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Increasing the solubility of levodopa and carbidopa using ionization approach

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ABSTRACT

The aim of this study is to increase the solubility of levodopa (LD) and carbidopa (CD) to improve loading quantity of both drugs for oral pullulan thin film preparation. Solubilization technique through ionization was employed to measure the solubility for pH range of 1.5–3.5 and molar concentration at 0.1 and 0.2. Quantitative determination of solubilized LD and CD in various solvents showed the highest solubility in pH 1.5, the lowest solubility in pH 3 and 3.5 since zwitterion characteristic of amino and carboxyl group of both drugs. High concentration of proton is able to from cation at carboxylic moiety after ionization. Higher level of molar concentration provided the higher solubility because of the increment of ionic strength. Therefore, suitable solvent for maximizing solubility was found to be either 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5 or 0.1 M hydrochloric acid/0.1 M citric acid pH 1.5. They were subsequently employed as the solvent of LD and CD for oral pullulan thin film preparation. Utilization of 0.1 M hydrochloric acid/0.1 M citric acid pH 1.5 provided less tackiness, thinner with less weight/unit film sheet compared to that of 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5. It might be due to the lower solid fraction of HCl provided the higher integrity of polymer network formation after drying.

Keywords: Solubility, Levodopa, Carbidopa, pH, Molar concentration

INTRODUCTION

Both youth and elderly patients mostly suffer from tablet administration, for example, trembling, choking including the need of water for intake. More efficient way to overcome above patient's noncompliance is the development of other solid dosage form like an oral thin film (OTF). An OTF definitely describes as the thin sheet of polymeric material that can easily disintegrate in oral cavity with the aid of small amount of saliva. In general, the size of OTF is properly defined at around 5 to 20 cm^2.[1] It should contain drugs together with other essential additives. OTF is commonly able to contain active ingredients in the range of 1–25% of its weight depending on the value of drug-polymer solubility. Sujaritnarakorn *et al*.[2] reported that pullulan OTF containing levodopa (LD) and carbidopa (CD) could be fabricated with the loading quantity of 40 mg and 4 mg of LD and CD in 90 cm^2. Such loading quantity was quite low due to the lower drug solubility. It was found to be the larger size range in which provided the difficulty of patient administration. Therefore, an increasing of higher loading quantity of both LD and CD in OTF that closes to tablet formulation should be achieved. Subsequently, the size of OTF should eventually be reduced according to higher drug loading. The commonly used techniques for increasing the loading quantity of drug in OTF are described herein as following:

- Increasing the quantity of polymer: The higher amount of polymer can hold higher drug content since the larger area from higher polymer allows more active ingredients to be distributed thoroughly.
- Changing the acidity of the formulation: If active ingredients can be dissolved well at a certain p

H. Such pH solution shall be used to be a solvent in the formulation.[3]

- Adding of surfactant and/or cosolvent: To increase the solubility of active ingredients, the solubilization techniques would be suggested.[3]

Since LD and CD have low intrinsic water solubility of 5000 mg/L and 3.8 mg/L, respectively.[4-6] Their loading quantity in hydrophilic polymer OTF like pullulan was consequently less than the target level. However, these two drugs were more dissolvable in acidic condition such as hydrochloric acid, acetic acid solution, and formic acid solution including buffer solution of citric acid.[2,4,5] It was due to the fact that LD and CD showed

the pKa of 1.65 and 2.35, respectively.[4,5] Therefore, both drugs provide more solubility in low pH conditions from drug ionization. In addition, the stability of both LD and CD in acid environment was well acceptable.[7] It should be clearly seen that the most suitable technique for increasing drug loaded in OTF is goes to the downward changing of pH.

Thus, the objective of this study is to increase the solubility of LD and CD to improve the loading capacity of both drugs for OTF preparation using the increment of proton concentration of solvent. In addition, the investigation of the effect of proton concentration and molar concentration was performed.

METHODS

Solubility Determination of LD and CD in Various Solvent Systems

The solvent systems of interest for LD and CD were determined by varying the type and pH including molar concentration. They were listed as following.

- 0.2 M hydrochloric acid/ 0.1 M citric acid pH 1.5
- 0.1 M hydrochloric acid/ 0.1 M citric acid pH 1.5
- 0.1 M hydrochloric acid/ 0.1 M potassium chloride pH 1.5
- 0.1 M citric acid pH 2
- 0.1 M citric/citrate buffer pH 2.5
- 0.1 M citric/citrate buffer pH 3.0
- 0.1 M citric/citrate buffer pH 3.5

Excess amounts of LD and CD powder were separately suspended in experimental solvents. They were then agitated at 300 rpm at controlled temperature of 30±2°C for 48 h.[8] They should also be protected from light. Clear supernatant of suspended sample was collected at 1, 2, 4, 8, 16, 32, and 48 h. It was further filtered through cellulose acetate membrane 0.45 μm. UV-spectrophotometry was used for quantitatively determination of solubilized drugs at the maximum wavelength of 280 and 279 nm for LD and CD, respectively. The relationship between drug concentrations versus time was constructed. Consequently, the saturated solubility was investigated by determining the concentration at plateau. The solvent systems that provided highest solubility of LD and CD were then employed as the platform for next study.

Preparation of OTF Containing LD and CD Using Appropriated Solvent System

Preliminary prototype formulation of OTF was modified based on Sujaritnarakorn *et al*.[2] It was composed of pullulan as a film former, glycerine as a plasticizer, ascorbic acid as antioxidant,[7] and suitable solvent. Final formulation is illustrated in Table 1.

Solvent casting method was utilized as the method of preparation of OTF. Briefly, LD 150 mg and CD 37.5 mg were separately dissolved in 12.5 and 6.25 ml of selected solvent, respectively. They were later well combined with continuous stirring. Ascorbic acid and glycerin were then introduced and vigorously agitated at 500 rpm for 30 min. Pullulan was later dispersed with continuous stirring until clear and homogeneous solution without any bubbles was obtained. The solution should be avoided from excessive heat and protect from light. Approximate 6 g of sample was poured gently into 30 cm^2 plastic mold (6 × 5 × 1.5 cm) for four pieces. It was subjected into hot air oven, which controlled the temperature at 40 ± 2°C. After 2 days, dry film is carefully removed from plastic mold and stored in aluminum bag.

Table 1: Pullulan OTF prototype containing LD and CD

Ingredients	Concentration (%w/w)
Pullulan	6.0000
glycerine	0.6000
LD	0.6250
CD	0.1563
Ascorbic acid	0.7980
Selected solvent qs to	100.0000

OTF: Oral thin film, LD: Levodopa and CD: Carbidopa

The dry film sample was characterized according to the physical aspects as followed. The characterizations were done in triplicate.

Physical Appearance

Film sample was inspected by sensory evaluation (Organoleptic test) for transparency, clarity, smoothness, fragility, tackiness, and further investigated the presence of insoluble precipitated solid particle by polarized light microscope.

Thickness

Film thickness was measured using Vernier caliper. The sample of 4 × 5 cm film was prepared and measured in triplicate at five different positions, that is, the four corners and a center of film sheet.[9]

Weight Variation

The sample of 4 × 5 cm film was weighed individually using analytical balance.

Moisture Content

Moisture content of 4 × 5 cm film was determined using moisture analyzer at 105°C.

In vitro Disintegration Time

Disintegration time of dry film sample was determined by mounting the 4 × 5 cm film to plastic frames. It was then attached to paddle of dissolution testing machine. The paddle was moving at the speed of 10 revolutions/minute. Disintegration test was performed in simulated oral cavity environment (simulated saliva fluid pH 6.8, 300 mm) at 37°C.[10]

Statistical Analysis

Comparative data analysis was performed using *t*-test at α-level 0.05.

RESULTS AND DISCUSSION

Solubility of LD and CD in Various Solvent Systems

The solubility measurement of LD and CD in either buffers or acid solutions of various pH at controlled temperature of 30 ±

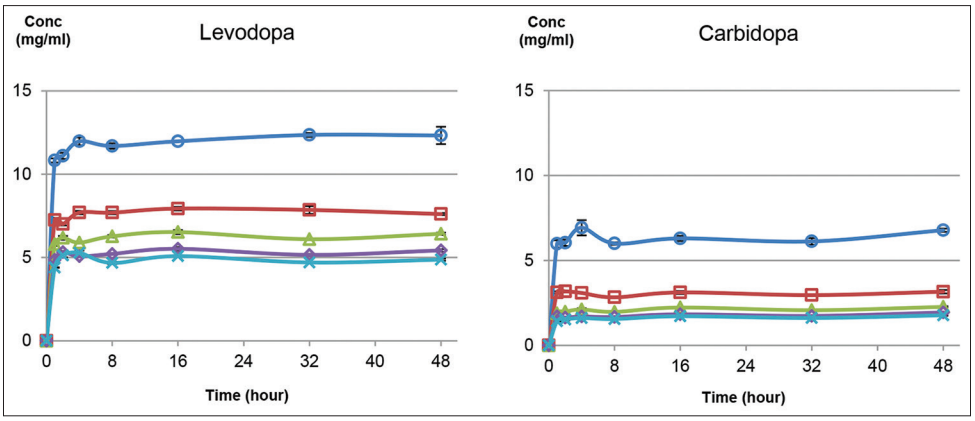

Figure 1: The solubility profile of drugs in acid and buffer solutions at the pH between 1.5 to 3.5; (—○—) 0.1 M hydrochloric acid/ 0.1 M citric acid pH 1.5; (—□—) 0.1 M citric acid pH 2; (—△—) 0.1 M citric/citrate buffer pH 2.5; (—◇—) 0.1 M citric/citrate buffer pH 3.0; (—×—) 0.1 M citric/citrate buffer pH 3.5

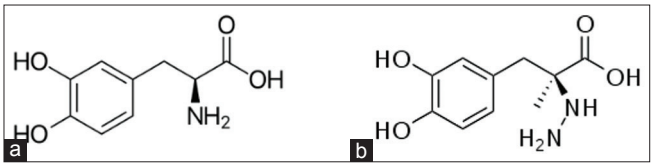

Figure 2: Molecular structure of (a) levodopa; (b) carbidopa

2°C is shown in Figure 1. The results revealed that the highest solubility of both LD and CD was found to be around 12 and 6 mg/ml at pH 1.5 acid solutions, respectively. Meanwhile, the lowest solubility of LD was around 5 mg/ml and 1.7 mg/ml for CD at the pH between 3 and 3.5. The solubility significantly increased at pH lower than 3. Moreover, the solubility of LD is commonly higher than CD at same pH condition. Therefore, the lower the pH the higher the solubility of both LD and CD. It was due to the zwitterions of LD and CD that contain primary amine and carboxyl group in the molecule as are shown in Figure 2. The higher concentration of proton or lower pH is able to form a cation at carboxylic group from the ionization. In addition, Tseng *et al.*[11] reported that the solubility of amino acid liked molecules commonly increased when the remarkable deviation of proton concentration from its isoelectric point occurred. It was the reasonable evidence to support the solubility increment of LD and CD under enriched proton condition.

In addition, different ion of solvent used in this study provided the significance different of solubility of LD and CD. Focusing on the system of pH 1.5, the solubility of both LD and CD in 0.1 M hydrochloric acid/0.1 M citric acid was significantly higher than that of 0.1 M hydrochloric acid/0.1M potassium chloride [Figure 3]. It was due to both ionic strength and common ion effect of chloride ion in the system. The solvent comprising potassium chloride shared common chloride ion with hydrochloric acid that eventually resulted in less soluble of LD and CD.

Not only the type and ion of solvent but also the molar concentration would play an important role on the solubility of LD and CD. The solubility of LD and CD in 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5 was significantly higher than 0.1 M hydrochloric acid/0.1 M citric acid pH 1.5 [Figure 4]. The result demonstrated that the higher molar concentration (0.2 M hydrochloric acid) provided the higher solubility of both LD and CD compared to 0.1 M hydrochloric acid because of the increasing of ionic strength (Tseng *et al.*, 2009). It was meant that if the concentration of hydrochloric acid increased the ionic strength also increased while the activity coefficient decreased. It would follow the Debye-Huckel relationship. Hence, higher molar concentration of 0.2 M hydrochloric acid should positively affected the solubility of LD and CD.

Preparation of OTF Containing LD and CD Using Appropriated Solvent Systems

The appropriated solvents used to maximize the solubility of LD and CD were found to be either 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5 or 0.1 M Hydrochloric acid/0.1 M citric acid pH 1.5. They were then employed as the solvent of LD and CD OTF preparation. OTF contained LD and CD using above different solvent systems were fabricated and characterized. OTF evaluation parameters and results were tabulated and shown in Table 2.

Both OTF samples were found to be clear and transparent without any precipitated solid crystal under polarized light microscope. It should be concluded that all components in the film formulation were molecularly miscible and eventually resulting in homogeneous polymeric thin film. However, they exhibited some different properties such as thickness, weight per unit, and tackiness of film. The utilization of 0.1 M hydrochloric acid/0.1 M citric acid pH 1.5 solvent systems provided the film with less tackiness than that of 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5. Film produced with 0.1 M hydrochloric acid/0.1 M citric acid pH 1.5 provided less tackiness, thinner film sheet, and less weight per unit. It was due to the higher solid content of hydrochloric acid in 0.2 M hydrochloric acid/0.1 M citric acid pH 1.5. The higher solid content from hydrochloric acid was directly involving on the formation of polymer network. Higher solid fraction was prone to effect on the completeness of polymer network formation after drying.[12] It was eventually resulted in less integrity of polymeric thin film in the case of higher solid content.

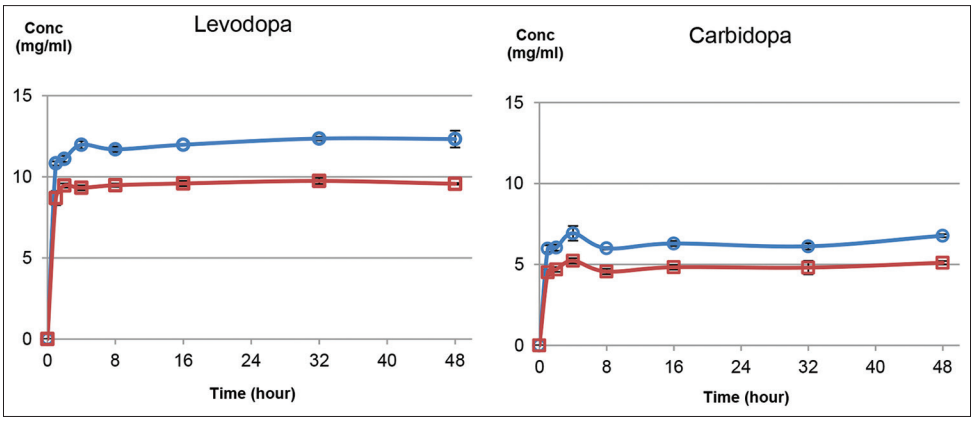

Figure 3; The solubility of drug in acid solutions at the pH 1.5; (⊖) 0.1 M hydrochloric acid/ 0.1 M citric acid pH 1.5; (⊟) 0.1 M hydrochloric acid/ 0.1 M potassium chloride pH 1.5

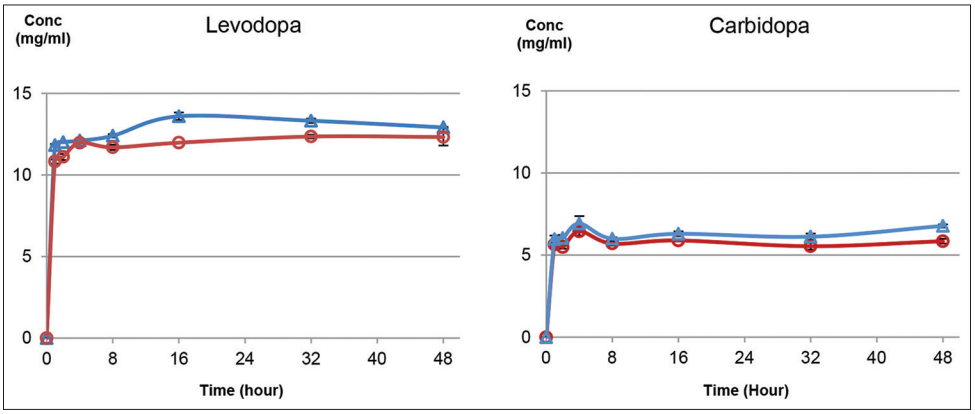

Figure 4: The solubility profile of drugs in hydrochloric acid and citric acid; (⊖) 0.1 M hydrochloric acid/ 0.1 M citric acid pH 1.5; (⊿) 0.2 M hydrochloric acid/ 0.1 M citric acid pH 1.5

Table 2: OTF evaluation parameters and results of oral pullulan thin film containing LD and CD

Characterization parameters	Solvent used	
	0.2M hydrochloric acid/0.1M citric acid pH 1.5	0.1M hydrochloric acid/0.1M citric acid pH 1.5
Physical appearance		
Transparency	Clear	Clear
Smoothness	Smooth	Smooth
Fragility	Unbreakable	Unbreakable
Precipitated solid particle	Not presence	Not presence
Tackiness of film	Tacky	Slightly-tacky
Thickness (millimeter)	0.115±0.009*	0.098±0.004*
Weight (gram)	0.336±0.004*	0.316±0.003*
Moisture content (%)	5.433±1.540	4.127±0.102
Disintegration time (second)	25.967±5.648	23.467±3.495

*$P<0.05$

CONCLUSION

The increasing of proton concentration and molar concentration of solvent plays a key role on the solubility of LD and CD at controlled temperature of 30±2°C. In particular, both ionization of carboxylic group of drug molecule and the increasing of ionic strength of the solvent used are well corresponded to the solubilization of LD and CD. Nevertheless, the effect of proton concentration and molar concentration are unable to elevate the loading quantity of both drugs compared to that of tablet formulation. Other solubilization approaches should be further observed.

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