapped substances is obtained when the loaded agent is physically encapsulated; any chemical bond between the carrier and the active agent leads to a delayed and partial release. Bromelain is an enzyme that presents many -OH and -NH groups that can form covalent bonds with the isocyanate groups during the carrier synthesis; there is the possibility of grafting the active agent to the carrier even though bromelain was acetylated before its encapsulation. This grafting leads to the modification of the carrier properties and many of these changes can be observed through electron microscopy. Figure 4 comparatively presents the SEM images of the carrier without and with bromelain. There is no important difference between the samples; both of them are polydisperse systems that contains large particles due to its medium tendency to form clusters (Zeta potential between 20-30 mV).

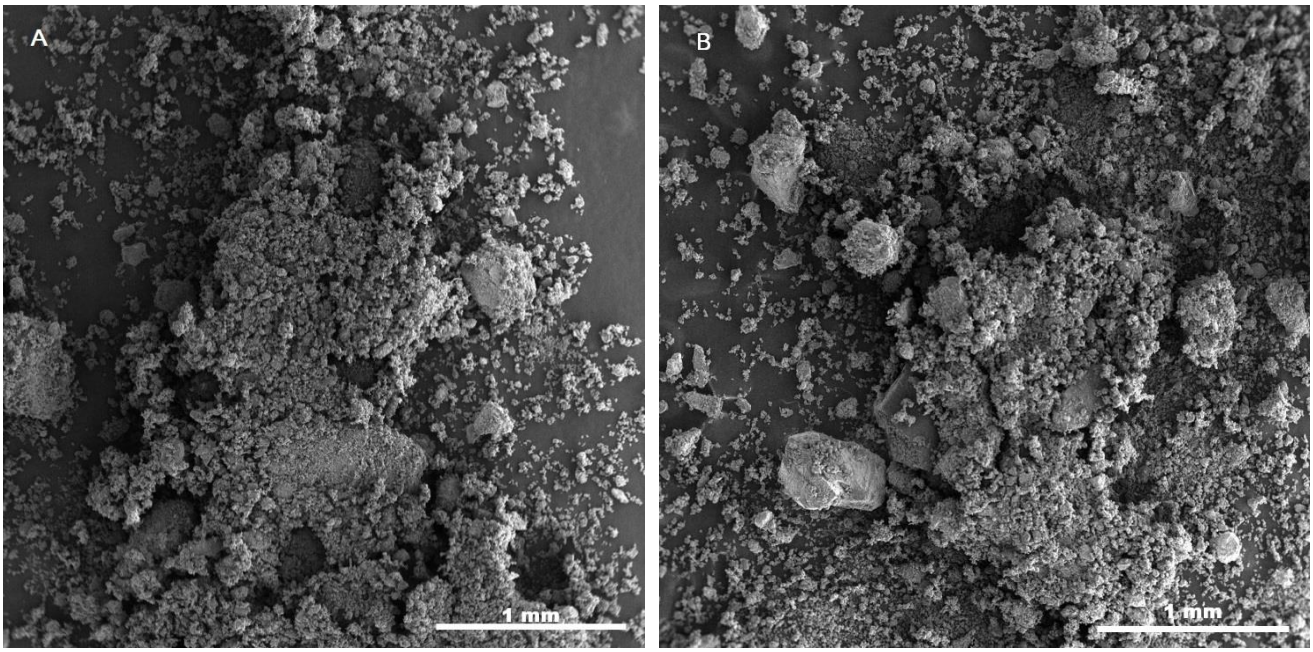


Figure 4. The aspect of the samples: A (PUM-0) and B (PUM-1)

The thermal resistance of the materials used in pharmaceutical industry can be used as a predictor for the compatibility between the active agent and the excipients or as a predictor for its shelf-life which determines the time when a product is considered to be safe and effective. The DSC curves (Figure 5) indicate that the synthesized samples are very similar and they are very stable in the studied temperature range. However, it is worth to mention that bromelain as an enzyme is a chemical compound that degrades at high temperatures as it appears in another study [33].

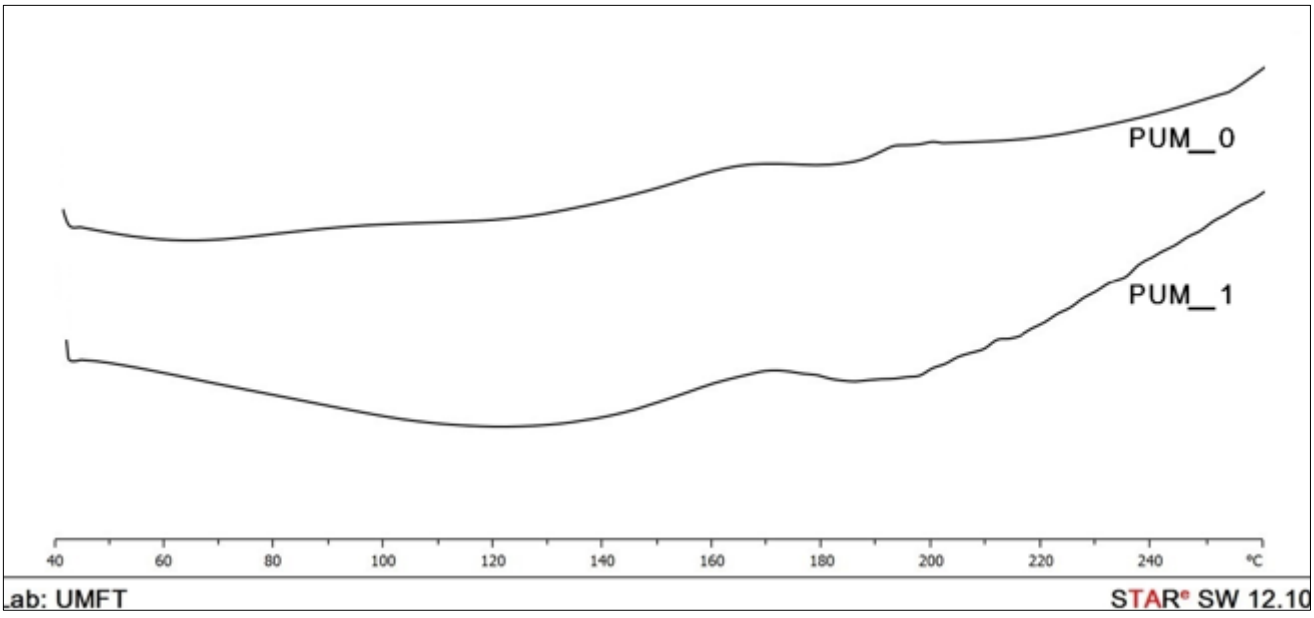


Figure 5. The thermal behavior of the samples

Cell cultures mimic the *in vivo* condition for studies on the chemical compounds and drugs toxicity studies and as efficacy markers; a huge number and types of cells lines are involved in various toxicity studies [34]. Primary Human Dermal Fibroblasts-adult (HDFa) are isolated from adult skin and they are often used in the research of skin diseases, melanoma, anti-aging efficacy or antimicrobial potential of new cosmetics, etc. Figure 6 comparatively presents the viability of HDFa at 24 and 48 h for the tested

samples. This assay revealed high cell viability values (more than 90% for 24 and 48 h); this result is probably due to the good biocompatibility and biodegradability of polyurethane samples which were mentioned previously in the literature [35,36]. Overall, the toxicity of the samples studied in this research, with or without bromelain is negligible ($p < 0.01$, one-way ANOVA, Tukey HSD test). Therefore, the samples are non-toxic and safe to be applied on the human skin.

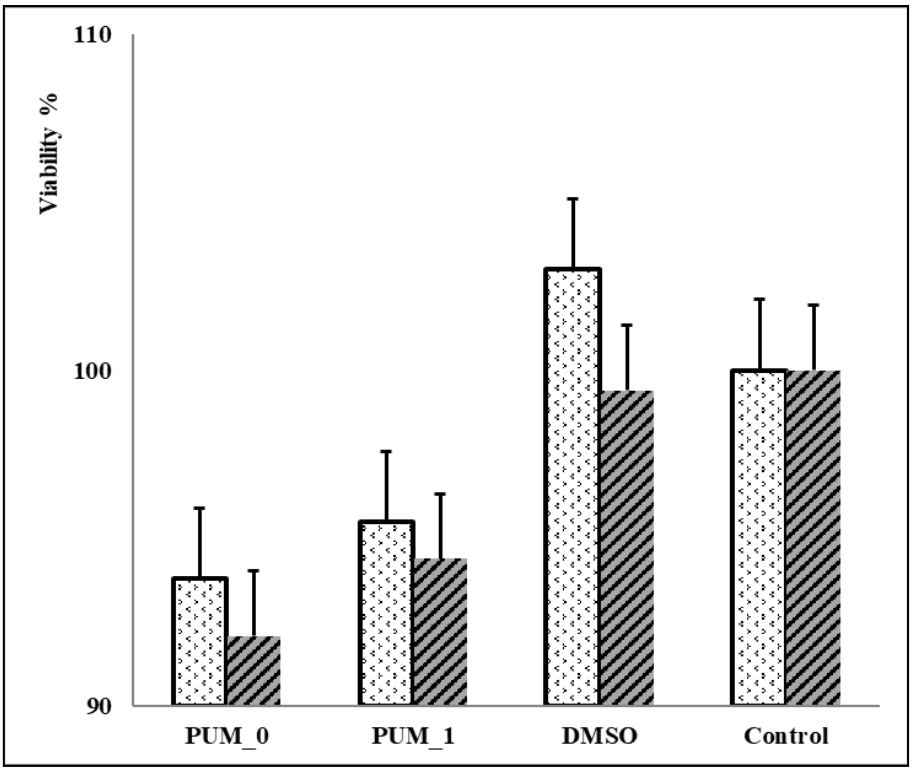
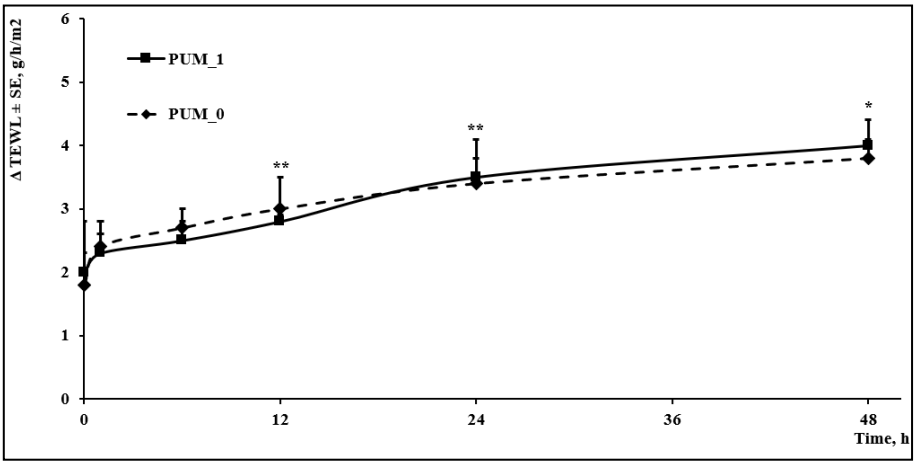


Figure 6. The cells viability, 24 and 48 h exposure on the tested compounds

Human and/or animal skin can be used to predict the irritation potential of new compounds and new pharmaceutical formulations. These tests are proper for many products intended to be used in different cutaneous applications, but they are proper for the investigation of oral delivery products that may irritate the oral cavity and oropharyngeal mucosa. Figure 7 presents the changes of the two main skin parameters that present important modifications during any irritation (TEWL and erythema).



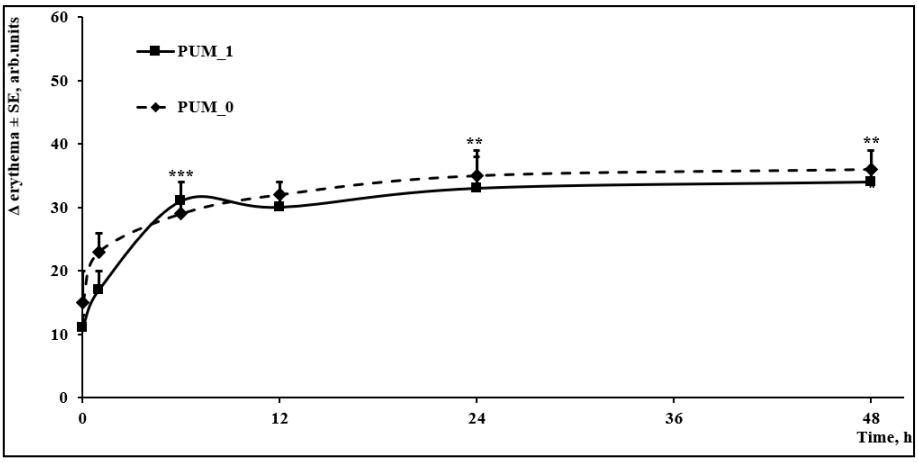


Figure 7. The evolution of the main skin parameters

Slight and similar increases of these two skin parameters were observed in the case of these two samples (PUM_0 and PUM_1). The increases of TEWL and erythema indicate unpleasant processes, but it is very important to compare the amplitude of these changes with those of a well-known irritative agent such as sodium lauryl sulfate used as reference in the literature [37]: they found TEWL increases approx. 11 g/h/m^2 (only 4 units in our case) and around 90 arbitr. units for erythema index (35 units in our case) using the same instruments in a shorter evaluation (only 25 h).

The assessment of the irritation potential of these samples (Figure 7) indicates the obtaining of a safe drug carrier that can be used for the transmembrane delivery of bromelain.

4. Conclusions

This paper describes a research on the encapsulation of bromelain inside polyurethane matrices which are used as a drug delivery system. The hydroxyl groups of bromelain were first protected by an esterification process in order to not influence the macromolecular chains in reactions with -NCO groups of isophorone diisocyanate. The obtaining of polyurethane particles around 330 nm was confirmed in SEM and Zetasizer analysis; it has been shown that the degradation of carrier increases from a simulated plasma (almost 30% after 120 h) to a simulated gastric medium (around 50% after just 8 h) and this is a normal situation due to the *p*H differences. The values of the viability of HDFa at 24 and 48 h on one hand, and the evolution of the main skin parameters on the other hand, reveal the fact that this drug carrier is safe to be used on human body, but clinical trials are necessary in the next step of the evaluation.

The results of the present and our previous studies on polyurethanes as drug delivery systems suggest that these materials are proper to be used as a bromelain carrier. Both *p*H and UV-Vis measurements show a good biocompatibility and an optimal release profile.

Acknowledgments: This article was supported by the grant 5EXP/1244/30.01.2020 from Victor Babes University of Medicine and Pharmacy, Timisoara, Romania.

References

1.SPROUSE, A.A., VAN BREEMEN, R.B., Pharmacokinetic Interactions between Drugs and Botanical Dietary Supplements, *Drug Metab. Dispos.*, **44**, 2016, 162-171, https://doi.org/10.1124/dmd.115.066902.

2.ATAIDE, J.A., DE CARVALHO, N.M., REBELO, M.A., CHAUD, M.V., GROTTO, D., GERENUTTI, M., RAI, M., MAZZOLA, P.G., JOZALA, A.F., Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties, *Sci Rep.*, **7**, 2017, 18031, https://doi.org/10.1038/s41598-017-18271-4.

3.SECOR, E.R.Jr., CARSON, W.F.4th, CLOUTIER, M.M., GUERNSEY, L.A., SCHRAMM, C.M., WU, C.A., THRALL, R.S., Bromelain exerts anti-inflammatory effects in an ovalbumin-induced murine model of allergic airway disease, *Cell. Immune.*, **237**, 2005, 68-75, https://doi.org/10.1016/j.cellimm.2005.10.002.
4. JUHASZ, B., THIRUNAVUKKARASU, M., PANT, R., ZHAN, L., PENUMATHSA, S. V., SECOR, E.R.Jr., SRIVASTAVA, S., RAYCHAUDHURI, U., MENON, V.P., OTANI, H., THRALL, R.S., MAULIK, N., Bromelain induces cardioprotection against ischemia-reperfusion injury through Akt/FOXO pathway in rat myocardium, *Am. J. Physiol. Heart Circulat. Physiol.*, **294**, 2008, H1365–H1370, https://doi.org/10.1152/ajpheart.01005.2007.
5.PASSALI, D., PASSALI, G.C., BELLUSSI, L.M., SARAFOLEANU, C., LOGLISCI, M., MANEA, C., IOSIF, C., PASSALI, F.M., Bromelain's penetration into the blood and sinonasal mucosa in patients with chronic rhinosinusitis, *Acta otorhinolaryngologica Italica*, **38**, 2018, 225-228, https://doi.org/10.14639/0392-100X-1693.
6.ONKEN, J.E., GREER, P.K., CALINGAERT, B., HALE, L.P., Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro, *Clin Immunol*., **126**, 2008, 345-352, https://doi.org/10.1016/j.clim.2007.11.002.
7.FERANCIKOVA, N., BUKOVCAN, P., SARKOZYOVA, N., DRAGUNOVA, J., CUCOROVA, V., KOLLER, J., Bromelain-based enzymatic debridement as a treatment of choice in high-risk patient with deep facial burns, a case report, *Int. J. Surg. Case Rep*., **71**, 2020, 6-10, https://doi.org/10.1016/j.ijscr.2020.04.052.
8. CHANG, T.C., WEI, P.L., MAKONDI, P.T., CHEN, W.T., HUANG, C.Y., CHANG, Y.J., Bromelain inhibits the ability of colorectal cancer cells to proliferate via activation of ROS production and autophagy, *PLoS One*., **14**, 2019, e0210274, https://doi.org/10.1371/journal.pone.0210274.
9. KWATRA, B., A review on potential properties and therapeutic applications of bromelain, *World J. Pharm. Pharm. Sci*., **8**, 2019, 488-500.
10.PAVAN, R., JAIN, S., SHRADDHA, KUMAR, A., Properties and therapeutic application of bromelain: a review, *Biotech. Res. Int.*, **2012**, 2012, 976203, https://doi.org/10.1155/2012/976203.
11. RATHNAVELU, V., ALITHEEN, N.B., SOHILA, S., KANAGESAN, S., RAMESH, R., Potential role of bromelain in clinical and therapeutic applications (Review), *Biomed. Rep.*, **5**, 2016, 283-288, https://doi.org/10.3892/br.2016.720.
12. BAGGA, P., ANSARI, T.M., SIDDIQUI, H.H., SYED, A., BAHKALI, A.H., RAHMAN, M.A, Khan, M.S., Bromelain capped gold nanoparticles as the novel drug delivery carriers to aggrandize effect of the antibiotic levofloxacin, *EXCLI J.*, **15**, 2016, 772-780, https://doi.org/10.17179/excli2016-710.
13. KUTLEHRIA, A., KAUSHIK, P., SHARMA, S., KAUR, A., Aquasomes as a carrier system for oral delivery of bromelain, *Int. Res. J. Pharm*., **9**, 2018, 123-129.
14.KOHJIYA, S., IKEDA, Y., TAKESAKO, S., YAMASHITA, S., Drug release behavior from polyurethane gel, *React. Polym*., **15**, 1991, 165-175, https://doi.org/10.1016/0923-1137(91)90160-P.
15.YUI, N., KATAOKA, K., YAMADA, A., SAKURAI, Y., Novel design of microreservoir-dispersed matrices for drug delivery formulations: Regulative drug release from poly(ethylene oxide)- and poly(tetramethylene oxide)-based segmented polyurethanes, *J. Control. Release*, **6**, 1987, 329-342.
16.BORCAN, F., SOICA, C.M., GANTA, S., AMIJI, M.M., DEHELEAN, C.A., MUNTEANU, M.F., Synthesis and preliminary in vivo evaluations of polyurethane microstructures for transdermal drug delivery, *Chem. Cent. J*., **6**, 2012, 87, https://doi.org/10.1186/1752-153X-6-87.
17.CAMPINEZ, M., AGUILAR-DE-LEYVA, A., FERRIS, C., DE PAZ, M.V., GALBIS, J., CARABALLO, I., Study of the properties of the new biodegradable polyurethane PU (TEG-HMDI) as matrix forming excipient for controlled drug delivery, *Drug Develop. Ind. Pharm.*, **39**, 2013, 1758-1764, https://doi.org/10.3109/03639045.2012.736516.
18. LOWINGER, M.B., BARRETT, S.E., ZHANG, F., WILLIAMS, R.O.3rd., Sustained Release Drug Delivery Applications of Polyurethanes. *Pharmaceutics*, **10**, 2018, 55, https://doi.org/10.3390/pharmaceutics10020055.

19.BORCAN, F., SOICA, C.M., DEHELEAN, C.A., GANTA, S., AMIJI, M.M., Size and Stability Optimization for Polyurethane Nanostructures used as Transdermal Drug Vehicle, *Rev. Chim.*, **63**(11), 2012, 1164-1166.
20.PROKS, M., BORCAN, F., CHEVERESAN, A., PINZARU, I., ALMAJAN GUTA, B., CORICOVAC, D., PAUNESCU, V., LAZUREANU, V., Study on the release and bioevaluations of green silver nanoparticles entrapped inside polymer-based nanovesicles, *Mater. Plast.*, **55**(4), 2018, 696-699.
21.CHANDRASEKARAN, A.R., JIA, C.Y., THENG, C.S., MUNIANDY, T., MURALIDHARAN, S., SO, D., Invitro studies and evaluation of metformin marketed tablets-Malaysia, *J. Appl. Pharm. Sci.*, **1**, 2011, 214-217.
22. ARANY, P., PAPP, I., ZICHAR, M., REGDON, G.Jr., BÉRES, M., SZALÓKI, M., KOVÁCS, R., FEHÉR, P., UJHELYI, Z., VECSERNYÉS, M., BÁCSKAY, I., Manufacturing and Examination of Vaginal Drug Delivery System by FDM 3D Printing, *Pharmaceutics*, **13**(10), 2021, 1714, https://doi.org/10.3390/pharmaceutics13101714.
23.CITU, I.M., TOMA, C., TRANDAFIRESCU, C., ANTAL, D., ZAMBORI, C., OPREAN, C., BOJIN, F., BORCAN, F., PAUNESCU, V., LAZUREANU, V., Preparation and Characterization of a Polyurethane Nanocarrier Used for Mixtures of Betulin and Fatty Acids, *Rev. Chim.*, **66**(3), 2015, 431-437.
24. BORCAN, F., PREDA, M., BORCAN, L.C., PINZARU, I., FLORESCU, S., SISU, E., POENARU, M., Comparative Characterization of Birch Bark Extracts Encapsulated Inside Polyurethane Microstructures, *Mater. Plast.*, **55**(3), 2018, 385-388.
25.SURBER, C., ABELS, C., MAIBACH, H., *p*H of the Skin: Issues and Challenges, *Curr. Probl. Dermatol.*, **54**, 2018, 143-151.
26. BOUCHEMAL, K., BRIANCON, S., PERRIER, E., FESSI, H., BONNET, I., ZYDOWICZ, N., Synthesis and characterization of polyurethane and poly(ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification, *Int. J. Pharm.*, **269**, 2004, 89-100, https://doi.org/10.1016/j.ijpharm.2003.09.025.
27. BORCAN, F., CHIRITA-EMANDI, A., ANDREESCU, N.I., BORCAN, L.-C., ALBULESCU, R.C., PUIU, M., TOMESCU, M.C., Synthesis and preliminary characterization of polyurethane nanoparticles with ginger extract as a possible cardiovascular protector, *Int. J. Nanomed.*, **14**, 2019, 3691-3703, https://doi.org/10.2147/IJN.S202049.
28.KOKUBO, T., TAKADAMA, H., How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials*, **27**, 2006, 2907-2915, https://doi.org/10.1016/j.biomaterials.2006.01.017.
29.WU, J., ZHANG, Z., GU, J., ZHOU, W., LIANG, X., ZHOU, G., HAN, C.C., XU, S., LIU, Y., Mechanism of a long-term controlled drug release system based on simple blended electrospun fibers, *J. Control. Release*, **320**, 2020, 337-346, https://doi.org/10.1016/j.jconrel.2020.01.020.
30. GHENSI, P., CUCCHI, A., CREMINELLI, L., TOMASI, C., ZAVAN, B., MAIORANA, C., Effect of Oral Administration of Bromelain on Postoperative Discomfort After Third Molar Surgery, *J Craniofac Surg.*, **28**(2), 2017, e191-e197, https://doi.org/10.1097/SCS.0000000000003154.
31.RACOVICEANU, R., TRANDAFIRESCU, C., VOICU, M., GHIULAI, R., BORCAN, F., DEHELEAN, C., WATZ, C., AIGNER, Z., AMBRUS, R., CORICOVAC, D.E., CIRCIOBAN, D., MIOC, A., SZUHANEK, C.A., SOICA, C., Solid Polymeric Nanoparticles of Albendazole: Synthesis, Physico-Chemical Characterization and Biological Activity, *Molecules*, **25**, 2020, 5130. https://doi.org/10.3390/molecules25215130.
32.GALLARDO, V., MORALES, M.E., RUIZ, M.A., DELGADO, A.V., An experimental investigation of the stability of ethylcellulose latex. Correlation between zeta potential and sedimentation, *Eur. J. Pharm. Sci.*, **26**, 2005, 170-175, htpps://doi.org/10.1016/j.ejps.2005.05.008.
33.JUTAMONGKON, R., CHAROENREIN, S., Effect of Temperature on the Stability of Fruit Bromelain from Smooth Cayenne Pineapple, *Kasetsart J. (Nat. Sci.)*, **44**, 2010, 943-948.

34.ASUZU, P.C., TROMPETER, N.S., COOPER, C.R., BESONG, S.A., ARYEE, A.N.A., Cell Culture-Based Assessment of Toxicity and Therapeutics of Phytochemical Antioxidants. *Molecules*, **27**(3), 2022, 1087, https://doi.org/10.3390/molecules27031087.
35.WENDELS, S., AVEROUS, L., Biobased polyurethanes for biomedical applications, *Bioactive Materials*, **6**(4), 2021, 1083-1106, https://doi.org/10.1016/j.bioactmat.2020.10.002.
36.ZHANG, X., BATTISTON, K.G., MCBANE, J.E., MATHESON, L.A., LABOW, R.S., SANTERRE, J.P., Design of biodegradable polyurethanes and the interactions of the polymers and their degradation by-products within in vitro and in vivo environments, In: COOPER, S.L., GUAN, J. (edts.) Advances in Polyurethane Biomaterials, Woodhead Publishing, 2016, 75-114, https://doi.org/10.1016/B978-0-08-100614-6.00003-2.
37.GURITA (CIOBOTARU), V.G., PAVEL, I.Z., BORCAN, F., MOACA, A., DANCIU, C., DIACONEASA, Z., IMBREA, I., VLAD, D., DUMITRASCU, V., POP, G., Toxicological Evaluation of Some Essential Oils Obtained from Selected Romania Lamiaceae Species in Complex with Hydroxypropyl - gamma-cyclodextrin, *Rev. Chim.*, **70**(10), 2019, 3703-3707, https://doi.org/10.37358/RC.19.10.7628.

Manuscript received: 08.10.2022