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In vitro small intestinal absorption and pH stability of tableted KIOM-C and KIOM-MA-128

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Abstract−Hot water extracts, KIOM-C and KIOM-MA-128, were formulated into tablets and three kinds of absorption enhancers were included in the tablets. The mixture of starch and lactose was used as an excipient, sodium carboxymethylcellulose as a disintegrating agent, and magnesium stearate as a binder. The weight ratio of water extract/excipient/disintegrating agent/absorption enhancer/binder was 80/7/10/2/1. The tablets were completely disintegrated in an aqueous solution in 40 min, and the disintegration rates were almost the same with all the tablets tested. Among the enhancers, EDTA was the most effective in enhancing the *in vitro* small intestine absorption of KIOM-C and KIOM-MA-128.

Key words: KIOM-C, KIOM-MA-128, Tablet, Disintegration, Small Intestine Absorption, pH Stability

INTRODUCTION

Gastrointestinal (GI) tract absorption is required to be high enough for the high efficacy of a biological active agent. Absorption enhancers, prodrugs, and nano carriers have been used for that purpose [1-3]. The enhancers have an effect on either paracellular absorption or transcellular absorption. Ethylene diamine tetra acetic acid (EDTA), sodium caprylate, saponin, taurine, diethyl maleate, azone and triglyceride are known to be paracellular absorption enhancers [4,5]. Since the paracellular space is filled with polar body fluid, they promote the absorption of polar drugs [6]. The tight junction can be loosened and the space between the endothelial cells of GI tract can be broadened by the enhancers, leading to a promoted absorption. On the other hand, sodium glycocholate, sodium deoxycholate, N-lauryl-β-D-maltopyranoside, and sodium caprate are known to be transcellular absorption enhancers [7]. Since the cellular membranes are lipidic, they promote the absorption of non-polar drugs [8]. The cellular membrane can be fluidized and the cellular glutathione can be exhausted by the enhancers, leading to a promoted absorption. Recently, monoelein cubic phase nanoparticles (so-called cubosome) were used to enhance *in vitro* small intestinal absorption of hot water extracts, KIOM-C and S-164 [9]. Monoolein can interact with the endothelial cellular membrane due to its cellular membrane affinity and disturb the packing. In addition, the lipidic nanoparticle can be up-taken by the endothelial cells and carry the water-soluble extract through the transcellular route [8]. Other prerequisites to obtain a high efficacy are that biological active agents release out of their dosage forms promptly and be chemically stable in the condition of GI tract fluid.

In this study, KIOM-C and KIOM-MA-128 were formulated into tablets and three kinds of absorption enhancers (namely EDTA, diethyl maleate, and sodium salicylate) were included in the tablets to enhance the small intestinal absorption. KIOM-C is a hot water extract from Scutellaria baicalensis George, Glycyrrhiza uralensis Fisch, Paeonia lactiflora Pall, Angelica gigas Nakai and is claimed to have a strong anti-virus (anti-influenza) activity and an immunity-boosting activity [10]. KIOM-MA-128 (MA-128) is a hot water-soluble extract from Glycyrrhiza uralensis Fisch, Sophora flavescens, Arctium lappa Linne, Cnidium officinale Makino and Reynoutria elliptica and is claimed to be efficacious in the treatment of atopic dermatitis [9,11]. EDTA was chosen as a paracellular absorption enhancer, and diethyl maleate and sodium salicylate as trans cellular absorption enhancer. The mixture of starch and lactose was used as an excipient, disintegrating agent, sodium carboxymethylcellulose as a disintegrating agent, and magnesium stearate as a binder. Tablets were prepared by pressing the mixture of water extract, excipient, disintegrating agent, absorbance enhancer, and binder by using a press. The disintegration rate was determined as a physical property of the tablets. The effect of absorption enhancers on the *in vitro* small intestinal absorptions of KIOM-C and KIOM-MA-128 were investigated using tablets containing different absorbance enhancers. And the pH stability of free water extracts and tableted ones in aqueous solutions of pH 2 and pH 7.4 was investigated for 24 hr by analyzing the residual amount using HPLC analysis. When tablets prepared in the present work are orally administered, they will contact stomach juices (strong acidic pH) and subsequently to small intestinal juices (neutral pH). This is the rationale for the pH stability test.

EXPERIMENT

1. Materials

Male Sprague-Dawley rats (200-230 g) were supplied by Orientbio Inc. (Gyonggi-do, Korea). Sodium carboxymethylcellulose, starch, EDTA, diethyl maleate (DM), sodium salicylate (SS) and lactose were purchased from Daejung Chemicals & Metals Co. (Siheung, Korea). Magnesium stearate was purchased from Junsei Chem-

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ical Co. (Tokyo, Japan). Water was distilled in a water purification system (Pure Power I$^+$, Human Corporation, Korea) until the resistivity was 18 MΩ/cm. All other reagents were of analytical grade. KIOM-C and KIOM-MA-128 were provided by Korea Institute of Oriental Medicine.

2. Preparation of Tablets

Water extract (either KIOM-C or KIOM-MA-128), excipient (starch/lactose, 1/1 (w/w)), disintegrating agent (sodium carboxymethylcellulose), binder (magnesium stearate) and/or absorption enhancer (either EDTA, DM or SS) were homogeneously mixed in a conical tube so that the weight ratio of water extract/excipient/disintegrating agent/absorbance enhancer/binder was 80/7/10/2/1. The mixture, 400 mg, was put in a press and tableted at a pressure of 9 tons.

3. Disintegration of Tablets

One tablet of KIOM-C, KIOM-C/SS, KIOM-C/DM, KIOM-C/EDTA, KIOM-MA-128, KIOM-MA-128/SS, KIOM-MA-128/DM, and KIOM-MA-128/EDTA was put in 7 ml of phosphate-buffered saline (PBS, 10 mM, pH 7.4) contained in 10 ml vials, and the disintegration was observed with time lapse.

4. *In vitro* Small Intestinal Absorption

In vitro small intestinal absorption of KIOM-C and KIOM-MA-128 contained in tablets was investigated, based on a procedure described in the literature [12]. Small intestines were obtained from rats weighting 205-220 g (Sprague Dawley) sacrificed under the atmosphere of diethyl ether. Residual diet and juices were removed from the small intestines by washing in a glucose-saline solution. The upper parts (about 4 cm-long) of the intestines were cut out, and the rest were cut into 10 cm-long pieces. After being turned inside out, the cut intestine was zipped at one end using a suture, and glucose-KRP medium, 1 ml, was put in the small intestine as a receptor solution. 1 ml of pipette tip was inserted in the open end of the small intestine with the tip facing inward the small intestine; it was put in a 1 ml of test tube with the mouth of tip and that of test tube directing upward. And then, 5 mL of HEPES buffer (30 mM, pH 7.4) was put as a donor solution in the space between the intestine and the test tube. A tablet was soaked in the donor solution, then 20 μl of receptor solution was taken at a given time for 24 h. The amount of KIOM-C was determined with a high performance liquid chromatography system (Agilent 1200 series) equipped with a UV detector (0.05 AFUS, 203 nm). Acetonitrile/water mixture was allowed to flow through a reversed phase column (4.6× 250 mm, C18 5 μm, Phenomenex Luna C18) at a flow rate of 1.0 mL/min in gradient elution mode. The volumetric ratio of water/MeOH was 75/25 in the time interval of 0-10 min, 70/30 in 10-30 min, 50/50 in 30-40 min, 30/70 in 40-60 min, 0/100 in 60-75 min, and 75/25 in 75-90 min. A calibration curve of KIOM-C was set up by plotting the area of peak observed at the retention time of 37.2 min vs. the concentration of KIOM-C solution in 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, and 0.0625 mg/ml. The amount of KIOM-MA-128 was determined under the same condition as adopted in quantifying KIOM-C except for the gradient elution mode. The volumetric ratio of water/CH_3CN was 95/5 in the time interval of 0-70 min, 0/100 in 70-85 min, and 95/5 in 85-100 min. The calibration curve of KIOM-MA-128 was set up by plotting the area of peak observed at the retention time of 27.5 min vs. the concentration of KIOM-MA-128 solution in 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, and 0.0625 mg/ml.

5. pH Stability of KIOM-C and KIOM-MA-128

320 mg of free water extracts (KIOM-C and KIOM-MA-128) and tableted ones were put in buffer solutions of pH 2.0 and pH 7.4 so that the concentration of water extract was 32 mg/ml. The mixture was allowed to stand at room temperature under dark condition, and an aliquot of the mixture suspension was taken at 12 hr and 24 hr for the determination of the water extract concentration using an HPLC system. The analytical condition of HPLC was the same as that adopted in the previous section. The water extract concentrations determined at the time lapse of 12 hr and 24 hr were represented as relative concentrations with respect to the initial concentrations.

RESULTS AND DISCUSSION

1. Disintegration of Tablets

Fig. 1 shows the photos of KIOM-C tablet, KIOM-C/SS tablet, KIOM-C/DM tablet, KIOM-C/EDTA tablet, KIOM-MA-128 tablet, KIOM-MA-128/SS tablet, KIOM-MA-128/DM tablet and KIOM-MA-128/EDTA tablet. The diameter was 1.3 cm and the thickness was 0.2 cm. The tablets were successfully prepared, and the water extracts and the absorption enhancers had no significant effect on the tablet dimension.

Fig. 2 shows the photos of the time-dependent disintegration of KIOM-C tablet, KIOM-C/SS tablet, KIOM-C/DM tablet, KIOM-C/EDTA tablet, KIOM-MA-128 tablet, KIOM-MA-128/SS tablet, KIOM-MA-128/DM tablet and KIOM-MA-128/EDTA tablet in PBS buffer solution (30 mM, pH 7.4). The tablets were somewhat dissolved, but they maintained their integrity and the aqueous media were still clear 10 min after being put in the buffer solution. The tablets were mostly dissolved and the aqueous media were turbid, but small residues of the tablets were found in 20 min. The tablets were completely disintegrated and no residues of the tablets were found at 40 min. The disintegration rate will depend mainly on the composition of table once tablets have been prepared under the same pressure. The weight ratio of water extract/excipient/disintegrating agent/absorption enhancer/binder was 80/7/10/2/1. Since water extract is the major component in all the tablets, it might have a great effect on the disintegration rate. But, whatever the extract is (KIOM-C or KIOM-MA-128), it is readily soluble in water so the kind of water extract will have little effect on the disintegration rate. The other difference among the tablets is the kind of absorption enhancer included. Even though EDTA, diethyl maleate and sodium salicylate

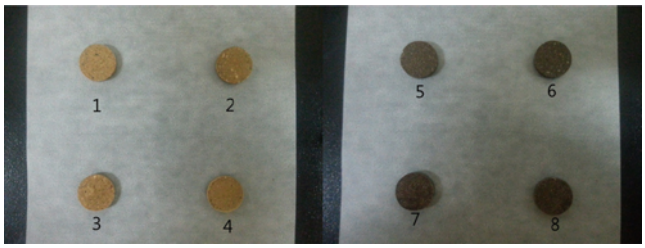

Fig. 1. Photos of KIC tablet (1), KIC/SA tablet (2), KIC/DM tablet (3), KIC/EDTA tablet (4), KIMA tablet (5), KIMA/SA tablet (6), KIMA/DM tablet (7), and KIMA/EDTA tablet (8).

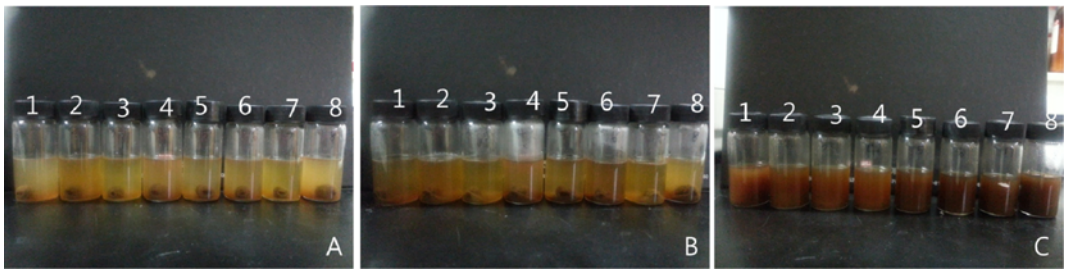

Fig. 2. Photos of time-dependent disintegration of KIC tablet (1), KIC/SA tablet (2), KIC/DM tablet (3), KIC/EDTA tablet (4), KIMA tablet (5), KIMA/SA tablet (6), KIMA/DM tablet (7), and KIMA/EDTA tablet (8) in PBS buffer solution (30 mM, pH 7.4) in 10 min (panel A), 20 min (panel B) and 40 min (Panel C).

have different physicochemical properties (e.g., polarity and solubility), they would little effect on the disintegration rate either due to their low contents in the tablet.

2. *In vitro* Small Intestinal Absorption

The standard curve of KIOM-C was Y=8.21X+0.016 (R^2=0.9999), where Y is the area of peak observed at the retention time of 37.2 min and X is the concentration of KIOM-C solution in mg/ml. Fig. 3 shows the effect of the adsorption enhancers on the small intestinal absorption of KIOM-C. When no absorption enhancer was included, the absorption increased with time lapse for the first 4 hr, thereafter there was no significant absorption for the rest 20 hr. It means that equilibrium between donor part (outside of small intestinal) and receptor part (inside of small intestinal) was reached in 4 hr. The maximum amount of KIOM-C permeated through the small intestine was around 4 mg. When sodium salicylate was included in the tablet, the effective absorption period was prolonged and the absorption was significantly promoted. The maximum amount of KIOM-C that permeated through the small intestine was around 6 mg. Sodium salicylate is known to interact with cell membrane proteins and increase the permeability of the cell membrane [13]. So it could interact with the endothelial cell protein and promote the intestinal absorption of KIOM-C through trans-cellular route. When diethyl maleate was included in the tablet, the absorption was also significantly promoted, and the maximum amount of KIOM-C permeated through the small intestine was almost the same as the value obtained with sodium salicylate. Diethyl maleate was reported to deplete cellular glutathione and increase the permeability of the cell membrane [14]. So it could exhaust endothelial cellular glutathione and promote the intestinal absorption of KIOM-C through trans-cellular routes. On the other hand, when EDTA was included in the tablet, the absorption was markedly promoted and the maximum amount of KIOM-C permeated through the small intestine was around 11 mg, almost two-times higher than the maximum amount obtained with sodium salicylate and diethyl maleate. EDTA is known to chelate Ca^{2+} of a tight junction connecting adjacent endothelial cells and broaden the space among the cells [8,15]. Thus, it could remove Ca^{2+} from the tight junction and promote the intestinal absorption of KIOM-C through paracellular route. Since KIOM-C is water-soluble, it will be hardly absorbed through the lipidic trans-cellular route; instead, it will be readily absorbed mainly through the water-filled paracellular route. This may account for why EDTA is more effective in promoting small intestinal absorption than sodium salicylate and diethyl maleate. The standard curve of KIOM-MA-128

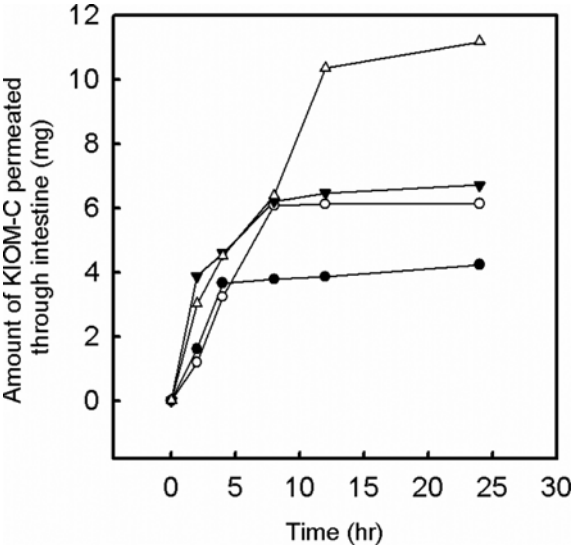

Fig. 3. Effect of the absorption enhancers on the small intestinal absorption of KIOM-C. KIC tablet (●), KIC/SA tablet (○), KIC/DM tablet (▼), KIC/EDTA tablet (△).

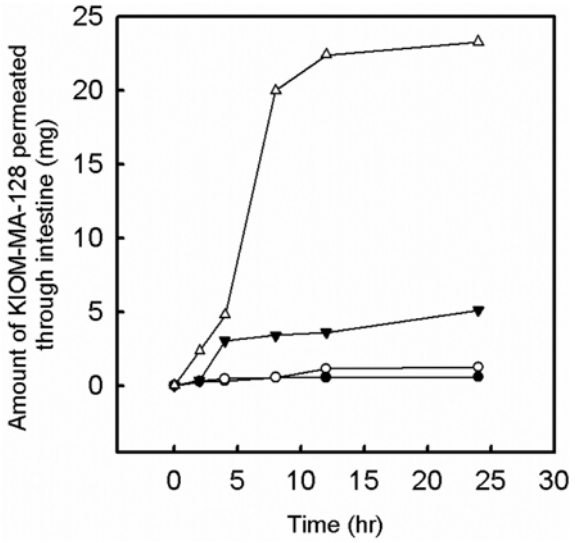

Fig. 4. Effect of the absorption enhancers on the small intestinal absorption of KIOM-MA-128. KIMA tablet (●), KIMA/SA tablet (○), KIMA/DM tablet (▼), KIMA/EDTA tablet (△).

was Y=10.75X−0.089 (R^2=0.9988), where Y is the area of peak observed at the retention time of 27.1 min and X is the concentration of KIOM-MA-128 solution in mg/ml. Fig. 4 shows the effect of the absorption enhancers on the small intestinal absorption of KIOM-MA-128. When no absorption enhancer was included, no appreciable absorption was detected for 24 hr. Unlike KIOM-MA-128, KIOM-C exhibited significant intestinal absorption without the aid of an absorption enhancer (Fig. 3). It is believed that KIOM-MA-128 is more polar, so it is harder to be absorbed into lipidic intestine. In fact, the solubility of KIOM-MA-128 in distilled water (adjusted to pH 7.4) (184 mg/ml) was much higher than that of KIOM-C (103 mg/ml), indicating that KIOM-MA-128 is more polar than KIOM-C. Sodium salicylate had little effect on the absorption. Diethyl maleate promoted the absorption but the effect was not marked. The little absorption-promoting effect is possibly because they are absorption enhancers for trans-cellular route, which is not a main transport route of water-soluble compounds. And, the absorption enhancers for trans-cellular route had less promoting effect on the absorption of KIOM-MA-128 than on the absorption of KIOM-C (Fig. 3 and Fig. 4). This could also be ascribed to that KIOM-MA-128 is more polar than KIOM-C. Since KIOM-MA-128 is more polar, it will be less affected by sodium salicylate and diethyl maleate which act on the non-polar trans-cellular route. On the other hand, EDTA markedly promoted the absorption of KIOM-MA-128. As described previously, it is an absorption enhancer for paracellular route, which is a main transport route of water soluble compounds. And, EDTA had greater promoting effect on the absorption of KIOM-MA-128 than on the absorption of KIOM-C (Fig. 3 and Fig. 4). This could also be attributed to the difference in the polarity between the two water extracts. Since KIOM-MA-128 is more polar than KIOM-C, it will be affected more greatly by EDTA, which can broaden the polar paracellular route.

3. pH Stability of Water Extracts

Fig. 5 shows the pH stability of free KIOM-C and tableted KIOM-C in aqueous phase of pH 2.0 and pH 7.4 for 24 hr. At pH 2.0, free KIOM-C lost about 8% of its content in 12 hr and more than 8% in 24 hr. At pH 7.4, it lost more than 10% in 24 hr. Tableted KIOM-Cs were chemically more stable than free KIOM-C. For example, at pH 2.0, KIOM-C of KIOM-C tablet lost less than 1% in 12 hr and lost less than 6% in 24 hr. And, at pH 7.4, KIOM-C of KIOM-C tablet lost only 2% in 24 hr. The same trend was obtained with KIOM-C/SS tablets and KIOM-C/DM tablets. The reason why tableted KIOM-C was more stable than free KIOM-C is possibly because KIOM is prevented from contacting water before tablets are disintegrated in aqueous solution. In fact, it took about 40 min for tablets to disintegrate. Another reason would be that excipient (starch/lactose) and disintegrating agent (sodium carboxymethylcellulose) can prevent KIOM-C from being chemically degraded even after disintegration. Among the tablets tested, KIOM-C/EDTA tablet exhibited the lowest effect in stabilizing KIOM-C. For example, at pH 2, KIOM-C of KIOM-C/EDTA tablet lost about 9.5% of its content in 24 hr. And, at pH 7.4, it lost about 8.5%. EDTA is a chelating agent so it will capture the sodium ion of disintegrating agent (sodium carboxymethylcellulose) and the magnesium ion of binder (magnesium stearate). As a result, carboxymethylcellulose and stearate will be negatively charged more strongly due to chelation so they could more effectively deteriorate KIOM-C.

Fig. 6 shows the pH stability of free KIOM-MA-128 and tableted KIOM-MA-128 in aqueous phase of pH 2.0 and pH 7.4 for 24 hr. At pH 2.0, free KIOM MA-128 lost about 4% of its content in 24 hr. At pH 7.4, it lost about 9% in 24 hr. Tableted KIOM-MA-128 were chemically somewhat more stable than free ones. For example, at pH 2.0, KIOM-MA-128 of KIOM-MA-128 tablet lost only 2% in 24 hr. And, at pH 7.4, it lost only 6% in 24 hr. Excipient and disintegrating agents are likely to prevent the chemical degradation of KIOM-C. As in the case of KIOM-C/EDTA tablet, KIOM-MA-128/EDTA tablet exhibited the lowest effect in stabilizing KIOM-MA-128, possible due to the sodium and magnesium-chelating effect of EDTA.

CONCLUSION

Tablets of KIOM-C and KIOM-MA-128 were successfully pre-

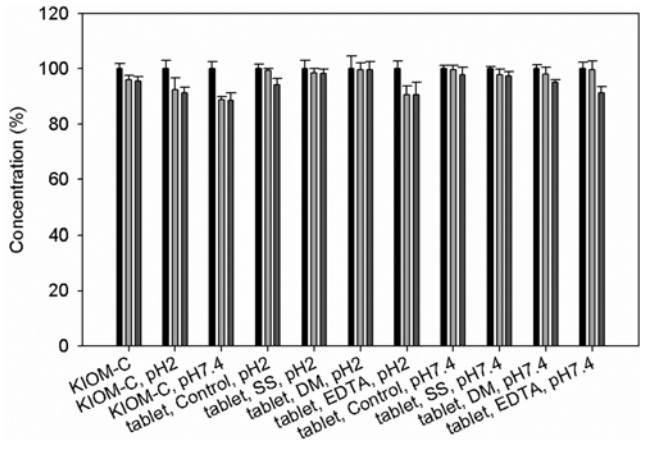

Fig. 5. pH stability of free KIOM-C and tableted KIOM-C in aqueous phase of pH 2.0 and pH 7.4 for 24 hr. The concentrations determined at the time lapse of 12 hr (▒) and 24 hr (■) were represented as relative concentrations when the initial concentrations (■) were taken as 100 %.

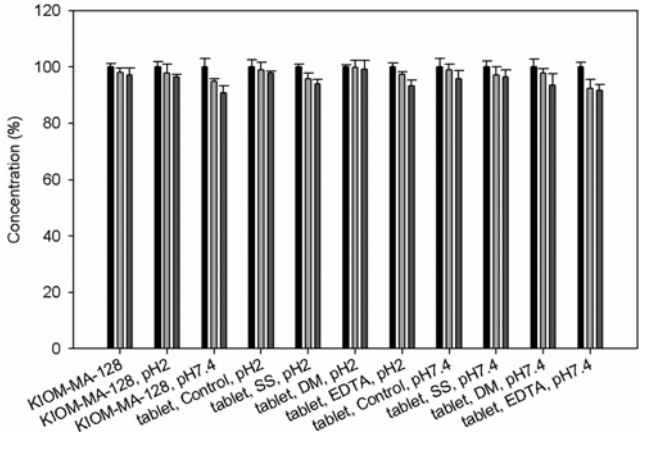

Fig. 6. pH stability of free KIOM-MA-128 and tableted KIOM-MA-128 in aqueous phase of pH 2.0 and pH 7.4 for 24 hr. The concentrations determined at the time lapse of 12 hr (▒) and 24 hr (■) were represented as relative concentrations when the initial concentrations (■) were taken as 100%.

pared using the mixture of starch and lactose as an excipient, sodium carboxymethylcellulose as a disintegrating agent and magnesium stearate as a binder. The complete disintegration of the tablets in an aqueous solution was observed in 40 min and the disintegration rates were independent on the tablet composition used in the present work. EDTA was found to be the most effective among the enhancers tested in enhancing the *in vitro* small intestine absorption of KIOM-C and KIOM-MA-128. And, diethyl maleate and sodium salicylate were found to be more effective enhancers for KIOM-C than for KIOM-MA-128. On the other hand, EDTA turned out to be more effective enhancer for KIOM-MA-128 than for KIOM-C. Tableted KIOM-C and KIOM-MA-128 were more chemically stable in an aqueous phase than free ones. Tablets containing EDTA showed the lowest stabilizing effect among the tablets tested. However, the effect of EDTA on the pH stability was not marked. Since EDTA was found to be the most effective among the enhancers tested in enhancing the *in vitro* small intestine absorption of KIOM-C and KIOM-MA-128 while deteriorating slightly the extracts, EDTA could be considered to be used in further *in vivo* testing. The KIOM-C tablets and KIOM-MA-128 ones prepared in the present study could be used as oral dosage forms in the treatment of influenza and atopic dermatitis, respectively.

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