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Application of validated spectrophotometric method to quantify metformin in the development of glucose-responsive microparticles loaded dissolving microneedles

Sumayya Binti Abd Azis [a], Nur Syafika [a], Hanin Azka Qonita [a], Tiara Resky Anugrah Mahmud [b], Ahmad Abizart [b], Andi Dian Permana [a,*]

[a] Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia
[b] Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia

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ABSTRACT

Metformin (MTF) is a first-line drug in the treatment of type 2 diabetes mellitus. Delivered through the oral route, MTF has several limitations, mainly due to the side effects in gastrointestinal, non-specific release and low intestinal permeability, resulting in the low bioavailability of MTF in the body. Here, we developed glucose-responsive microparticles (GR-MP) containing MTF delivered via dissolving microneedles (DMNs) to overcome these limitations. To support the development of the formulation, in this study, a simple analytical method was developed using a UV–visible spectrophotometer. The method was validated in four different media, namely PBS, PBS containing 1 % w/v glucose, 2 % w/v glucose and 4 % w/v glucose, to mimic the normal and hyperglycemic condition. The method was further validated as per International Conference Harmonization (ICH). This analytical method was applied to quantify the amount of MTF in the GR-MP preparation, in vitro release, drug content in DMNs and, importantly, ex vivo permeation study in in vitro hyperglycemic conditions. The results exhibited that the calibration curves in all media showed a correlation coefficient (R) of 0.998, indicating the linearity of the method. Moreover, LLOQ values in the four different media were 2.23 μg/mL, 1.95 μg/mL, 1.94 μg/mL, and 2.88 μg/mL, respectively. Importantly, the method was precise and accurate with desired dilution integrity according to ICH, implying the validity of the methods. Finally, the method was successfully applied in the development of DMNs containing GR-MP of MTF, showing that the incorporation of MTF into this combination approach could selectively control the release of the drug according to the glucose concentration both in in vitro release and ex vivo permeation studies. Therefore, this approach could be a favorable system to solve the oral administration of MTF. Further in vivo analytical methods should now be developed to explore the effectiveness of this system in a suitable animal model.

1. Introduction

One of the biggest causes of death in the world is diabetes mellitus (DM). According to data from the International Diabetes Federation, 537 million people had DM in 2021, and it was estimated to increase to 783 million in 2045. DM causes various complications that were the direct cause of 1.5 million deaths worldwide in 2019. Specifically, approximately 90 % of DM cases are type 2 DM (T2DM) [1].

The first-line treatment for T2DM is metformin (MTF) tablets administered orally. MTF has been shown to be most effective in lowering blood glucose levels. MTF works in lowering blood sugar levels through various mechanisms. Consequently, the use of MTF in high doses and in the long term can potentially increase the risk of hypoglycemia. Research shows that 112 out of 4072 cases of MTF overdose could trigger hypoglycemia, which could potentially lead to intolerance to MTF [2]. Other side effects associated with the oral MTF therapy can cause undesired impacts on the gastrointestinal tract. MTF also has low permeability to cell membranes and, therefore, the absorption of MTF given orally does not occur optimally [3]. This causes an increase in the accumulation of drugs in the intestines, resulting in some dangerous side effects [4].

To overcome these problems, it is crucial to design a smart delivery

* Corresponding author.
E-mail address: andi.dian.permana@farmasi.unhas.ac.id (A.D. Permana).

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system to deliver MTF. Recently, the development of a glucose-responsive delivery system has attracted the interest of numerous researchers to selectively control the release of antidiabetic drugs. To the best of our knowledge, there has been no glucose-responsive system developed for MTF. In this study, we presented microparticles with glucose-responsive ability that could release MTF in the presence of glucose. Accordingly, this could be beneficial in preventing hypoglycemia [5]. Some compounds have been explored to possess this characteristic, including glucose oxidase (GOD), concanavalin A (Con A), and phenylboronic acid (PBA) [6]. Among these three compounds, the use of PBA is more frequent because it is lower cost, biodegradable, and easy to fabricate compared to GOD and Con A. Importantly, since PBA is not a protein like GOD and Con A, disadvantages such as poor volatile inactivation, and the high cost can be avoided [7].

In designing a controlled release form of drug delivery, the choice of polymer is one of the crucial things to consider [8]. There are many polymers that can be used in designing controlled release systems in the form of microparticles, one of which is a synthetic polymer in the form of poly(lactic) acid, which is a polymer with great potential, but controlling the particle size and drug adsorption efficiency of this polymer is quite difficult, and initial burst release may occur [9,10]. Another polymer that is usually used in the manufacture of microparticles is ethyl cellulose. However, the structure of ethyl cellulose which does not have a carboxyl group, makes PBA compounds unable to be linked to gelatin polymers and form polymer complexes that are responsive to glucose [11].

In this study, a polymer in the form of chitosan (CS) was chosen due to several benefits, namely being non-toxic, biodegradable, and biocompatible. These polymers also have unique physical and chemical characteristics, such as intermolecular hydrogen bonding and their polycationic charge under acidic conditions [12]. This leads us to the binding of the hydroxyl and amino groups present in the CS chain, which has a strong affinity for PBA. This binding resulted in a decrease in the pKa value of PBA and led to the manufacture of pH-responsive compounds, which has broad application prospects, causing PBA-CS combinations to achieve glucose sensitivity below the physiological pH of the human body [13]. Previously, several studies have been carried out in formulating metformin in the form of microparticles. One of them is research conducted by Avram et al 2017 who formulated MTF in the form of microparticles using a syringe technique using chitosan polymer [8,14]. However, the particles were not developed for selective delivery for hyperglycemic conditions. Therefore, further development is required to selectively release MTF based on glucose concentration.

As previously explained, the oral administration of MTF resulted in several side effects. As a result, modified transdermal delivery of MTF was chosen to overcome the side effects of MTF related to the low permeability of MTF to cell membranes, resulting in reduced bioavailability [15]. The choice of subcutaneous administration of antidiabetic drugs is commonly used, such as insulin and GLP-1 agonist drugs. Subcutaneous administration of drugs can certainly help overcome previous MTF problems, but new problems arise, such as discomfort to the patient, bleeding, infection at the injection site, and many more [16].

As an innovative delivery system, dissolving microneedles have been widely explored as an alternative delivery system to the injection route [17–19]. This system is applied intradermally which would dissolve when applied and release the active substances [20]. Therefore, the fabrication of DMN as a drug delivery system is an interesting solution for the oral therapy of MTF. The use of DMN as a drug delivery system not only solves the problems of administration using injection, but can also reduce sharp object waste after use because the needle used can dissolve due to its fabrication using water-soluble polymers [21]. However, the use of the DMN system can have an impact on the difficulty of drug encapsulation and dose control. Therefore, the DMN system could be collaborated with GR-MP in order to obtain an efficient drug delivery system and controlled drug release [22]. In this study, MTF-loaded-GR-MP was further incorporated into DMN for a selective and efficient delivery system in the *in vitro* mimicking diabetes environment.

In the development of a new drug delivery system, various tests and characterizations are required. One of the critical points is analyzing the active compounds. In this study, with regard to the analysis process, MTF was analyzed in the development of GR-MP, *in vitro* testing, and *ex vivo* permeation test. With respect to the media used, in this study phosphate buffered saline (PBS) and PBS containing glucose media represented normal and diabetes conditions. Previously, there were studies that carried out the determination of pure MTF and from tablets quantitatively using the reversed phase-high performance liquid chromatography (RP-HPLC) method [23] and Ultra Violet (UV) spectrophotometer. However, the chromatographic method has drawbacks, such as requiring large costs, a lot of solvents, reliable power, and expensive instruments [24]. This could limit the application of the analytical method in the several laboratories which do not have access to use the HPLC. These studies also analyzed the MTF of tablet dosage forms and did not use specific media. It has been reported previously that the results of research conducted by Georgia et al., 2021 in quantifying irinotecan from human plasma using UV–vis spectrophotometric techniques show that this technique was still relevant and valid in analyzing drugs from blood plasma [25].

Considering several aspects mentioned previously, in this study, an analytical method of MTF was developed from GR-MP-DMN preparations in PBS, PBS containing 1 % w/v glucose, 2 % w/v glucose and 4 % w/v glucose mediums using a UV–vis spectrophotometer. This analytical method has been widely used in the determination and has proven to be an analytical method that is simple, easy, and provides precise results in determining the number of samples [26]. Importantly, the application of a UV–vis spectrophotometer has been widely used in almost all scientific laboratories, making it a versatile tool in the drug development. To ensure that the developed analytical method provides appropriate results, this study was also conducted involving the validation of the analytical method based on the International Conference Harmonization (ICH) guidelines. Method validation parameters such as linearity, accuracy, precision, limit of detection (LOD), and the determined limit of quantification (LOQ), and were extensively applied in the determination of entrapment efficiency and drug loading in MP, drug content in DMN preparations, and *in vitro* and *ex vivo* permeation profiles.

2. Material and methods

2.1. Materials

Metformin HCl was obtained from Tokyo Chemical Industry Co., Ltd, Tokyo, Japan. Chitosan (medium molecular weight), glutaraldehyde polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), potassium dihydrogen phosphate (KH_2PO_4), glucose, potassium chloride (KCl), disodium phosphate (Na_2HPO_4) and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Singapore). All other reagents used in this study were analytical grade,

2.2. Preparation of PBS and PBS containing glucose

PBS was prepared by dissolving 0.2 g of KCl, 8 g of NaCl, 2.4 g of KH_2PO_4, and 1.44 g of Na_2HPO_4 with ± 800 mL CO_2-free water. Following the solubilization, the solution pH was set to 7.4. Finally, CO_2-free water was added to make up the final volume to 1 l. To prepare PBS containing glucose media, glucose with concentrations of 1 % w/v, 2 % w/v and 4 % w/v were dissolved using PBS.

2.3. Preparation of MTF stock solution

The stock solution was prepared by dissolving 10 mg of MTF into 10 mL of different media separately to achieve 1000 μg/mL of MTF

solution.

2.4. Determination of maximum UV absorption, preparation of calibration solution, and quality control solution

Initially, the stock solution of MTF in the respective media was diluted to achieve a concentration of 20 μg/mL. The determination of the maximum UV absorption in MTF in all media was carried out using a UV–vis spectrophotometer (Dynamica, HALO XB-10). Thereafter, the calibration solution in all media was prepared by diluting MTF stock solution using the respective media to achieve the serial concentrations of 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL, 1 μg/mL and 0.5 μg/mL.

Quality control solutions were prepared in three different concentrations, such as 12 μg/mL for high-quality control (HQC), 7.5 μg/mL for medium-quality control (MQC), and 4 μg/mL for low-quality control (LQC).

2.5. Validation method

The UV–vis spectrophotometer validation method was carried out by measuring the validation parameters, such as linearity, specificity, LOD and LOQ, dilution integrity, as well as accuracy and precision.

2.5.1. Linearity

Determination of linearity in the method validation was carried out by plotting the absorbance of three replications of the MTF calibration solution in three different media. From the curve results obtained, the correlation coefficient (R) was calculated. Linear parameters are considered valid if the value of R is close to 1 [27].

2.5.2. Specificity

The specificity needs to be known to ensure that there is no interference from other materials in the sample [27]. Specificity was determined by comparing the UV spectra of GR-MP blank, DMN blank, and MTF in both GR-MP and DMN system. The UV spectra was scanned between 200 and 400 nm.

2.5.3. Limit of detection (LOD)

The detection limit (LOD) was investigated to determine the smallest amount of analyte that could show absorption or absorbance in the instrument without having accuracy and precision criteria. LOD was calculated by using Eq. (1). In the equation, 3.3 represents the factor for LOD, SD is the standard deviation of the blank, and b is the slope of the blank regression line [28].

$$LOD = \frac{3.3 \times SD}{b} \tag{1}$$

2.5.4. Lower limit of quantification (LLOQ)

LOQ is the smallest amount of analyte that can still be measured for its absorbance using an instrument and has accuracy and precision criteria. LLOQ can be calculated by using Eq. (2). In the equation, 10 represents the factor for LLOQ, SD represents the standard deviation of the blank, and b represents the slope of the regression line [28].

$$LLOQ = \frac{10 \times SD}{b} \tag{2}$$

2.5.5. Accuracy

Accuracy is a parameter that shows the degree of closeness of the analysis results to the actual analyte content. Accuracy is expressed as the percent recovery of the added analyte. The accuracy test was carried out by comparing the MTF concentration in LLOQ, LQC, MQC and HQC solutions from the absorbance measurement results with the theoretical concentration, then the relative standard deviation (% RSD) was calculated. the %RSD value should not be more than 15 % of the theoretical concentration [27]. Measurements were done intra-day and inter-day.

2.5.6. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision test was the same as in the previous accuracy test, where the concentrations of the absorbance measurement results of LLOQ, LQC, MQC, and HQC solutions were compared with the theoretical concentrations. The relative error value (%RE) was calculated, and the results obtained should not be more than 15 % of the coefficient of variation (CV) [29]. Measurements were carried out intra-day and inter-day.

2.5.7. Dilution integrity

Dilution integrity was carried out by preparing 75 μg/mL MTF in all media. Then each solution was diluted 5 and 10 times, the experiment was carried out in triplicate, and the absorbance of the analyte was observed [30].

3. Application

3.1. Microparticle formulation

Microparticles were prepared using CS. In this study, 5 formulations were prepared containing 100 mg of MTF with different amounts of CS, namely 100 mg, 150 mg, 200 mg, 250 mg and 300 mg for F1, F2, F3, F4 and F5, respectively. GR-MTF-MP were prepared by mixing MTF and CS with 5 mg of EDTA, and added to 3 mL of acetic acid solution in water (1 % v/v) under the stirring condition at 500 rpm at room temperature. After that, 6 mL of ethanol was added to make a cloudy solution which indicated the formation of MPs. After that, 50 μL of glutaraldehyde (25 %) solution were added as a crosslinker by forming a reaction between the aldehyde group and the amino group of the MP. Furthermore, the MP formed were centrifuged at 3000 rpm for 20 min, and the sediment obtained was washed using distilled water to obtain pure MP CS [31].

To prepare GR-MP, PBA solution (11.2 mg) was dissolved in 1 mL of DMSO, and reacted with EDC.HCl (15.5 mg) and NHS (9.3 mg) for 30 min (mixture 1). After that, the mixture 1 solution was added to 5 mL of MP CS solution, while stirring at 37 °C for 24 h. Then, the PBA-decorated MP CS (MP PBA-CS) was dialyzed in distilled water for 48 h to remove unreacted PBA [31]. MP CS containing MTF was referred to as MP CS-MTF, and PBA-CS MPs containing MTF were referred to as MP PBA-CS-MTF. Particle size and polydispersity index (PDI) were all calculated.

3.2. Determination of entrapment efficiency and drug loading

The entrapment efficiency (EE) of MTF in MP was determined using the indirect method. In the washing steps, the supernatant was taken, and the concentration of MTF was calculated using a validated analytical method. Furthermore, the drug loading (DL) determination was carried out by mixing 50 mg of the formulation with 10 mL methanol. The mixture was sonicated for 30 min and diluted with PBS. ED and DL were calculated using the following calculations [32]:

$$\%\,EE = \frac{(Weight\ of\ initial\ drug - Weight\ of\ free\ drug)}{Weight\ of\ initial\ drug} \times 100 \tag{3}$$

$$\%\,DL = \frac{Amount\ of\ entrapped\ drug\ in\ microparticle}{Total\ weight\ of\ microparticle} \times 100 \tag{4}$$

3.3. In vitro release test

The *in vitro* release profile of MTF from MP was investigated using dialysis membrane method [20,32,33]. Briefly, MP formulations equal to 10 mg of MTF were placed inside dialysis membrane (Spectra-Por®,

12,000–14,000 MWCO dialysis membrane). The membrane was further immersed into 100 mL of release media. Three different media were used, namely PBS, PBS containing 1 % w/v glucose, 2 % w/v glucose and 4 % w/v glucose. The study was carried out in an orbital shaker at 100 rpm at 37 °C. The media (1 mL) was sampled at certain time intervals, and then the concentration of MTF was determined using a UV–vis spectrophotometer. Fresh media was added after the sampling to ensure the sink condition during the study. The drug release mechanism was then analyzed using a variation of the mathematical kinetic model [32].

3.4. Mathematical modelling for in vitro release test

The data obtained from the *in vitro* assays were then fitted into five different mathematical models to determine the release kinetics of MTF from MP. The models applied were zero-order kinetics (Z0), first-order kinetics (F0), Krosmeyer-Peppas (KP), Higuchi, and Hixson-Crowell (HC). The equations of each model are described below:

$$Zero\ order\ kinetics: C_t = C_0 + K_0 t$$

$$First\ order\ kinetics: lnC_t = lnC_0 + k_1 t$$

$$Krosmeyer - Peppas\ model: C_t = k_{KP} t^n$$

$$Higuchi\ model: C_t = k_H \sqrt{t}$$

$$Hixson - Crowell\ model: C_t^{\frac{1}{3}} = C_0^{\frac{1}{3}} k_{HC} t$$

C_t represents the concentration of MTF at time t, C_0 represents the initial concentration of MTF in the medium (t = 0), k_0 represents the zero-order constant, k1 represents the first-order constant, k_{KP} represents the Korsmeyer–Peppas constant, k_H represents the Higuchi constant, and k_{HC} represents the Hixson constant. - Crowell. All calculations were performed using the DD-solver software. The release kinetics is determined from the correlation coefficient value (R) obtained [30].

3.5. DMN fabrication and determination of drug content

In this study, two-layered DMNs containing MP CS-MTF and MP PBA-CS-MTF were fabricated using the centrifugation method [34]. The formulation contained the aqueous gel of 15 % w/w of PVA (31–50 kDa) and 25 % w/w of PVP (58 kDa) in distilled water mixed with 30 % w/w of MP. Initially, 100 mg formulation was poured on the top of DMN MN silicon mould (needle density 10 × 10, the pyramidal needle with 700 μm of high and 200 μm wide on the base and 200 μm of spacing). Thereafter, the mould was centrifuged at 3000 rpm for 15 min at room temperature. The excess of the formulation was removed and dried for 6 h. Following this, an aqueous gel containing 15 % w/w of PVA (31–50 kDa) and 25 % w/w of PVP (58 kDa) was poured as a second layer. The formulation was dried at room temperature for 24 h and removed from the mould. It was important to note that the DMNs used in this study possessed adequate mechanical and insertion properties.

In an attempt to measure the MTF content in DMNs, the formulation was initially dispersed in 5 mL of distilled water. Afterwards, the dispersion was mixed with 10 mL of methanol and sonicated for 30 min. The mixture was then centrifuged for 10 min at a speed of 5000 rpm. The supernatant was collected, and the absorbance was measured using the UV–vis spectrum.

3.6. Ex vivo permeation studies

Ex vivo permeation studies were performed using Franz diffusion cells, using rat skin [35–37]. PBS, PBS containing 1 % w/v glucose, 2 % w/v glucose and 4 % w/v glucose were used as the release medium. Prior to the experiment, skin was washed and soaked in the release medium for 30 min. Afterwards, the surface of the skin was dried, and the skin was placed between the donor and recipient compartments. The experiment was conducted at 100 rpm at 37 °C. During the study, the sampling was carried out at several time intervals, starting from 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 h by taking 1 mL in the receiving compartment, then replaced with the same volume of new media. The samples were then analyzed using UV–vis spectrophotometry.

3.7. Statistical analysis

All data obtained were expressed in mean ± standard deviation (SD), all values were obtained using Microsoft excel® 2019 software (Microsoft Corporation, Redmond, USA). Graphpad Prism® version 6 (GraphPad Software, San Diego, California, USA) was used to analyze the data statistically, where statistical significance was indicated by p value less than 0.05.

4. Results and discussion

4.1. Validation of analytical method

4.1.1. Determination of maximum UV absorption and specificity

The purpose of this study was to validate the UV–vis spectrophotometer as an instrument that allow the quantification of MTF in the development of a new dosage form, namely in the form of GR incorporated into DMN. Measurement of MTF levels was carried out in PBS media as a model medium for normal condition and PBS containing different concentration of glucose media as a model medium for hyperglycemia condition [38]. The results showed that MTF exhibited maximum absorption at 234 nm in all media (Fig. 1). Accordingly, the wavelength was used in the further steps in this study.

Specificity parameter was intended to ensure that the MTF analysis using a UV–vis spectrophotometer from the MP and DMN formulations did not experience interference from other compounds. As shown in Fig. 2, the measurement results of the blank MP and MN showed a peak in the range of 210–220 nm, and did not indicate a possible interference at the MTF peak at 234 nm. In addition, the MTF peaks in both the MP and DMN formulas showed the same peaks as the pure MTF solution, which indicated that there was no peak shift due to additives or solvents used in the formulation. Therefore, the method developed in this study has been specific to the appropriate wavelength.

4.1.2. Linearity, LOD and LOQ

Linearity parameter is one of the characteristics required in the validation process of an analytical method. Based on the ICH guidelines, linearity needs to be evaluated by plotting a function of analyte concentration with the absorbance results obtained, then evaluated by mathematical modeling. Determination of linearity parameters from the validation of this method was carried out by measuring the absorbance of MTF in the concentration range of 0.5–16 μg/mL in all media. The spectrum of MTF in all media tested in various concentrations are depicted in Fig. 3. The linearity acceptance criteria for active pharmaceutical ingredients is R greater than 0.998 [39]. The results of the linearity measurement of MTF showed the correlation coefficient value of 0.9983 for PBS media, 0.9991 for PBS containing 1 % w/v of glucose media, 0.9991 for PBS containing 2 % w/v of glucose media, and 0.9981 for PBS containing 1 % w/v of glucose media which of these four values met the criteria for linearity parameters.

The LOD and LLOQ values in all media were calculated from the calibration data, as shown in Table 1. The LOD MTF values in PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media and PBS containing 4 % w/v of glucose media were found to be 2.23 μg/mL, 1.95 μg/mL, 1.94 μg/mL and 2.88 μg/mL respectively. LLOQ MTF values PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media and PBS containing 4 % w/v of glucose were calculated as 0.74 μg/mL, 0.64 μg/mL, 0.64 μg/mL, and 0.95 μg/mL respectively.

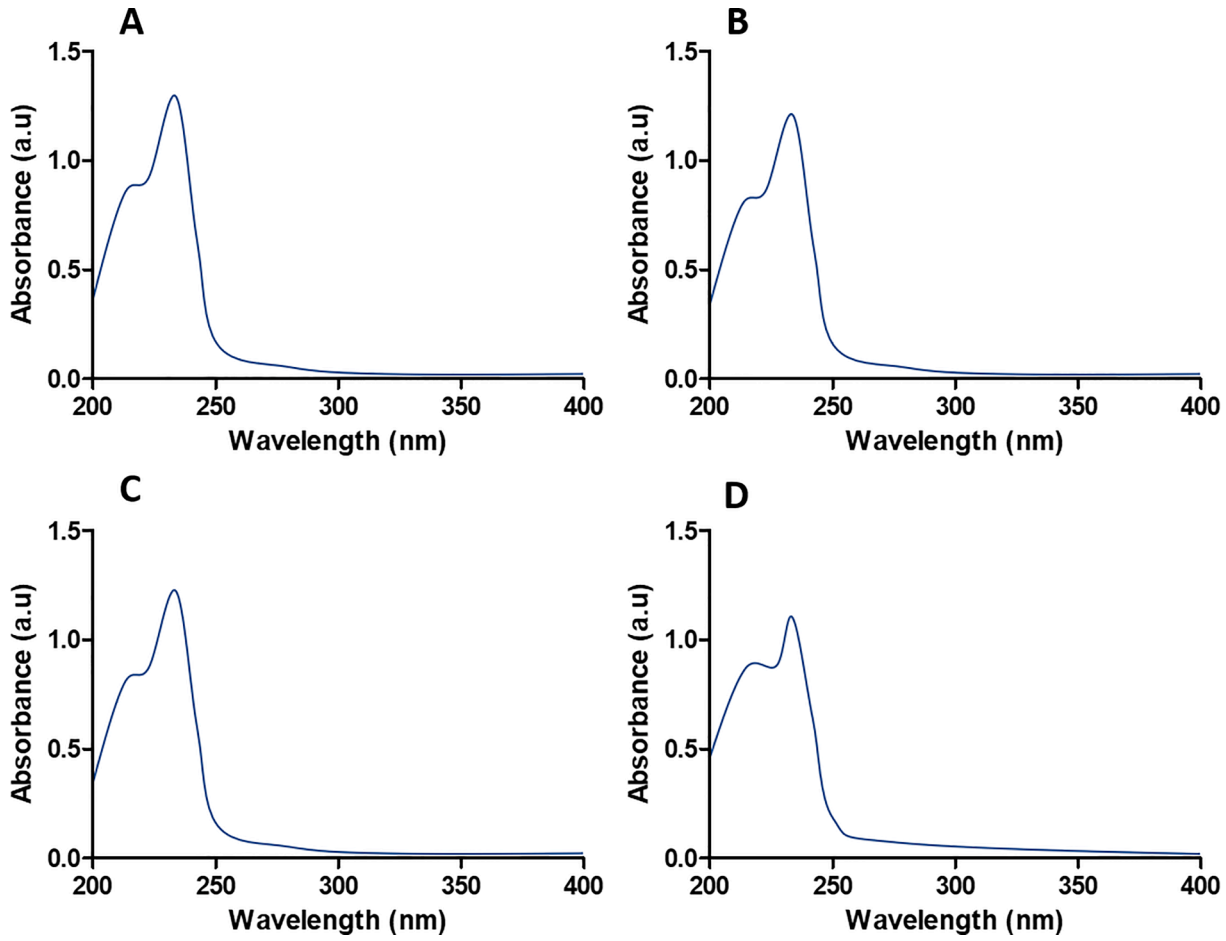

Fig. 1. Maximum absorbance of MTF in PBS media (A); PBS containing 1 % w/v of glucose media (B); PBS containing 2 % w/v of glucose media (C); PBS containing 4 % w/v of glucose media (D).

4.1.3. Precision and accuracy

Precision and accuracy were assessed for intra-day and inter-day. Intra-day determination was carried out to evaluate the repeatability of this analytical method, whereas inter-day determination was conducted to investigate the variation of the day to the measurement. Precision and accuracy were analyzed in four different concentrations consisting of QC sample (HQC, MQC, and LQC) and LOQ sample which were measured in three replications respectively in one day for inter-day determination and three replications in three days for intra-day determination.

Precision was aimed to evaluate the closeness between a series of measurements from the homogenous sample under the same condition. The results were presented as %RSD values and are shown in Table S1, Table S2, Table S3, and Table S4 for PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media, and PBS containing 4 % w/v of glucose media, respectively. It was found that all of the %RSD values were less than 15 % and, thus, fulfilled the requirement from ICH [29,40,41]. As the analytical method was considered to be precise when %RSD is less than 15 %, this UV–vis spectrophotometry method was therefore considered to have precision values to quantify MTF in all media used in this study.

Accuracy was carried out to evaluate the closeness between true value and value found from measurements. The accuracy values were reported as %RE. Tables S1–S4 show the accuracy of UV–vis spectrophotometry method for MTF in PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media and PBS containing 4 % w/v of glucose media, respectively. The analytical method was considered to be accurate when the %RE values are ± 15 % [27]. All the values were found to be ± 15 %; therefore, the analytical method was considered accurate.

4.1.4. Dilution integrity

Dilution integrity is the ability rate of the dilution process performed during the validation process as accurate, precise, and reliable [27]. Based on the dilution integrity data obtained in Table 2, it was found that the results showed the satisfactory results, where the dilution integrity bias on all media was less than 15 %. The precision parameter observed from the %RSD with values ranging from 1.62 %−4.68 % indicated that the dilution in this validation method was accurate and precise. In addition, it also implied that the analysis using this method can still be carried out on MTF with concentrations higher than the upper range of the calibration standard by using the appropriate dilutions.

4.2. Application of the analytical method

4.2.1. Entrapment efficiency and drug loading

Following the successful validation of the spectrophotometric method, it was applied to characterize the MP's EE and DL capacities. In this study, in an attempt to achieve optimum parameters, we investigated five different concentrations of CS. The results of the characterization are depicted in Fig. S1.1. With regard to the particle size, it was found that the increase of CS concentration could increase the particle size of the formulation. It might be due to the increased CS concentration resulting in a higher viscosity of the medium, leading to the decrease of the energy to break the droplet into a smaller size. This phenomenon was

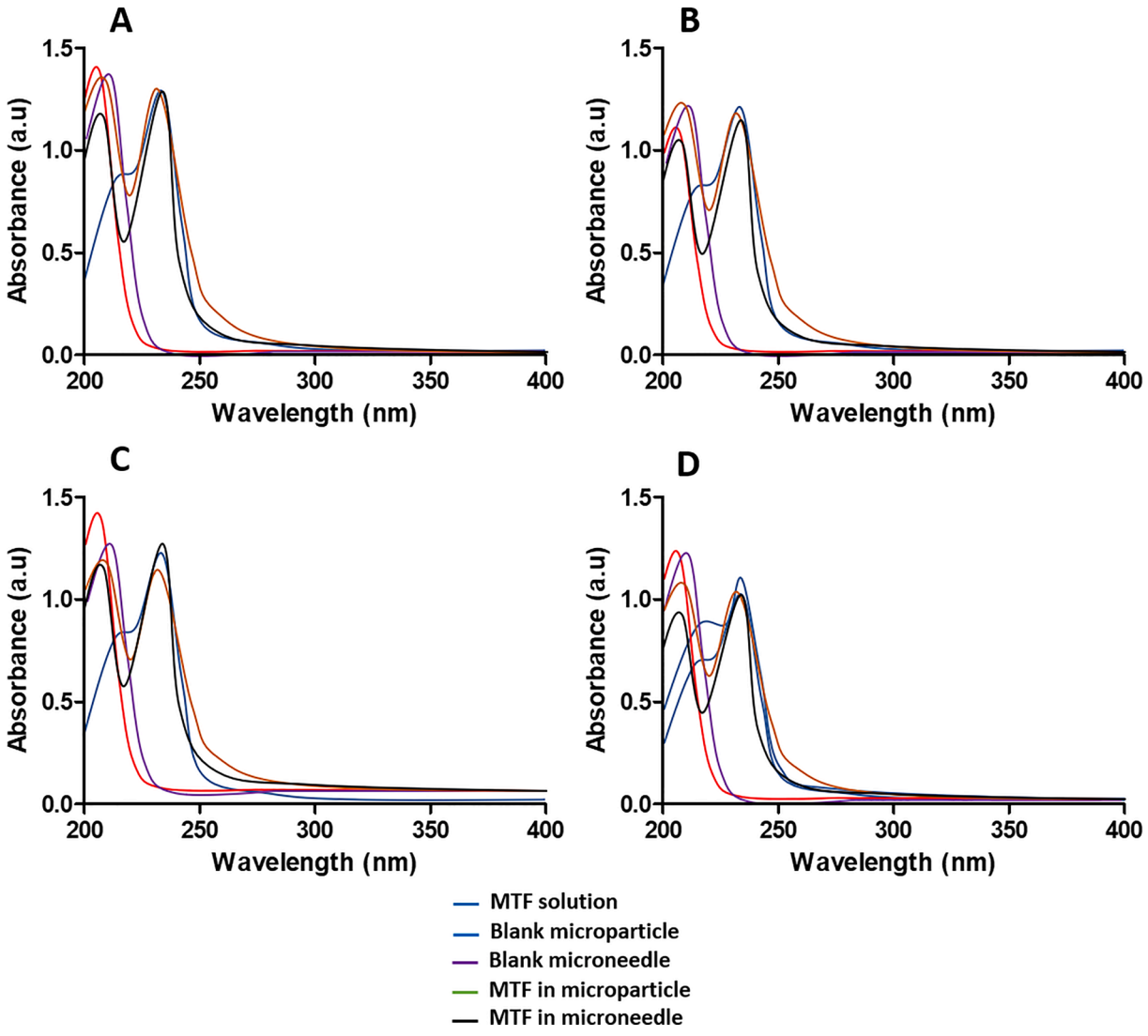

Fig. 2. Representative UV-Spectra of pure MTF, blank MP, blank DMN, MTF in MP, and MTF in DMN in PBS media (A); PBS containing 1 % w/v of glucose media (B); PBS containing 2 % w/v of glucose media (C); PBS containing 4 % w/v of glucose media (D).

also observed in numerous studies investigating the concentration of the polymer in the size of micro/nanoparticles [29,42]. The results showed that the particle size of F1, F2, F3, F4 and F5 were 2.87 ± 0.21 μm, 3.09 ± 0.28 μm, 4.87 ± 0.31 μm, 7.09 ± 0.59 μm and 10.19 ± 0.92 μm, respectively. Regarding the PDI values, despite the difference in size, all formulations exhibited a narrow distribution pattern. Furthermore, the EE evaluation results showed the improvement of EE values following the increment of the CS concentrations. Following the application of the validated method, it was calculated that EE values were 27.49 ± 3.12 % for F1, 39.16 ± 4.01 % for F2, 53.19 ± 5.84 % for F3, 55.18 ± 4.14 % for F4, 58.42 ± 5.19 % for F5. Furthermore, the DL values were 12.19 ± 1.31 %, 13.92 ± 1.44 %, 15.01 ± 1.59 %, 14.39 ± 1.48 % and 12.01 ± 1.37 % for F1, F2, F3, F4 and F5, respectively. Analyzed statistically, statistical differences (p less than 0.05) between the EE and the DL values in F1, F2 and F3 showed that the increase in CS concentration could potentially increase the EE and the DL of MP. However, the improvement of CS concentration in F3 and F4 did not significantly increase both parameters. Accordingly, considering the less amount of CS used in F3 compared to F4 and F5, based on the parameters evaluated here, F3 was considered as the optimum MP formulations.

Furthermore, Fig. S1.2 shows the microscopy images of F3 MP. Additionally, the formulation of F3 without PBA was also prepared. The images show the spherical shape of the MP. Importantly, the sizes of the microscopy images were in good agreement with the results from the particle size determination.

4.2.2. *In-vitro release assay*

A further validated UV–vis spectrophotometer analysis method was applied to determine the amount of MTF released in an *in vitro* release assay. This test was carried out on GR-MP-MTF formulas F1, F2, F3, F4, F5 (the formulations with PBA), MP-MTF (formulation without PBA), and free MTF solution in PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media and PBS containing 4 % w/v of glucose media. The results are revealed in Fig. 5.1, showing that after 24 h, the release of MTF in PBS medium was 13.87 ± 1.25 %, 5.94 ± 0.53 %, 5.77 ± 0.52 %, 4.93 ± 0.44 %, 4.02 ± 0.36 % for the GR-MP-MTF formula F1, F2, F3, F4, F5, respectively, and 99.19 ± 10.91 % and 98.19 ± 9.03 % for pure MTF solution and MP-MTF formula (F3 without PBA), respectively. Specifically, the release of MTF in PBS medium from free MTF solution reached almost 100 % in just 2 h. The MTF release from MP formula without glucose responsive polymer reached almost 100 % after 24 h. On the other hand, the MTF release from GR-MP in all formulations was only less than 15 % MTF after 24 h. Importantly, the increased glucose concentration in the release medium led to the enhancement of release of MTF from the MP-MTF formulation, indicating the successful development of GR-MP. As shown in Fig. 4.1,

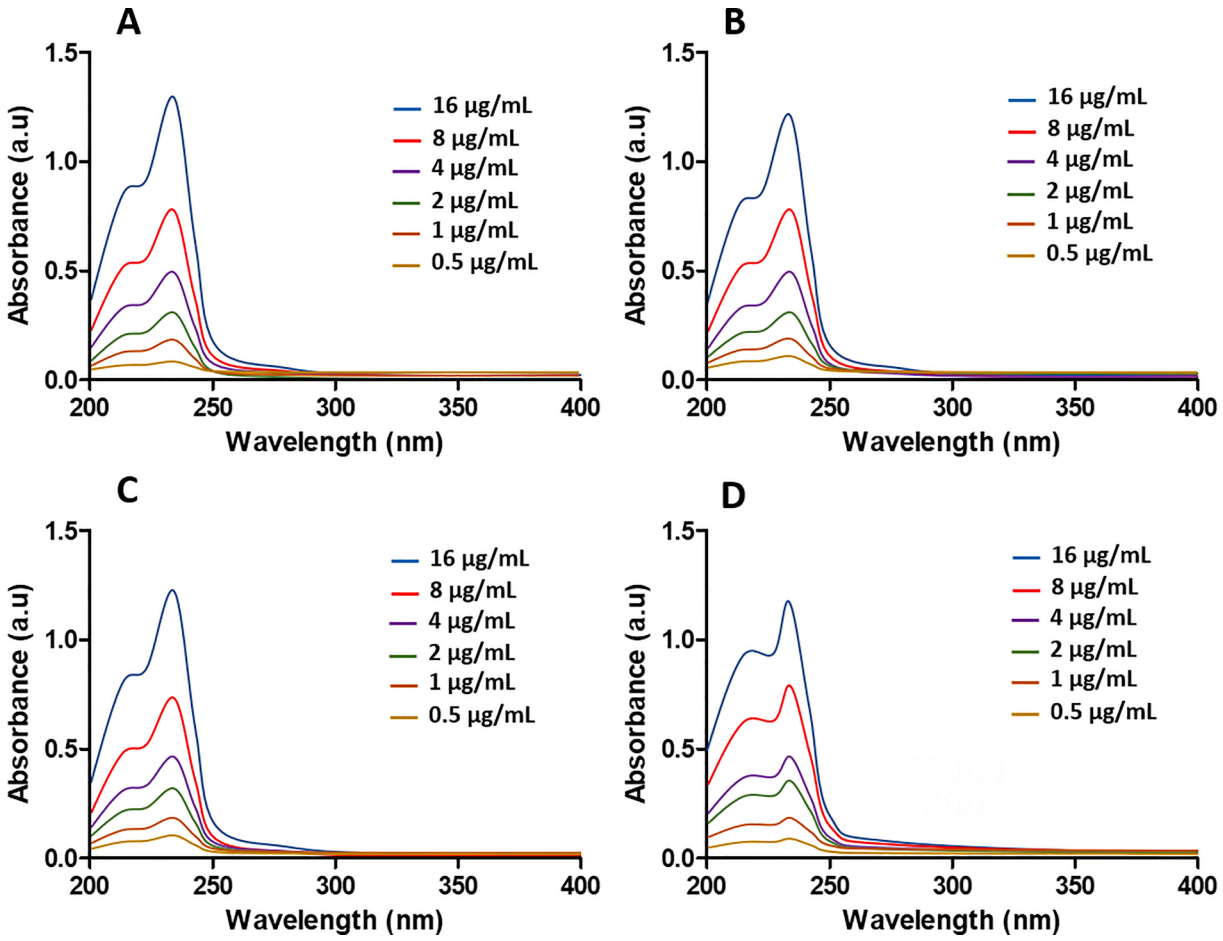

Fig. 3. Spectrum of MTF standard solution in PBS media (A); PBS containing 1 % w/v of glucose media (B); PBS containing 2 % w/v of glucose media (C); PBS containing 4 % w/v of glucose media (D).

Table 1
The calibration curve properties of MTF in different media with LOD and LOQ values of MTF.

Media	Concentration range (µg/mL)	R	LOD (µg/mL)	LLOQ (µg/mL)
PBS	0.5–16	0.9989	0.74	2.23
PBS with 1 % glucose	0.5–16	0.9991	0.64	1.95
PBS with 2 % glucose	0.5–16	0.9991	0.64	1.94
PBS with 4 % glucose	0.5–16	0.9981	0.95	2.88

Table 2
Dilution integrity data of UV–vis spectrophotometry method for MTF in all media (mean ± SD, $n = 3$).

Media	Dilution tested	Concentration added (µg/mL)	Concentration found (µg/mL) ± SD	% RSD	%RE
PBS	10	7.5	8.26 ± 0.15	1.87	−0.39
	5	15	15.36 ± 0.51	3.30	0.31
PBS with 1 % of glucose	10	7.5	7.28 ± 0.30	4.11	−1.62
	5	15	14.25 ± 0.52	3.64	3.45
PBS with 2 % of glucose	10	7.5	7.75 ± 0.36	4.68	−0.11
	5	15	14.76 ± 0.71	4.79	3.47
PBS with 4 % of glucose	10	7.5	7.49 ± 0.12	1.62	1.66
	5	15	15.09 ± 0.64	4.23	3.22

after 24 h, MTF released from all GR-MP-MTF formulations reached almost 100 %, namely 99.91 ± 8.99 %, 99.98 ± 9.00 %, 99.18 ± 8.93 %, 91.24 ± 8.21 %, and 79.54 ± 7.16 % for F1, F2, F3, F4, and F5, respectively. This amount was not significantly different (p greater than 0.05) from MTF released from MP-MTF without PBA and pure MTF solution. The difference in the amount of MTF released in the GR-MP-MTF and MP-MTF without PBA in the PBS and the PBS with glucose media was due to the presence of PBA in the formulation. PBA is a GR material that is sensitive to changes in glucose levels where the increment of glucose levels causes a break in the bond between the phenylboronic-diol in PBA, which in turn causes the expansion of the polymer that binds to PBA and releases the MTF contained in the formulation [43]. Among all the MP formulations, with respect to the release pattern, F3 was considered as the optimum formulation. This was because due to the findings that F3 could control the release of MTF for up to 24 h, reaching almost 100 % of MTF released. On the other hand, F4 and F5 could only release around 80 % of MTF after 24 h.

The results obtained from the *in vitro* release test were further fitted to five mathematical kinetic models to determine the MTF release model from the formulation. The values of the correlacy coefficient for the *in vitro* released of MTF from F3 in PBS were 0.8257, 0.8193, 0.4580, 0.9988, and 0.8215 for Zero order (ZO), First order (FO), Higuchi (H), Korsmeyer-Peppas (KP), and Hixson-Crowell (HC), respectively. In PBS containing 1 % w/v glucose, the value of the correlacy coefficient were 0.9916, 0.9772, 0.7090, 0.9980, and 0.9829 for ZO, FO, H, KP, and HC, respectively. Regarding the release in PBS containing 2 % w/v glucose,

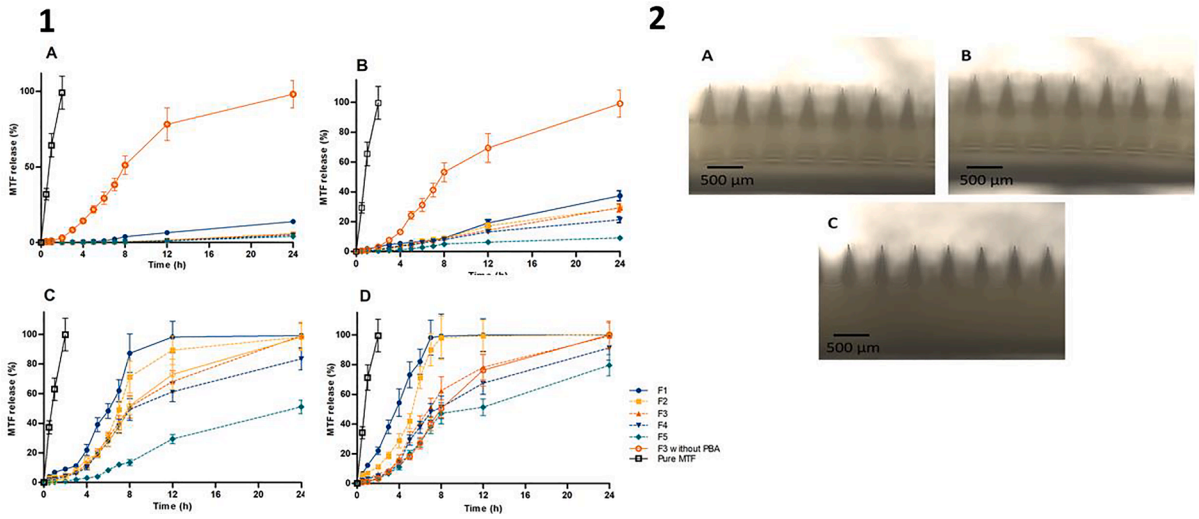

Fig. 4. *In vitro* release profile of MTF from MP in PBS media (A); PBS containing 1 % w/v of glucose media (B); PBS containing 2 % w/v of glucose media (C); PBS containing 4 % w/v of glucose media (D) data (mean ± SD, $n = 3$) (1). The microscope images of DMN containing F3 with PBA (A); F3 without PBA (B); and pure MTF (2).

the values of the correlacy coefficient were found to be 0.9306, 0.9015, 0.7633, 0.9403, and 0.9333 for ZO, FO, H, KP, and HC, respectively. Finally, in PBS containing 4 % w/v glucose, the values of the correlacy coefficient were 0.8391, 0.9099, 0.7995, 0.9006, 0.9358 for ZO, FO, H, KP, and HC, respectively. The result obtained show that *in vitro* release of MTF in PBS, PBS containing 1 % w/v of glucose media and PBS containing 2 % w/v of glucose media followed Korsmeyer-Peppas model. This kinetic model described the mechanism of drug release from the polymeric matrix. Meanwhile, *in vitro* release of MTF in PBS containing 4 % w/v of glucose media followed Hixson-Crowell, which kinetic model to describe drug release from systems that has a change in surface area and diameter of the particle, in the case of hydrophilic matrix swelling and erosion of the polymer occurs simultaneously [44].

4.2.3. DMN fabrication and drug content in MNs

Following the successful development of GR-MP, the formulations were further incorporated into DMNs to facilitate dermal delivery of MTF. Using the mixture of the aqueous gel of 15 % w/w of PVA (31–50 kDa) and 25 % w/w of PVP (58 kDa), DMNs containing MPs possessed a complete and sharp needle shape (Fig. 4.2) with adequate mechanical and insertion properties. As a control, MP without PBA and free MTF-loaded DMNs were also prepared. The combination of these polymers has shown the effectiveness of the DMNs formulation in numerous studies [45,46]. The validated spectrophotometer UV–vis analysis method was further used to determine MTF content in the formula MP-MTF-PBA and MP-MTF without PBA. As shown in Fig. 5.1, the percentage of MTF recoveries was found to be 98.12 ± 1.13 %, 98.09 ± 1.59 %, and 99.23 ± 2.01 % for MP-MTF-PBA, MP-MTF (without PBA) and pure MTF. This result indicated that the formulation of MTF in MP form and combined with PBA did not affect the concentration of MTF in the DMN formulations. The recovery percentage of all formulas also fulfilled the acceptable recovery percentage from ICH, which is 95–105

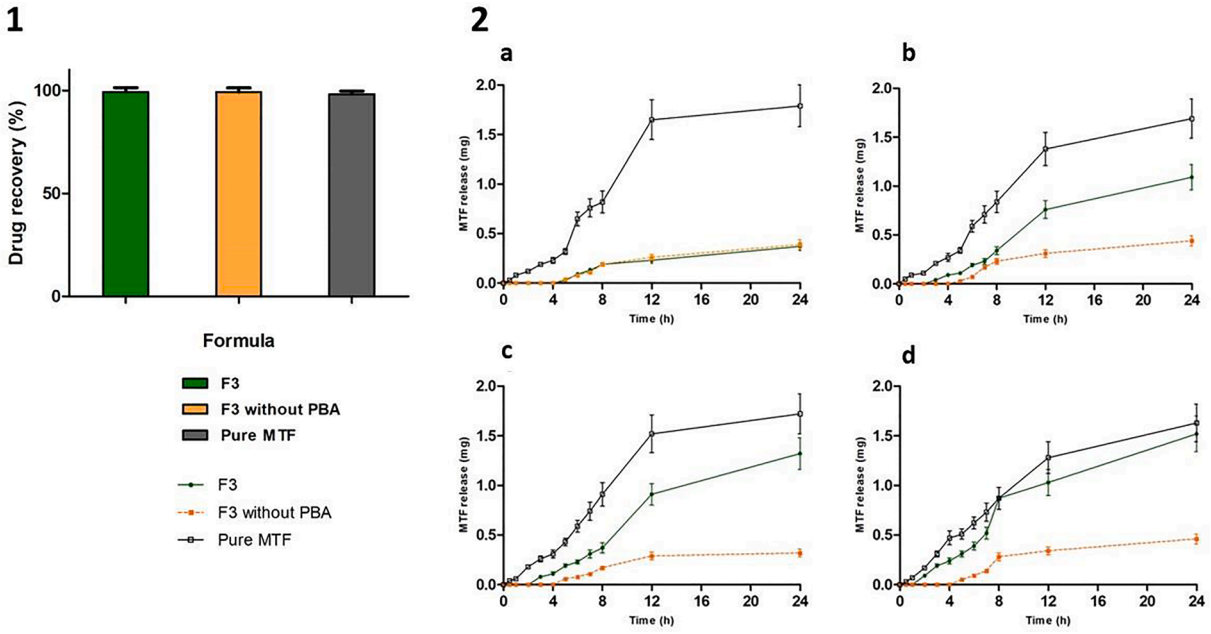

Fig. 5. MTF recovery (%) from MN (1); Ex vivo permeation profile of MTF from DMN (B) in PBS media (a); PBS containing 1 % w/v of glucose media (b); PBS containing 2 % w/v of glucose media (c); PBS containing 4 % w/v of glucose media (d) data (mean ± SD, $n = 3$) (2).

% [27].

4.2.4. Ex vivo permeation study

Ex vivo permeation test was carried out to investigate the penetration ability of MTF formulated into GR-MP-MTF, MP-MTF (without PBA), and pure MTF delivered using DMN. This evaluation was carried out to ensure the controlled release of MTF in the GR-MP-DMN formulation in *in vitro* hyperglycemic modeling media. Based on the results of the *ex vivo* permeation test in the four media presented in Fig. 5.2, it was observed that the MTF permeation continued to increase with time. Specifically, the DMN containing the MP-MTF formulation (without PBA) showed a significant and similar increase in permeation in all media, indicating the non-specific release pattern of this formulation. Moreover, the DMNs containing pure MTF also showed non-specific release behavior, with the highest amount of MTF released over 24 h. On the other hand, importantly, the formulation of GR-MP into DMNs could potentially control the permeation of MTF. After 24 h, the MTF permeation in PBS media, PBS containing 1 % w/v of glucose media, PBS containing 2 % w/v of glucose media and PBS containing 4 % w/v of glucose media were 0.37 ± 0.04, 1.09 ± 0.13, 1.32 ± 0.16, and 1.52 ± 0.18 mg. This increase in the amount of permeable MTF indicated the successfulness of the GR-MP-MTF formulation in controlling the release of MTF according to the amount of glucose contained in the media as a hyperglycemic model This shows that the modification of the formula in the form of CS-MP can help the controlled release system in MTF [12].

Here, for the first time, we successfully applied a simple validated analytical method using UV–vis spectrophotometer to quantify MTF in the development of GR-MP loaded DMNs. The method was found to be valid with desired accuracy, precision and dilution integrity results. Importantly, the application of the method indicated the successfulness of the selective delivery the approach in the *in vitro* hyperglycemic condition. Accordingly, this approach could be an alternative of the oral administration of MTF. Moving forward, the effectiveness of this system should be evaluated in appropriate in vivo models with suitable analytical models.

5. Conclusion

This research was conducted with the aim of developing and validating a UV–vis spectrophotometric method for the analysis of MTF in the development of GR-MP loaded DMNs. The method used was validated with the parameters of selectivity, accuracy, precision, linearity, LOD and LLOQ, and dilution integrity. The method was validated in in vitro normal physiological and hyperglycemic conditions using PBS, PBS containing 1 % w/v glucose, 2 % w/v glucose and 4 % w/v glucose. Based on all the validation parameter tests, this method was found to meet the requirements of the ICH guidelines, indicating that this analytical method was valid for the application of the drug development of MTF. Specifically, the validated analytical method was successfully applied to evaluate EE, DL, in vitro release profile in MP system, drug recovery and ex vivo permeation in DMNs system. The results from the application of the method showed that the incorporation of MTF into the combination of GR-MP and DMNs could potentially improve the selective delivery and control the release as well as the permeation profile in in vitro hyperglycemic conditions, making it as an innovative approach to overcome the problems in oral administration of MTF. To further evaluate the efficacy, as the next step, in vivo analytical method must now be developed.

CRediT authorship contribution statement

Sumayya Binti Abd Azis: Conceptualization, Methodology, Funding acquisition, Writing – original draft. **Nur Syafika:** Methodology, Writing – original draft. **Hanin Azka Qonita:** Methodology, Writing – original draft. **Tiara Resky Anugrah Mahmud:** Data curation, Validation. **Ahmad Abizart:** Methodology, Data curation. **Andi Dian Permana:** Conceptualization, Project administration, Funding acquisition, Validation, Supervision.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.108051.

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