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Research Article

DEVELOPMENT OF TRANSDERMAL DOSAGE FORM USING COPROCESSED EXCIPIENTS OF XANTHAN GUM AND CROSS-LINKED AMYLOSE: *IN VITRO* AND *IN VIVO* STUDIES

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ABSTRACT

Objective: A transdermal hydrogel dosage form consists of a three-dimensional polymer network that binds water in large quantities and is used for drug delivery. The study's aim was to prepare coprocessed excipients as a matrix for a transdermal hydrogel containing diclofenac sodium and examine *in vitro* and *in vivo* drug penetrations.

Methods: Four types of coprocessed excipients were produced using two methods that combined crosslinking and coprocessing steps. The produced excipients were formulated as transdermal gels containing sodium diclofenac. An *in vitro* penetration test was then performed using a Franz diffusion cell to pass the drug through a rat skin membrane. An *in vivo* penetration test was performed by applying the hydrogel to the abdominal skin of male Sprague-Dawley rats and then measuring the plasma drug concentration.

Results: *In vitro* penetration results showed that the flux from Co-CLA6-XG 1:2, Co-CLA12-XG 1:2, CL6-Co-A-XG 1:2, and CL12-Co-A-XG 1:2 transdermal hydrogels was 655.23±116.43 μg·cm^{-2}/h, 569.08±26.58 μg·cm^{-2}/h, 867.42±101.27 μg·cm^{-2}/h^{-1}, and 736.99±15.39 μg·cm^{-2}/h^{-1}. The *in vivo* study resulted in area under the curve for the Co–CLA6–XG 1:2, Co–CLA12–XG 1:2, CL6–Co–A–XG 1:2, and CL12–Co–A–XG 1:2 transdermal hydrogels was 32.08±5.40 μg·ml^{-1}·h, 34.27±8.34 μg/ml·h, 6.20±2.90 μg/ml·h, and 14.38±2.38 μg/mL·h, respectively.

Conclusion: The study results showed that the excipients could be processed to form a matrix within a transdermal hydrogel formulation and deliver sodium diclofenac into systemic circulation in a controlled release manner.

Keywords: Amylose, Xanthan gum, Coprocessed excipient, Transdermal hydrogel, *In vitro* penetration, *In vivo* penetration.

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INTRODUCTION

One aim of research and development of drug delivery systems is to minimize adverse side effects. A common type of pharmaceutical preparation is a transdermal drug delivery system, which delivers a drug through the skin into the systemic circulation. This system has many advantages over oral or injectable drug delivery systems, including avoidance of first-pass metabolism so that a drug can be administered with small therapeutic doses [1]. Moreover, transdermal delivery systems also can reduce undesirable side effects, such as ulceration of the gastrointestinal tract, provide easier administration, and increase patient compliance because of reduced dosing frequency [2]. A type of dosage form that can be used in transdermal drug delivery systems is a hydrogel.

Hydrogels consist of a three-dimensional structure that is able to absorb water or biological fluid in large amounts [1]. Moreover, hydrogels can contain drug molecules in a way that allows the drug to be released slowly [3]. To produce a hydrogel formulation, an excipient is required that is capable of forming a three-dimensional structure that is strong, flexible, and can modulate drug release. Excipients with these functional properties can be obtained through chemical and physical modification of the excipients. One excipient that can prolong drug release is a coprocessed excipient. Coprocessing is a method of mixing an excipient that aims to eliminate unwanted functional properties and maintain or improve the desired functional properties [4]. A coprocessed excipient is obtained by incorporating one of the excipients into the structure of other excipient particles through a physical process, such as codrying. Among the examples of excipients that can be used for controlled drug release formulations in the form of a hydrogel are high amylose starch and xanthan gum [5,6].

A study conducted by Surini described the development of amylose and xanthan gum excipients as a matrix for sustained-release tablet formulation [7]. The development included modification in crosslinking and coprocessing of the excipients. The resulting excipient was Co-CLA-XG, which is a coprocessed excipient of xanthan gum and amylose that was previously cross-linked by sodium trimetaphosphate (STMP). In addition, CL-Co-A-XG was produced, which is a cross-linked excipient of coprocessed amylose and xanthan gum. The study used STMP as a crosslinking agent at concentrations of 6% and 12% [7]. In addition, a different variation of amylose and xanthan gum, namely, 1:1, 1:2, and 2:1 were used. As a result, modified cross-linked and coprocessed excipients with ratio (amylose: xanthan gum) of 1:1, 1:2, and 2:1 for each Co-CLA6-XG, Co-CLA12-XG, CL6-Co-A-XG, and CL12-Co-A-XG (12 excipient types) were obtained. The Co-CLA-XG excipients were a rough powder (such as fiber) that was voluminous, odorless, and slightly yellowish-white. The CL-Co-A-XG excipients were fine granules, odorless, white, and slightly yellowish. From those results, the CL-Co-A-XG excipients were characterized by higher viscosity and flow properties than those of Co-CLA-XG. The 6% concentration of the cross-linked agent gave a greater gel strength of excipient than that of the 12% concentration. However, both types of excipients were assessed as suitable for controlled release of drugs because such excipients are characterized by high viscosity, high gel strength, and sufficient expansion ability [7].

In this study, the excipients of Co-CLA6-XG, Co-CLA12-XG, CL6-Co-A-XG, and CL12-Co-A-XG were used with a 1:2 ratio of amylose and xanthan gum because that ratio is expected to form a solid compact gel. Evaluations of the transdermal hydrogel included physical organoleptic observations, preparation acidity (pH), preparation consistency and thickness, and determination of drug levels in the hydrogel. We also performed *in vitro* and *in vivo* penetration tests. The *in vitro* penetration test was performed using Franz diffusion cells to assess drug penetration through the skin [8]. The *in vivo* penetration test was performed in male Sprague-Dawley rats as the most representative method to assess drug incorporation into the systemic circulation.

METHODS

Materials

Amylose (Shangqiu Kangmedia Bio-Tech, China), xanthan gum (Cargill Bioengineering, Canada), diclofenac sodium (Yung Zip Chemical, Taiwan), STMP (Shangqiu Kangmedia Bio-Tech, China), sodium hydroxide (Merck, Germany), hydrochloride acid (Merck, Germany), sulfuric acid (Merck, Germany), nitric acid (Merck Germany), ascorbic acid (Takeda, Japan), orthophosphate acid (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany), ammonium molybdate tetrahydrate (Merck, Germany), triethylamine high-performance liquid chromatography (HPLC) grade (Merck, Germany), methanol HPLC grade (Merck, Germany), and diltiazem hydrochloride (Piramal Healthcare, India) were used. Other chemicals and solvents were of analytical grade and purchased from commercial suppliers. Sprague-Dawley rats were obtained from Institut Pertanian Bogor (Bogor Agricultural University, Bogor, Indonesia).

Synthesis of Co-CLA6-XG and Co-CLA12-XG (1:2) (method A)

Synthesis method of cross-linked amylose and coprocessed excipient to give a cross-linked amylose-xanthan gum-based on Cury *et al*. [9], with modification as stated in Surini *et al*. [7]. The first stage was synthesis of cross-linked amylose by reacting amylose with STMP as a crosslinking agent. Amylose was dispersed in distilled water while stirring with a magnetic stirrer until perfectly dispersed. Sodium hydroxide 10 N solution was dripped slowly into the mass to maintain the pH at 11–12 during the reaction. In another flask, STMP solutions were prepared to produce 6% b/v for CLA6 and 12% b/v for CLA12. Thereafter, the STMP solution was slowly added to the dispersion of amylose while stirring using a homogenizer (CKL Machinery, Malaysia) at a speed of 3000 rpm for 4 h and then allowed to stand for 12 h to ensure completion of the reaction. After that, the suspension of CLA was neutralized by hydrochloride acid 7N until a pH of six was achieved. The suspension washed with ethanol 96% v/v until the filtrate gave a negative result with ammonium molybdate reagent. The residues were dried at room temperature for 48 h. The dried residues were then sieved through a 35-mesh (500-µm) sieve.

The second stage was coprocessing for each 3% CLA (CLA6 and CLA12) with 3% xanthan gum in distilled water in 1:2 ratio using a homogenizer (CKL Machinery) at 3000 rpm for 30 min. Thereafter, the mass was dried in a drum drier (R. Simon Dryers, UK) at a temperature of 109–112°C. The produced mass was mashed and sieved by a 35-mesh (500-µm) sieve.

Synthesis of CL6-Co-A-XG and CL12-Co-A-XG (1:2) (Method B)

The first stage was a physical modification by coprocessing amylose with xanthan gum at a concentration of 3% for each in distilled water in 1:2 ratio. The mass was mixed in a homogenizer (CKL Machinery) at 3000 rpm for 30 min to obtain a homogeneous mass. The homogeneous mass was dried in a drum drier (R. Simon Dryers) at a temperature of 109–12°C. The produced mass was mashed and sieved with a 35-mesh (500-µm) sieve. The second stage was crosslinking of the coprocessed amylose-xanthan gum using the same method as the first stage of method A.

Substitution degree (SD) of the excipients

The SD was determined by the colorimetry method [10] using two different solutions. The A solution was ascorbic acid 10%, and the B solution was ammonium molybdate tetrahydrate 0.42%. The sample was placed in a crucible and heated at 600°C for 3 h. The ash from the sample was dissolved in sulfuric acid 0.1 N solution and boiled for 10 min. The solution was then filtered by Whatman 40 paper and diluted with aquadest-sulfuric acid 0.1 N solutions (1:1). Then, the A and B solutions were added to the sample solution. A solution of the sample was mixed and incubated at 45°C for 20 min in a water bath; it was then immediately analyzed using a visible spectrophotometer at a wavelength of 820 nm. The interpretation of the phosphate SD was made by comparison with a calibration curve. The phosphate SD was calculated according to the following equation:

$$DS=\frac{162P}{3100-102P}$$

where P is the percentage of phosphate from cross-linked high amylose [11], 162 is the molecular weight of a glucose unit, 3100 is the molecular weight of phosphate, and 102 is the molecular weight of phosphate as $-PO_3Na$.

Preparation of transdermal hydrogels

The formulas of Co-CLA6-XG 1:2, Co-CLA12-XG 1:2, CL6-Co-A-XG 1:2, and CL12-Co-A-XG 1:2 transdermal hydrogels are shown in Table 1. The transdermal hydrogels containing diclofenac sodium were prepared by dissolving diclofenac sodium in propylene glycol, then adding it to polyethylene glycol (PEG) 400 and demineralized water. The hydrogel was mixed on a homogenizer at 1000 rpm until it became clear. The drug solution was homogenized in a sonicator for 10 min. Each excipient was dispersed in the drug solution and allowed to stand for 15 min. Subsequently, the mass was mixed in a homogenizer at 1000 rpm and 50°C for 15 min to give a homogenous mass. Then, up to 75 g of the homogenous mass was placed into a 10×7.5-cm mold and dried in an oven at 50°C for 5 h. After cooling, the hydrogel was cut into 1×1-cm sections.

Evaluation of transdermal hydrogels

Evaluation of the transdermal hydrogels included surface pH, thickness, consistency, and drug content. The surface pH of the hydrogel was measured by placing the hydrogel in demineralized water for 2 h. The electrode of the pH-meter was then placed on the surface of the hydrogel until it reached equilibrium. The thickness of a transdermal hydrogel section was measured using a caliper. The consistency of the hydrogel was determined using a penetrometer (Herzoo, Germany).

Table 1: Formulation of transdermal hydrogel containing diclofenac sodium

Composition	F1	F2	F3	F4	F5	F6	F7	F8
Diclofenac sodium (g)	5	0.5	5	0.5	5	0.5	5	0.5
Co-CLA6-XG (g)	5	5	-	-	-	-	-	-
Co-CLA12-XG (g)	-	-	5	5	-	-	-	-
CL6-Co-A-XG (g)	-	-	-	-	5	5	-	-
CL12-Co-A-XG (g)	-	-	-	-	-	-	5	5
Polyethylene glycol 400 (g)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Propylene glycol (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Demineralized water ad (g)	100	100	100	100	100	100	100	100

Table 2: Substitution degree of the excipients

Excipients	Substitution degree
CLA6	0.08±0.01
CLA12	0.10±0.00
CL6-Co-A-XG	0.10±0.00
CL12-Co-A-XG	0.17±0.04

Each point represents mean±standard deviation (n=3)

Table 3: Properties of the transdermal hydrogels

Hydrogel	Formula	Surface pH	Thickness (mm)	Consistency (yield value)	Drug content in each hydrogel (mg)
Co-CLA6-XG	F1	7.52±0.05	8.18±0.12	5537.71±102.09	48.15±0.59
	F2	7.21±0.04	7.20±0.38	5679.59±434.79	5.36±0.05
Co-CLA12-XG	F3	7.48±0.30	8.00±0.27	6150.01±171.85	48.03±1.45
	F4	6.96±0.16	7.15±0.05	6002.12±422.85	5.19±0.13
CL6-Co-A-XG	F5	7.18±0.12	7.55±0.20	5752.06±109.18	50.74±2.82
	F6	7.35±0.14	7.40±0.33	6858.55±103.08	5.06±0.06
CL12-Co-A-XG	F7	7.25±0.02	7.07±0.09	5578.43±207.51	50.82±0.88
	F8	7.33±0.02	7.27±0.15	6386.91±614.70	5.06±0.06

Each point represents mean±standard deviation (n=3)

The diclofenac sodium content in the hydrogel was determined using an ultraviolet (UV) spectrophotometer. Each hydrogel was dissolved in phosphate buffer pH 7.4. The solution was then homogenized in a sonicator to ensure that the diclofenac sodium was completely dissolved in the phosphate buffer pH 7.4. Hydrogel F1, F3, F5, and F7 were diluted to 1/100 of their initial concentrations with phosphate buffer pH 7.4, and F2, F4, F6, and F8 were diluted to 1/10 of their initial concentrations with phosphate buffer pH 7.4. Each sample solution was measured by UV spectrophotometry at a wavelength of 276 nm. Each hydrogel was assayed 3 times.

***In vitro* penetration study**

The methods used for animal sacrifice for *in vitro* and *in vivo* penetration studies were approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (approval no. 224/UN2. FI/ETIK/2015). Sprague-Dawley rats were housed under standard conditions of temperature, relative humidity, and light. Unless otherwise specified, food, and water were given *ad libitum*.

The *in vitro* penetration test method was based on that of Klimes *et al*. [12] with slight modifications. The penetration of diclofenac sodium through the skin was measured using Franz diffusion cells. The receptor compartment was filled with a solution of phosphate buffer pH 7.4, and the temperature was maintained at 37±0.5°C. The speed of the magnetic stirrer was set to 250 rpm. The abdominal skins were collected from the Sprague-Dawley male rats, which were approximately 8 w old and weighed ±200 g. The skins were placed between the donor and the receptor compartment with the dermal side in contact with the receptor medium. The hydrogels (F1, F3, F5, and F7) were placed in the donor compartment. As much as, 0.5 ml of the sample solutions was withdrawn at each time interval from the receptor and immediately replaced with the same amount of pH 7.4 phosphate-buffered solution. The amount of diclofenac sodium in the sample was then measured using a UV spectrophotometer (Shimadzu, Japan) at a wavelength of 276 nm.

***In vivo* penetration study**

The method was based on Sintov and Botner [13] with slight modifications. The experiment used 8-w old Sprague-Dawley male rats (n=6) for each hydrogel (F2, F4, F6, and F8). The rats were anesthetized by intra-peritoneum injection of urethane (1000 mg/kg). The abdominal skins of the rats were shaved, and each hydrogel (F2, F4, F6, and F8) was placed on skin with plaster to keep the hydrogel on the surface of the abdomen during the test. As much as, 0.5 ml of a blood sample was collected from the sinus orbital of the eye at each time interval and then inserted into a microtube that had been loaded with heparin. Blood samples were centrifuged at 10,000 rpm for 20 min to separate the blood plasma from blood cells.

Analysis of diclofenac sodium in rat plasma

Diclofenac sodium in rat plasma was analyzed by HPLC (Shimadzu) equipped with a photometric diode array detector at a wavelength of 283 nm. The 250×4.6 mm C_{18} reversed-phase column had a particle size of 5 µm. The mobile phase was a mixture of methanol: bidistilled water containing 1% triethylamine pH 6.5 by adding orthophosphoric acid (80:20) at a flow rate of 0.5 ml/min. Before analysis, as much as 250 µl of plasma sample was added with 100 µl of internal standard (diltiazem hydrochloride 100 ppm) and mixed on a vortex mixer for 10 s. Thereafter, the sample was added to 250 µl methanol HPLC grade and mixed on a vortex mixer for 2 min and centrifuged at 10,000 rpm for 10 min to precipitate the proteins. Furthermore, as much as 20.0 µl of supernatant was injected into the HPLC instrument under the selected conditions. The method was partially validated by the European Medicine Agency [14].

Table 4: Flux values and the cumulative amount of the penetrated drug

Hydrogel	Formula	J (µg.cm^{-2}/h^{-1})	Q (µg.cm^{-2})	%Q
Co-CLA6-XG	F1	655.23±116.43	7.629±711	23.66±4.10
Co-CLA12-XG	F3	569.08±26.58	6.842±467	19.24±0.81
CL6-Co-A-XG	F5	867.42±101.27	10.438±390	35.41±9.29
CL12-Co-A-XG	F7	736.99±15.39	8,994±105	31.26±0.33

Each point represents the mean±standard deviation (n=3). J represents the transdermal flux, and Q is the cumulative amount of drug penetrated through the rat skin

RESULTS AND DISCUSSION

SD of the excipients

The SD was calculated as the degree of substitution of hydroxyl groups by phosphate groups from STMP in the amylose and xanthan gum. Inorganic phosphate was produced by heating the excipient to 600°C. Inorganic phosphate was reacted with ammonium molybdate tetrahydrate in acid solution to produce a phosphomolybdate complex, which was then reduced by ascorbic acid to produce a blue color [15].

Table 2 shows the differences in the SD between the excipients. The SD values were determined for CLA6, CLA12, CL6-Co-A-XG, and CL12-Co-A-XG. An SD of 0.08 indicated substitution of eight phosphate groups for hydroxyl groups in every 100 anhydroglucose units of amylose and/or xanthan gum. An SD of 0.10 indicated a substitution of 10 phosphate groups for hydroxyl groups in every 100 anhydroglucose units of amylose and/or xanthan gum. CL6-Co-A-XG has ten phosphate groups, and CL12-Co-A-XG had an SD of 0.17 indicating 17 substituted hydroxyl groups in every 100 anhydroglucose units of amylose and/or xanthan gum. From this data, we concluded that increasing the concentration of STMP increased the SD of the excipient. The SD of each excipient did not change its physical characteristics but did affect its functional characteristics, such as the swelling index, gelling strength, and viscosity, which could affect drug release [7].

Evaluation of transdermal hydrogels

Table 3 shows the evaluation results of the transdermal hydrogels' properties, including surface pH, hydrogel thickness, consistency value, and drug content in each hydrogel. The surface pH of the transdermal hydrogel was in the range of the limits of skin pH (5.6–7.5), which indicated that the transdermal hydrogel would not cause local irritation of human skin [16]. The surface pH of the transdermal hydrogel also was affected by the other components, such as diclofenac sodium, PEG 400, and propylene glycol. As shown in Table 3, there is a slight

Table 5: Pharmacokinetic parameters of the transdermal hydrogels containing diclofenac sodium

Hydrogel	Formula	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-12} (µg/ml h)	K_e (h^{-1})	$t_{1/2}$ (h)	MRT (h)
Co-CLA6-XG	F2	4.35±0.94	1.00±0.00	32.08±5.40	0.10±0.03	11.47±4.04	16.66±5.86
Co-CLA12-XG	F4	4.87±1.06	1.00±0.00	34.27±8.34	0.12±0.03	7.96±1.90	11.89±2.67
CL6-Co-A-XG	F6	2.87±0.48	0.50±0.11	6.20±1.19	0.18±0.04	5.29±1.50	4.82±0.74
CL12-Co-A-XG	F8	4.34±0.58	0.50±0.00	14.38±2.38	0.10±0.02	8.48±1.57	11.23±2.00

AUC: Area under the curve, MRT: Mean residence time, K_e: Elimination constant. Data are expressed as mean±standard error, n=6

difference in thickness among the hydrogels, which is associated with water content in each hydrogel (data not shown). In addition, the produced transdermal hydrogels had a hard consistency according to Sherman [17]. The hardest consistency was observed for CL6-Co-A-XG followed by CL12-Co-A-XG, Co-CLA12-XG, and Co-CLA6- XG. The produced transdermal hydrogels showed rigid form but still contained water. The drug content of diclofenac sodium in the transdermal hydrogel was evaluated by UV–visible spectrophotometry at 276 nm. Compared with the drug content in the initial formula, the transdermal hydrogels contained 95–105% of diclofenac sodium, because the F1, F3, F5, and F7 should contain ±50 mg for *in vitro* penetration study and the F2, F4, F6, and F8 should contain ±5 mg for *in vivo* penetration study.

***In vitro* penetration study**

Fig. 1 shows the penetration profiles of diclofenac sodium from the transdermal hydrogels across through the rat skin membrane. The flux value and cumulative amount of drug that penetrated through the skin among the four hydrogels, comprising different polymers, are shown in Table 4. The diffusion of diclofenac sodium through the skin was passive with the concentration gradient as the driving force. The diffusion was affected by many factors, such as the solubility of diclofenac sodium and the thickness of the stratum corneum [18]. Diclofenac sodium in water solution can be hydrolyzed to diclofenac acid, which will increase the partition coefficient of the active substance so that the penetration of the drug can also be [14]. The mechanism of drug permeation through the skin begins by swelling of the hydrogel. The receptor compartment, which consists of phosphate buffer pH 7.4, caused hydration of the rat skin. The transdermal hydrogel, which consisted of a polymer matrix, could be wetted by absorbing fluid as it contacts the skin. This process causes the hydrogel to swell so that diclofenac sodium diffuses through the three-dimensional network of the polymer. During that process, the gradient concentration between the rat skin and hydrogel is high, which causes diclofenac sodium to permeate through the skin.

The highest cumulative amount of drug penetrated through the skin was achieved by the CL6-Co-A-XG 1:2 hydrogel (F5). The crosslinking in CL6-Co-A-XG (1:2) may occur between amylose and xanthan gum. The more crosslinking indicates high crosslinking density, which means the network was closely woven. In this case, the chain sections of the cross-links were short and anchored by many points. Therefore, the polymer became less flexible and more rigid [19]. This characteristic makes diffusion of the drug easier through the matrix.

The Co-CLA6-XG 1:2 (F1) and Co-CLA12-XG 1:2 (F3) hydrogels gave levels of penetration of diclofenac sodium <30% because the three-dimensional networks of hydrogels F1 and F3 consisted of some closed-hydrogen bonds and high gel strength that slowed drug release [7]. Dumoulin *et al*. stated that drug release could be controlled by crosslinking amylose, the degree of which was limited in CLA-3 to CLA-11, and the maximum drug release time was observed in CLA-6 (as long as 20–24 h) [20]. Furthermore, Lanaerts *et al*. [20] stated that the increase in the degree of crosslinking from CLA-6 to CLA-20 correlated with the decrease in crystal properties, which can lead to a decreasing ability to form a double-helix structure and acceleration of matrix hydration [20].

The percentage of cumulative drug penetrations of all hydrogels through the skin was <50%, which was caused not only by the ability of the polymer to slow drug release but also the characteristics of the

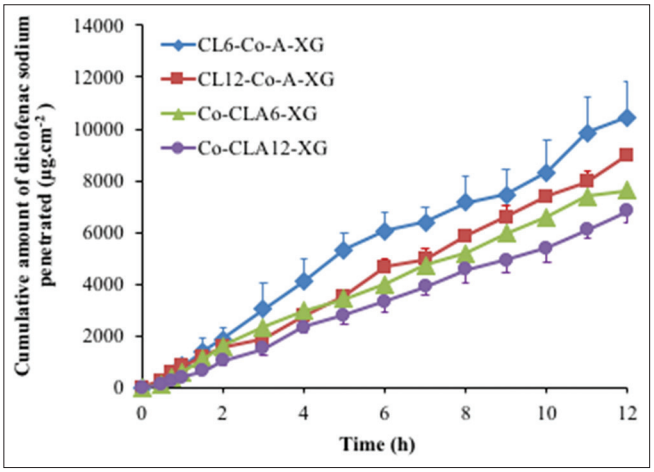

Fig. 1: *In vitro* percutaneous penetration profiles of diclofenac sodium through excised rat skins after application of transdermal hydrogel prepared from different excipients. Green, purple, blue, and red lines represent F1, F3, F5, and F7, respectively. Each point represents the mean±standard deviation; n=3

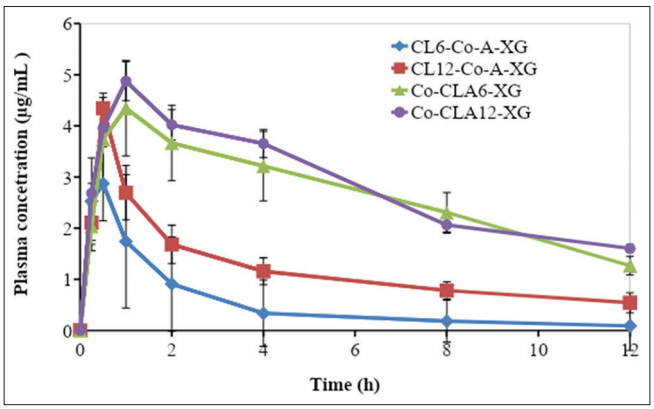

Fig. 2: Plasma concentration-time profiles after transdermal administration of diclofenac-containing hydrogel for Co-CLA6-XG (F2), Co-CLA12-XG (F4), CL6-Co-A-XG (F6), and CL12-Co-A-XG (F8), as the matrix. Green, purple, blue, and red lines represent F2, F4, F6, and F8, respectively. Each point represents the mean±standard error; n=6

diclofenac sodium as a drug model. The low partition coefficient of sodium diclofenac (log p=0.70) was an important factor related to drug permeation because the partition coefficient indicates the ability of a drug to partition into hydrophobic and hydrophilic phases [21].

***In vivo* penetration study**

Fig. 2 shows the mean of the plasma concentration-time profiles of diclofenac after transdermal administration for 12 h. The results show that the highest area under the curve (AUC) was achieved by F4 (Co-CLA12-XG hydrogel), followed by the Co-CLA6-XG (F2), CL12-Co-A-XG (F8), and CL6-Co-A-XG (F6) matrices. However, the AUC value of F4 was not significantly different than that of F2, (p>0.005). As presented

in Table 5, the AUC, C_{max}, T_{max}, $t_{½}$, and mean residence time (MRT) values of the transdermal diclofenac hydrogel, comprising Co-CLA6-XG as a matrix (F2), showed no significant difference ($p>0.005$) with F4, comprising Co-CLA12-XG. In contrast, the AUC of the transdermal hydrogel F6 and F8, comprising CL6-Co-A-XG and CL12-Co-A-XG, respectively, significantly decreased. The results indicated that the crosslinking degree of amylose and the method of preparation the excipient affected their drug delivery. In addition, the MRT and K_e values of the transdermal hydrogels of F2, F4, and F8 indicated that the produced excipients can be used as a transdermal matrix with controlled and prolonged release behavior.

CONCLUSION

For diclofenac sodium as the model drug, the *in vitro* and *in vivo* penetration study results indicated the feasibility of developing hydrogels using Co-CLA6-XG, Co-CLA12-XG, CL6-Co-A-XG, or CL12-Co-A-XG as a matrix former that could be used as a good transdermal dosage form. The results also show the ability of these hydrogels to provide good controlled release permeation of the active model drug. Comparison of the *in vivo* and *in vitro* skin penetration data in this study was difficult, but the results of both studies suggested that the hydrogel formulations would provide good controlled drug permeation through the skin. Further studies are needed to determine if these hydrogels can be used for humans in clinical studies.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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