Materials Letters 348 (2023) 134686

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Immobilization of saponin on chitosan Milli-particles for Type II diabetic treatment

Maxine Yew [a,1], Chang Nong Lim [b,1], Boon Aik Liam [c], Kai Seng Koh [c,*], Siew Shee Lim [d], Billie Hiew [c], Ju Yen Fu [e]

[a] Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, PR China
[b] Department of Chemical and Environmental Engineering, The University of Nottingham, University Park, Nottingham NG7 2RD, UK
[c] School of Engineering and Physical Sciences, Heriot-Watt University Malaysia, No.1, Jalan Venna P5/2, Precinct 5, Putrajaya 622000, Malaysia
[d] Department of Chemical and Environmental Engineering, University of Nottingham Malaysia, Jalan Broga, 43500 Semenyih, Selangor, Malaysia
[e] Nutrition Unit, Product Development and Advisory Services Division, Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

ARTICLE INFO

Keywords:
Milli-fluidic
Chitosan
Saponin
Diabetes
Biomaterial
Surfaces

ABSTRACT

Type II diabetes mellitus (T2DM) has caused an adverse impact on the health of more than a tenth of the Malaysian population. Saponin is a traditional herbal extract found to be effective in treating T2DM with milder toxicity, which makes it a good alternative to conventional anti-diabetic drugs with side effects. This research develops an effective drug delivery vehicle to transport the saponin extract via chitosan milli-particles (CPs) for targeted release in the digestive system. Saponin was loaded onto the CPs via electrostatic interaction following the surface charge alteration of the CPs. Swelling tests and an *in vitro* study based on simulated gastrointestinal conditions were conducted on the saponin-immobilized CPs (SICPs). The release of saponin from its carrier is attributed to Fickian diffusion and swelling of the chitosan milli-particle matrix at pH 1.2 and 6.8.

1. Introduction

An estimated 463 million people (approximately 9.3% of the global adult population) are living with diabetes globally in 2019. The number of diabetic patients is expected to increase to 578 million by 2030 and 700 million in 2045 [1]. In Malaysia alone, more than one-tenth of its population are plagued by Type II diabetes mellitus (T2DM). Conventional treatment for T2DM such as insulin injections and oral administration of anti-diabetic drugs, may result in complications including hypoglycaemia and increased risk of heart failure. With an increasing number of potential diabetic patients, there is therefore the need for more alternating treatments, especially less aggressive methods that may reduce side effects and complications in patients.

Saponin, an extract from plants such as bitter gourd, offers a natural way to reduce blood sugar levels for patients with mild diabetes [2]. The antihyperglycemic and antihyperlipidemic functions of saponins have been validated in many published literatures to date [3]. Pure saponin, however, should not be ingested excessively as it may cause hypoglycaemia and irritation to the skin and respiratory tract. Moreover, direct ingestion may cause its anti-diabetic properties to be damaged by the intense gastrointestinal metabolism during digestion [4]. To control the dosage and preserve the structure of saponin effectively during ingestion, antacid carriers such as chitosan are needed to ensure the drug enters the gastrointestinal tract for maximal drug effect [5].

Chitosan is a natural polymer, derivable from chitin found in fungi cell walls and exoskeletons of crustaceans and insects [6]. Since the 2000 s, due to its biodegradability and non-toxic properties, it has been widely used in medicinal applications, for instance drug and vaccine delivery, and tissue engineering [7,8]. Chitosan has successfully encapsulated many different drugs in the lab scale including anti-diabetic agents i.e., Metformin hydrochloride [9]. By regulating the size of the chitosan particles at sub-milli and -micron size, the surface area to volume ratio increases drastically, thus improving the diffusivity of drugs, reducing drug loading and accelerating drug release [10]. Despite chitosan's frequent use as carrier for various active ingredients, there has not been any reported work on its combination with saponin for T2DM treatment. Thus, the purpose of this research is to immobilize saponin on chitosan carriers as potential diabetic remedy, via a milli-

* Corresponding author.
E-mail address: k.koh@hw.ac.uk (K.S. Koh).
[1] Equal contributors.

https://doi.org/10.1016/j.matlet.2023.134686
Received 2 April 2023; Received in revised form 29 May 2023; Accepted 2 June 2023
Available online 5 June 2023

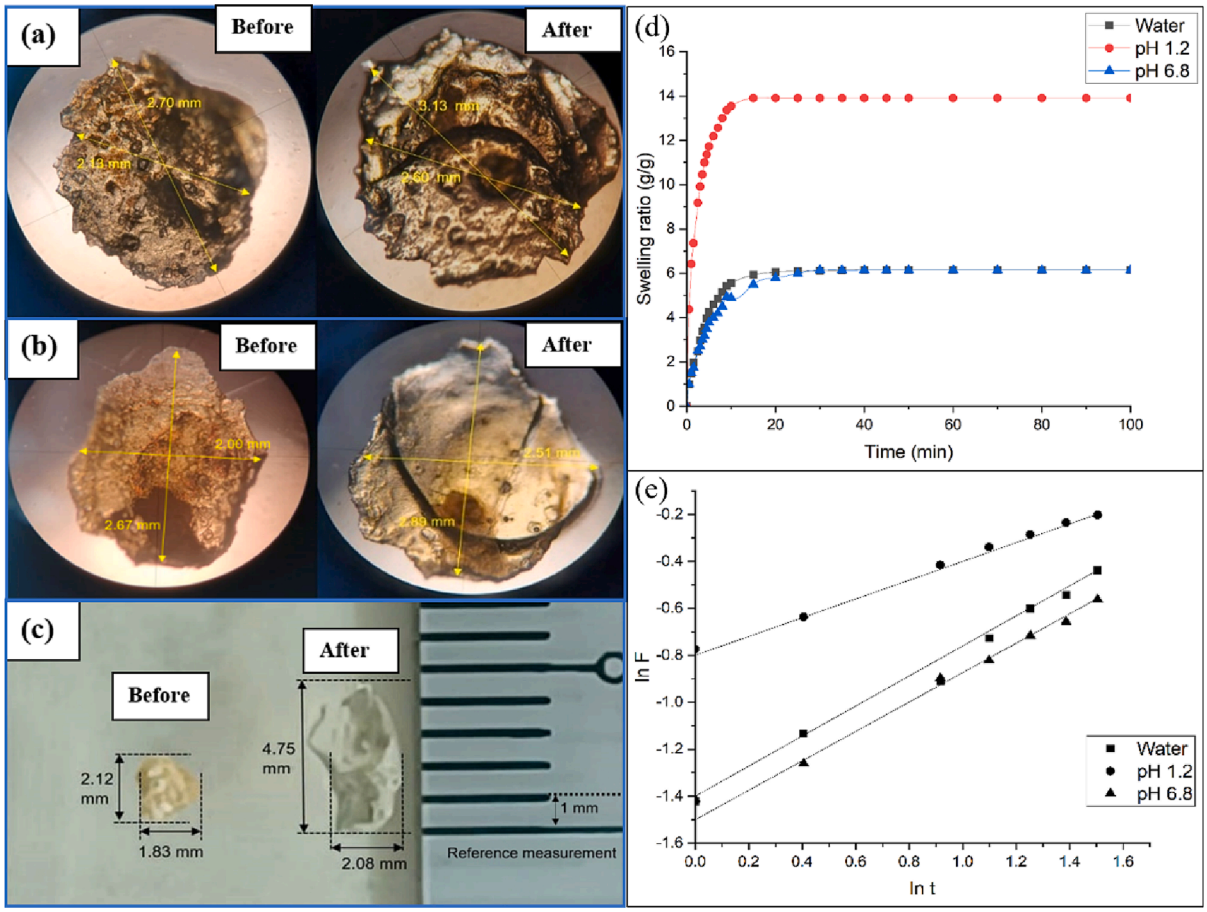

Fig. 1. Measurement of Feret diameter of SICPs before and after immersion in (a) distilled water, (b) buffer solution of pH 6.8, and (c) pH 1.2. (d) The maximum swelling percentage for SICPs was 18.6% for distilled water, 72.9% for buffer at pH 1.2, and 15.6% at pH 6.8. (e) A plot of ln F vs ln t, the slope and intercept correspond to the diffusion exponent and swelling constant.

fluidic approach. The chitosan milli-particles (CPs) coated with saponin biomolecules were within a size range of 1.1 to 1.5 mm. The effectiveness of the milli-particles in transporting and releasing saponin was evaluated by studying the polymer swelling characteristics in a simulated gastrointestinal system.

2. Materials and methods

CPs and SICPs were synthesized based on our previous method [11] with an off-the-shelf milli-fluidic device. The CPs were formed from the dripping of 2 wt% chitosan solution off a needle tip into sodium hydroxide (NaOH) solution which induced instantaneous precipitation. The CPs were then soaked in phosphate buffer solution (PBS) for surface electrostatic charges modification prior to the immobilization of negatively charged saponin onto the cationic CPs. The dried CPs were left to soak in 0.5 wt% saponin in ethanol solution for 6 h under continuous agitation to allow saponin immobilization. The swelling behaviour of the SICPs was assessed by determining the Feret diameter and change in weight after soaking them in deionized water (control), buffer solutions of pH 1.2 and 6.8. An *in vitro* digestion study was conducted to study the release of saponin by subjecting the SICPs in simulated gastrointestinal system at pH 1.2 and 6.8 respectively.

3. Results and discussion

Chitosan particles of 1.1 to 1.5 mm were obtained through free fall off a dispensing needle in this study. The CPs were collected and solidified in NaOH solution bath. FESEM-EDX elemental analysis validated the adsorption of saponin onto the chitosan surface as the amount of oxygen in SICPs increased to 34% while in blank CPs, carbon and oxygen were approximately 85 and 15 wt%.

4. Swelling of chitosan matrix

The changes in Feret diameters and weight of SICPs were determined and tabulated as swelling ratios, as shown in Fig. 1. The swelling behaviour of SICPs agrees well with reported work, as SICPs immersed in a buffer solution of pH 1.2 exhibited the highest swelling percentage. It is reported that chitosan hydrogels have higher swelling rate at low pH, attributed to a higher rate of protonation of the amine group of chitosan under acidic condition, and consequently higher rate of dissociation of hydrogen bonding between polymer chains [12]. At higher pH conditions, faster percolation is observed in chitosan hydrogel [13]. Thus, due to a greater number of crosslinked bonds at higher pH of 6.8, swelling was impeded in SICPs. In retrospect, corresponding to a lower number of cross-linked bonds, the chitosan particles have lower elastic moduli and mechanical strength at pH of 1.2 and consequently, higher equilibrium swelling ratio [13].

The diffusion mechanism of SICPs in media of different pH levels was investigated using the natural logarithm form of Fick's law ($lnF = nlnt + lnk$) to describe water diffusion into dried polymer [14]. The swelling and diffusion exponent, n and the swelling constant, k were evaluated from the plot in Fig. 1e. Result shows n for SICPs in water, pH 1.2 and pH 6.8 was 0.64, 0.45 and 0.63 respectively, thus suggesting the transport across SICPs was anomalous (non-Fickian), where n is typically > 0.43 and < 1 [15].

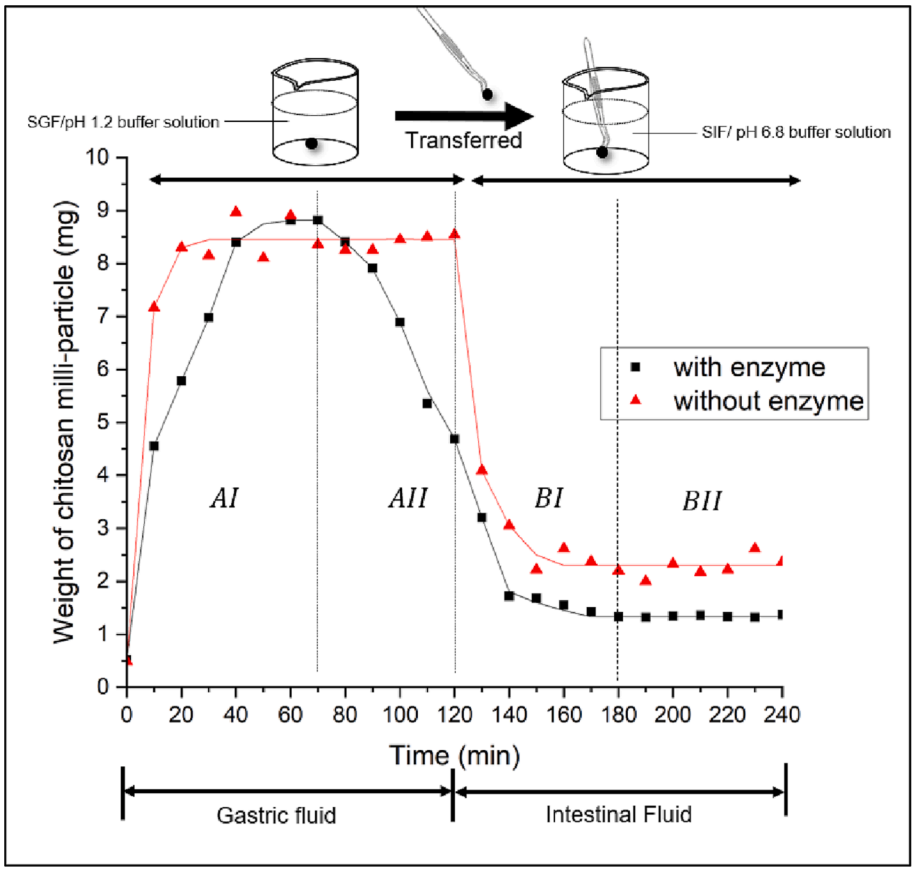

Fig. 2. A comparison of SICPs weight in buffers with and without enzymes.

5. *In vitro* digestion study

A realistic model of the ingestion of SICPs into the gastrointestinal system was developed by consecutively immersing the saponin immobilized particles in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) containing gastrointestinal enzymes and steroids of pepsin, pancreatic lipase, and bile salt, for 2 h each. The experiment was repeated without the enzymes. The saponin digestion analysis was briefly categorized into 4 regions of AI, AII, BI and BII as depicted in Fig. 2. An initial increase in SICP weight was noted in AI, followed by a 30-minute plateau which corresponded to the swelling of the polymer until it achieved equilibrium. In AII, chitosan weight declined progressively into BI as the SICP was removed from the SGF and transferred to the SIF, inferred as the effect of enzymic degradation as SICP reached its maximum swelling capacity. According to the percolation theory, the SICP shrank when the transfer took place into the SIF at higher pH condition [13]. In region BII, no weight change was recorded, and SICP was deemed to have gained sufficient mechanical strength from the re-emergence of cross-linked bonds and stronger bonding, at reduced enzymic degradation activity.

Generally, both curves exhibited similar trend in SICPs weight change, except a steeper gradient was noted for the experiment without enzymes, especially in region A1 and A2. These results suggested that enzymic degradation occurred simultaneously with the swelling of the polymer, with the degradation being more apparent in region AII. In comparison, SICPs in buffer containing enzymes would undergo higher rate of degradation due to the enzymic activities, as reflected in the greater weight loss shown in Fig. 2.

6. Conclusion

For the first time saponin immobilized chitosan milli-particles were produced and investigated as an alternative T2DM remedy. The synthesis of the SICPs was straightforward, and a preliminary study simulating the human gastrointestinal system confirmed the degradation and release of saponin from the chitosan carriers due to intrinsic polymer swelling pattern which has been shown to be non-Fickian in nature. More extensive future study should be conducted in view of the great medicinal benefits of plant extracted saponin, especially in treating diabetes mellitus.

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.

References

[1] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, D. Bright, R. Williams, Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition, Diabetes Res Clin Pract 157 (2019), 107843, https://doi.org/10.1016/j.diabres.2019.107843.

[2] O.O. Elekofehinti, Saponins: Anti-diabetic principles from medicinal plants - A review, Pathophysiology 22 (2015) 95–103, https://doi.org/10.1016/j.pathophys.2015.02.001.

[3] J. Xu, S. Wang, T. Feng, Y. Chen, G. Yang, Hypoglycemic and hypolipidemic effects of total saponins from Stauntonia chinensis in diabetic db/db mice, J Cell Mol Med 22 (2018) 6026–6038, https://doi.org/10.1111/jcmm.13876.

[4] G.R. Waller, K. Yamasaki, Saponins used in traditional and modern medicine, Springer Science & Business Media, 2013.

[5] Z.A. Raza, S. Khalil, A. Ayub, I.M. Banat, Recent developments in chitosan encapsulation of various active ingredients for multifunctional applications, Carbohydr. Res. 492 (2020), 108004, https://doi.org/10.1016/j.carres.2020.108004.
[6] D. Elieh-Ali-Komi, M.R. Hamblin, Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials, Int J Adv Res (Indore) 4 (2016) 411–427.
[7] J.A. Jennings, J.D. Bumgardner, Chitosan Based Biomaterials Volume 1: Fundamentals, Woodhead Publishing (2016).
[8] R. Jayakumar, M. Prabaharan, R.A.A. Muzzarelli, Chitosan for biomaterials I, Springer, 2011.
[9] M. Szekalska, K. Sosnowska, A. Zakrzeska, I. Kasacka, A. Lewandowska, and K. Winnicka, The Influence of Chitosan Cross-linking on the Properties of Alginate Microparticles with Metformin Hydrochloride—In Vitro and In Vivo Evaluation.
[10] D.S. Kohane, Microparticles and nanoparticles for drug delivery, Biotechnol Bioeng 96 (2007) 203–209, https://doi.org/10.1002/bit.21301.
[11] J. Yong, K.S. Koh, V.L. Wong, S.S. Lim, Formulation of Chitosan-Saponin Microbeads using Microfluidic Technology, IOP Conference Series: Materials Science and Engineering 1257 (2022), 012030, https://doi.org/10.1088/1757-899X/1257/1/012030.
[12] R. Hejazi, M. Amiji, Chitosan-based gastrointestinal delivery systems, J. Control. Release 89 (2003) 151–165, https://doi.org/10.1016/S0168-3659(03)00126-3.
[13] F.J. Vernerey, S. Bryant, The role of percolation in hydrogel-based tissue engineering and bioprinting, Current Opinion in Biomedical Engineering 15 (2020) 68–74, https://doi.org/10.1016/j.cobme.2020.01.005.
[14] M.T.a. Ende and N.A. Peppas, Transport of ionizable drugs and proteins in crosslinked poly(acrylic acid) and poly(acrylic acid-co-2-hydroxyethyl methacrylate) hydrogels. II. Diffusion and release studies, Journal of Controlled Release 48 (1997) 47-56.
[15] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release I, Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs, Journal of Controlled Release 5 (1987) 23–36, https://doi.org/10.1016/0168-3659(87)90034-4.