CHAPTER 5

Discussion

5.1 Formulation of tranexamic acid patches

The hydrogels were observed for appearance 24 hours after preparation (day 1). The different gelling agents effected the texture and colour of hydrogel. Formula M35 prepared with HPMC (Methocel®) E4M was more rigid than formula J33 and M38. The presence of acrylax® 1061 in formula M38 yielded the white turbid and sticky gel. The optimum ratio of carbopol® 980 NF and HPMC E50 in formula J33 achieved good texture, clear and flexible gel, as well as better skin adhesion than other two formulae.

5.2 The pH measurement

The pH of hydrogel products (table 4.2) was in the range of 3.03-6.70. Neutral pH was observed in formulae M35, M38, P7 and P8. Acidity of hydrogels in formula P1 and P4 was due to lactic acid. The pH of formulae J33, P9, P10 and P11 were in the range of 5.1-5.4, which is close to the pH of skin surface. The low pH was due to the acidity of carbopol in the formulations. In presence of NMP, its alkalinity yielded higher pH hydrogels. Higher pH was observed in formulae M35, P7 and P8.

5.3 Spectrofluorimetric determination of tranexamic acid

After 5 minutes, the derivatization was complete at room temperature and well-lit conditions. It is suggested that the derivatization conditions were mild and not highly restrictive. The derivatives tended to be stable for more than 30 minutes. However, leaving the mixture for longer period of time before performing the measurement may not be beneficial due to the excess

CN^- ion can slowly react with the excess NDA (20). The fluorescent intensity of the reagent blank was also observed and it was negligible comparing with the intensity of NDA/CN^- /tranexamic acid derivative (figure 4.2). The fluorescence spectra (figure 4.3) was found to exhibit two peaks of excitation wavelengths (λ_{ex} = 420 and 440 nm) and one broad peak of emission wavelengths (λ_{em} = 460-480 nm) corresponding with the maxima of NDA/primary amine derivatives reported in literature (8).

5.3.1 Assay validation

The linearity of this method was valid ($R^2 > 0.999$) (37). Considering the derivatization procedure of tranexamic acid with NDA/CN^-, it was found that reaction process was not complicate and can be performed in ambient conditions (room temperature and unnecessary light protection). Although, the reaction was complete after 10 minutes, in this study the fluorescent intensity was measured after only 5 minutes after derivatization and yet stills obtained valid data. However, a consistent reaction time is necessary for reproducible results. Therefore, this spectrofluorimetric method is suitable for analysis of tranexamic acid in pharmaceutical preparations that requires no remarkably high sensitive detection.

5.4 Quantitative analysis of tranexamic acid in hydrogels

5.4.1 Examination of gel base influence

Statistical data showed significantly difference between the drug content obtained by standard solution and spiking M35s solution (ANOVA, Tukey tests, $P = 0.011$) and J33s solution ($P = 0.002$). The difference could be partly due to the analytical error which normally allowed ± 2% variation and possible adsorption of tranexamic acid to the hydrogel. More variation were observed when the gel was added, particularly in formula J33s. However, the drug-gel incorporated method was thought to be more realistic than spiking method because the drug had already existed in homogeneous matrix while the solution prepared from spiking method,

separate drug addition, might not yield a complete mixture, resulting in the detection error. However, no clear reason proved that the gel caused the interference of fluorescence detection.

5.4.2 Tranexamic acid content of hydrogels

Tranexamic acid contents of three patch formulae investigated after 24 hours of storage (day1) were in a range of 4.14-4.16 % drug content. The drug was found to be unremarkably lost due to it was in the range of 98.81-99.12 % recovery.

5.4.3 Tranexamic acid release study

5.4.3.1 Tranexamic acid release on day 1

Hydrogel formula J33 was examined for the reproducibility. All release profiles were similar (significantly indifference, ANOVA, Tukey tests, $P>0.05$). This formula was considered to be reproducible. The highest release of tranexamic acid from hydrogel matrix was obtained from the formula M35 containing HPMC as the hydrophilic polymer while the formulae M38 and J33 showed the similar release profiles. The HPMC, a nonionic polymer, does not affect the diffusion and release of the drug molecules. The low tranexamic acid released of formula M38 could be due to the steric effect of acrylax and some tranexamic acid-acrylax interaction. There may be the aminolysis of an acrylic ester with tranexamic acid, a primary amine, by the fact that an ester is naturally converted to an amide by nucleophile such as amine (38). The amides generated on the macromolecules cannot release while the free tranexamic acid molecules released in low amounts. The low tranexamic acid released was also due to the negative charge presenting on carbopol molecules, the amphoteric molecules like tranexamic acid may took their positive charges to bind with the negative charges of carbopol. Thus, less free drug molecules diffused and released through out the matrix. The slopes of Higuchi profiles represented the release rate of tranexamic acid and the hydrogel formulae M35 and J33 showed the linear relations between percentage drug released and square root of time. The slopes of triplicate

release profiles of J33 were similar. This Higuchi plot confirmed the reproducibility of J33 hydrogel patch.

5.4.3.2 Tranexamic acid release from hydrogel in presence of releasing accelerants

Lactic acid was added to the formula M35 as a releasing accelerant since it performed skin softening and replenishment of the stratum corneum (18) that probably increased the skin permeation of the drug. However, the cellophane membrane was used as a barrier between gel matrix and receiver fluid in diffusion cell for this experiment. The results investigated from cellophane barrier were expected to be the primary data for percutanious evaluation carried out in further study. It was found that the release from hydrogels consisted of lactic acid (formulae P1 and P2) were lower than a control formula (M35). Since the pH of hydrogel consisted of lactic acid was dropped to 3.0-3.9 that was below the pka_1 of tranexamic acid (pK_{a1}=4.3 and pK_{a2}=10.6) (39). The cationic form of tranexamic acid, an ammonium ion, was only presented in hydrogel formulation and then diffused from a matrix to receiver fluid during the release study. The fact that a zwitterion of tranexamic acid molecule occurs in a range of its pka_1-pka_2 values, tranexamic acid was also present predominantly in its amphoteric form where the pH of the buffer in receiver fluid was 7.4. As for the ionic forms, the carboxyl ion of tranexamic acid has stronger hydrogen bond with water than its ammonium ion in the same molecule. Therefore, the tendency for water solubility of a carboxylic ion was more than an ammonium ion, as less cationic forms of tranexamic acid were soluble, correspondingly, the less amounts were detected.

There was no study of lactic acid in carbopol polymer (in formula J33) because the good consistency gel cannot be achieved by this combination. The toughness lost in carbopol resulted from its intolerance in an acid system (20).

NMP was used to modify the release of tranexamic acid from the hydrogel patches and it was found to be a good releasing accelerant for formula P7 containing the drug and NMP in a ratio of 1:1. Considering the polar and hygroscopic activity of NMP (14,15), the aqueous medium in receiver cell that was mainly water was attracted by NMP and moved into the matrix during the diffusion process, resulting the gel to swell promptly and easily and the drug then released faster. However, when the amounts of drug:NMP was 1:2 (P8), the lower drug released

was observed. There could be the strong hydrogen bonding of NMP and proton site (amino group of tranexamic acid) (40), resulted in less free molecules of tranexamic acid released.

Due to the low polarity of dimethicone, it would significantly inhibit the water-NMP attraction into the gel matrix of formulae P9, P10 and P11. The amount of NMP in a ratio of 1:1 (P11) was thought to be insufficient for the swelling gel. When the NMP was presented in a ratio of 1:2 (P9), it was enough for good swelling of the hydrogel. As for the formula P10 (drug:NMP = 1:3), its release result was discussed as well as the result of formula P8.

5.5 Stability evaluations

5.5.1 Hydrogel appearance in long-term storage

There was some gel of formula M35, stored at 4°C, exuded through the backing membrane, which is made of well-absorbed material resulting only small amount of gel remained on a patch surface. The hygroscopic property of polyvinylpyrrolidone (PVP) and propylene glycol (PG) could play an important role for moisture absorption of hydrogel even in the low temperature condition with sufficiently high relative humidity such as in refrigerator storage. PG had the competition of water attraction inside hydrogel with the polymer (HPMC). It was suggested that too much aqueous content might not be an appropriate amount in M35 formula for long term storage. However, the result of the 4°C storage showed that formula M38 was more physically stable than M35 since less amounts of PVP, PG and water were used. A prominently unstable gel appearing on the 45°C-stored patch, a dry gel with a lot of small flakes on a patch (figure 4.22), was caused by water evaporation from the hydrogel. The presence of acrylax ® ,an acrylic ester polymer, also concomitantly caused the hydrogel to dry due to its poor weathering, limitation of heat resistant and high shrinkage (40). It was found that the colour of formula J33/45°C-stored hydrogel became darker comparing with a day1 hydrogel preparation. High temperature exposure of carbopol was the main factor for the discolouration (18).

5.5.2 Tranexamic content in long-term storage

The decrease in tranexamic acid content of hydrogel formula M35 stored at room temperature for 120 days was negligible. Nevertheless, the drug content of formulae M38 and J33 stored in room temperature was remarkably decreased (6.28 and 6.49%, respectively), reflecting the decrease in chemical stability. The high drug contents were found in every formula stored at 45°C due to the moisture depletion in hydrogel leading to an increase in drug concentration per unit weight. The approximate shelflife of active ingredient in hydrogel patches can be primarily assessed by accelerated test. It was found that tranexamic acid content of M35, M38 and J33 remained within an acceptable value (less than 11% decrease) during 3 months of 45°C storage. Therefore, these patches have an effective period of 2 years (47).

5.5.3 Tranexamic acid released on day 120

Tranexamic acid released from hydrogel formula M35

There was 49.88% of tranexamic acid released within 180 minutes from the hydrogel in day1 patch while 33.64% of tranexamic acid released from the hydrogel stored at 4°C for 120 days. This was due to the changes in physical appearance of the patch such as thickness, texture and moisture content. However, the amount of drug content determined from a 4°C-stored patch was highest among the patches stored in the other 3 conditions. This gel was suitable to be evaluated for the drug content due to its poor stability.

The comparison between the 120th day patches stored at RT and 45°C showed similar release profiles. However, the drug released from the RT-stored hydrogel at the initial period was higher than the 45°C-stored hydrogel due to the changes in physical properties of the hydrogel. Considering the swelling property of the gel, a hydrogel in 45°C-stored patch that had shrunk due to hot condition storage required longer time to reach the thermodynamic equilibrium. The swelling of gel compound corresponds to the elongation of polymer (α) in the equation (41)

$$f^* = kT\,(v/V)\,(\alpha - \alpha^{-2}) \qquad [5.1]$$

where f* is the stress, v/V is the density of network chains, k is Boltzmann constant and T is absolute temperature. The density of network chains is directly related to the stress whereas it is inversely related to the elongation of the polymer. The dry gel was thought to be the tightly packed macromolecules, so it had high density of network chains. The higher the density, the lower the elongation ability of the polymer. Additionally, according to Fujita equation (42),

$$D_{s,A} = A_d \exp(-B_d/v_f) \qquad [5.2]$$

where $D_{s,A}$ is the self-diffusivity of penetrant, v_f is the fractional unoccupied volumes, A_d and B_d are two constants for an exponential relation. High value of v_f reflects the tightly packed chains of the polymer and is related to the poor self-diffusivity of penetrant into the matrix. Therefore, the swelling behavior of a matrix in the 45°C-stored patch caused the matrix takes longer time for its thermodynamic equilibrium.

Tranexamic acid released from hydrogel formula M38

There were a high tranexamic acid released from the day120 hydrogel patches stored at RT and 45°C (table 4.9 and figure 4.10). It was found that the hydrogel in day1 patch released less than the hydrogel in day120 patch stored at RT. However, there was no significant difference between the 2 profiles (Repeated Measurement, Tukey tests, $P>0.05$). Even though the hydrogel in 45°C-stored patch released more drug than a day1 hydrogel, the release property of the unstable hydrogel could not be clearly explained and compared with the freshly prepared one. The high drug release from the 45°C-stored hydrogel was assumed that some amount of the drug leaked out of a shrunken matrix and rearranged into the disheveled crystal forms on the surface of a patch. The unstable matrix was no longer able to be a drug reservoir. Therefore, the unusual release of this dry hydrogel was found.

Tranexamic acid released from hydrogel formula J33

The percentage content of tranexamic acid representing A in Higuchi equation which demonstrates the drug release from a homogeneous matrix is shown by this relationship (43);

$$Q = [D_t(2A - C_s)C_s]^{1/2} \qquad [5.3]$$

where Q is the amount of drug release after time t per unit exposed area, D is the diffusivity of the drug in the homogeneous matrix media, A is the total amount of drug present in the matrix

per unit volume and C_s is the solubility of the drug in the matrix substance. A hydrogel consisted of higher drug content had consequently higher release. The day120 hydrogel stored at RT showed the lowest release due to its low drug content as shown in tables 4.6 and 4.18. Statistical comparison showed no difference within the release profiles in this study (Repeated Measurement, Tukey tests, $P>0.05$). Higuchi plots indicated that all of the release profiles (from day1 and day120 hydrogels) were first order kinetics.

5.5.4 Physical properties evaluations

The alteration of hydrogel appearance affected its release property that was discussed in 5.5.1. Thickness, skin adhesion time and friction efficiency (μ) of M35 patch were reported (table 4.16). Adhesion time which is directly related to μ was the results of the patches stored in every condition except for a 4°C-stored patch. The flat and exuded gel could be more pliable to cover the skin because of its thinness and moisture rich content. Considering to the thickness, skin adhesion time and μ evaluations of M38 patch (table 4.17), the day120 patch stored at RT had poor adhesive property that was probably caused by poor weathering and high shrinkage in long-term storage of acrylax®. The rigidity and shrinkage can be observed on long-term storage and caused the hydrogel to be lack of moisture and stickiness. The 4°C-stored hydrogel had least μ while its adhesion time was much more than the RT-stored hydrogel. The same reason for the 4°C-stored hydrogel of formula M35 was used for the formula M38. The thickness, skin adhesion time and μ of formula J33/day120 patches stored in 3 conditions were similar to a day1 patch (table 4.18). Even though the colour of 45°C-stored hydrogel became swarthy comparing with a day1 hydrogel preparation, but the drug content, texture and flexibility were considered to be the same as the hydrogel stored in another condition. Moreover, the adhesive property had decreased negligibly. Therefore, the formula J33 is considered to be a highly stable hydrogel that is able to keep in cold and even hot conditions and it is more stable than other two formulae (M35 and M38).

The effect of packaging

The laminated aluminum foil packaging of the hydrogel patches showed more protection to the air and temperature exposures than the zip-locked plastic bags. Therefore, formula J33 also showed higher stability than other two formulae as seen from the adhesive measurement available on the 90th day of storage in zip-locked plastic envelope while the two formulae had limited measurement.

5.6 Skin irritation test

The irritation test of tranexamic acid hydrogel patches showed that no irritation to the skin in every volunteer from the patch formula J33. Five out of nine volunteers had the skin reaction to the patches consisting of NMP. The irritation happened during the early occlusion and no severe skin reaction evaluated by visual scoring occurred on the volunteers after 24 hours of occlusion. The erythema and little scaling without itchiness were observed from those five volunteers only on day1. Then became a very weak erythema on day2. The itchiness investigated from the formula P9, P10 and P11 was assumed to be the irritation effect of NMP.