

# Influence of Natural Gum on Curcumin Supersaturation in Gastrointestinal Fluids

Patcharawalai Jaisamut, Kamonthip Wiwattanawongsa, Ruedeekorn Wiwattanapatapee

**Abstract**—Supersaturation of drugs in the gastrointestinal tract is one approach to increase the absorption of poorly water-soluble drugs. The stabilization of a supersaturated state was achieved by adding precipitation inhibitors that may act through a variety of mechanisms. In this study, the effect of the natural gums, acacia, gelatin, pectin and tragacanth on curcumin supersaturation in simulated gastric fluid (SGF) (pH 1.2), fasted state simulated gastric fluid (FaSSGF) (pH 1.6), and simulated intestinal fluid (SIF) (pH 6.8) was investigated. The results indicated that all natural gums significantly increased the curcumin solubility (about 1.2-6-fold) when compared to the absence of gum, and assisted in maintaining the supersaturated drug solution. Among the tested gums, pectin at 3% w/w was the best precipitation inhibitor with a significant increase in the degree of supersaturation about 3-fold in SGF, 2.4-fold in FaSSGF and 2-fold in SIF.

**Keywords**—Curcumin, Solubility, Supersaturation, Precipitation inhibitor.

## I. INTRODUCTION

**C**URCUMIN, a hydrophobic polyphenol obtained from the rhizomes of *Curcuma longa* Linn., has a wide variety of biological activities and pharmacological actions. It is chemically known as diferuloylmethane or 1,7-bis (40-hydroxy-30-methoxyphenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane [1]. Curcumin has a melting point of 183 °C and a molecular weight of (M.W.) 368.38. It is insoluble in water and ether, but dissolves in ethanol, acetone, dimethylsulphoxide and glacial acetic acid [2]. In addition, it undergoes photodegradation when exposed to light in solution and in a solid form [3] and is unstable at a neutral and basic pH value [4]. The key problem of curcumin is its low solubility in water (30 pmol/mL) [5] and soluble curcumin molecules are extremely sensitive to precipitation at a physiological pH. Drug solubility and drug dissolution are of crucial importance for orally administered drugs. To enable them to achieve desirable systemic exposure after oral dosing, the active drug substance should be in solution in the aqueous acidic environment of the stomach and the more neutral intestine in order to cross the luminal wall and enter the circulation. Poor water solubility results in limited absorption and numerous attempts have been made to try to circumvent this problem to improve its bioavailability [6], [7]. Various

approaches in developing new drug formulations have been directed at increasing the dissolution rate and improving drug solubilization in the gastrointestinal tract. It should be recognized, however, that the intraluminal concentration of a drug is not necessarily limited by its solubility in gastrointestinal fluids. Drugs may be in solution at a concentration above their saturation solubility, that is, in a state of supersaturation [8]. To stabilize this supersaturation state upon transfer to the intestine, inclusion of precipitation inhibitors (PPI) in their formulations, such as polymers, may be required.

Natural gums are categorized based on their origins, behaviors and chemical structures. Gums are often complex polysaccharides obtained from many sources where they perform a number of structural and metabolic functions e.g. fruits peel (e.g. pectin), plant exudates (e.g. acacia, tragacanth), tree or shrub exudates (e.g. gum Arabic, tragacanth), bacteria (e.g. xanthan gum), and animal sources (Gelatin) [9]-[13]. The polysaccharide gums are biosafe, biosustainable and biodegradable. Gums are being used for diverse applications in pharmacy. Among the various polymers, gums are widely accepted as carriers for the sustained release of orally administered drugs as they are simple and easy to formulate. The use of natural polymers and their semi-synthetic derivative in drug delivery continues to be an area of active research. Drug-release retarding polymers are the key performers in supersaturation systems.

The purpose of this study was to investigate the effect of various natural gums and their concentrations on the inhibition of precipitation of curcumin in gastrointestinal fluid. The relationship between drug solubility and degree of supersaturation over a period of time was also presented.

## II. MATERIALS

Curcumin, taurocholic acid sodium salt hydrate, pepsin from porcine gastric mucosa L-alpha phosphatidylcholine were from Sigma Aldrich. Acacia, gelatin, pectin and tragacanth were from the PC Drug Center Co., Ltd. (Bangkok, Thailand). Sodium chloride, hydrochloric acid, acetonitrile and methanol (HPLC grade) were from RCI Labscan (Bangkok, Thailand). All other chemicals were of analytical grade.

## III. METHOD

### A. Solubility Measurement

The equilibrium solubility, i.e., the concentration of a compound in a saturated solution when an excess of solid was present and the solution and solid were at equilibrium, is

P. Jaisamut is with the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla 90112 Thailand (e-mail: jaisamut.p@gmail.com).

R. Wiwattanapatapee is with the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla 90112 Thailand (corresponding author to provide phone: 667-428-8915; fax: 6674 428 148; e-mail: ruedeekorn.w@psu.ac.th).

generally used to define the solubility of a compound [14], [15]. A conventional shake flask method was utilized to determine the equilibrium solubility of curcumin in the fasted state simulated gastric fluid (FaSSGF) (pH 1.6), simulated gastric fluid without pepsin (SGF) (pH 1.2) and simulated gastric fluid (SIF) (pH 6.8) with or without different polymeric precipitation inhibitors (PPI) (acacia, gelatin, pectin and tragacanth) at a concentration of 0.5, 1 and 3% (w/w). FaSSGF was prepared as described by Vertzoni et al. [16]. SGF and SIF were prepared according to the United States Pharmacopeia (USP) protocol [17]-[18]. An excess amount of curcumin was added to each tube containing 1mL of the media. The sample was vortexed at a maximum speed for 10 min by using a mixer (Vortex-gene 2, Becthai Bangkok Equipment & Chemical, Thailand) then allowed to equilibrate in a water bath shaker (Heto Lab, Scientific Promotion, Thailand) (37°C at 100 rpm), with the time for achievement of equilibration set at 48h. Solid phase separation was achieved using centrifugation (30min, 1,000 rpm at 37°C) and filtration (through a 0.2µm Polyvinylidene Difluoride (PVDF) filter). The supernatants were collected and diluted with methanol for quantification of curcumin by an HPLC method. Detection of curcumin from the HPLC analysis was by measuring its absorbance at 425nm. The mobile phase consisted of a mixture of acetonitrile and 2% aqueous acetic acid (v/v) in the ratio of 70:30. All solubility experiments were performed in triplicate [17], [19].

#### B. Supersaturation Assay

From the solubility data, a degree of supersaturation (DS) was induced in the media by means of a solvent shift method using DMSO as the organic phase. The tube containing 30mL FaSSGF, SGF and SIF in the absence or presence of 1 % (w/w) PPI (acacia, gelatin, pectin and tragacanth), was equilibrated in a water bath shaker (37°C at 100 rpm). Curcumin stock solutions in dimethylsulfoxide (DMSO) were prepared to induce an initial degree of supersaturation (DS) equal to 30 based on the determined solubility in each of the media. After adding the DMSO stock solutions to the test medium, samples (1mL) were taken at 5, 15, 30, 45, 60, 90, 120 and 240min and centrifugation (30min, 1,000 rpm at 37°C). Samples were filtered by using a 0.2µm PVDF filter and directly diluted in MeOH. Diluted samples were collected into glass vials and stored until required for HPLC analysis. Three replicates of each supersaturation experiment were carried out for each test media, and the data presented as a mean ± SD [17], [19].

#### C. Data Analysis and Presentation

The supersaturation data were presented as the degree of supersaturation-time profiles (DS-time profiles). The DS was calculated by dividing the concentration measured at a particular time point by the equilibrium solubility of curcumin in the same medium. For example, a DS equal to 30 means that 30 times the amount of curcumin was dissolved compared to the equilibrium solubility in the corresponding medium. The degree of supersaturation can be expressed by (1):

$$DS = C/C_{eq} \quad (1)$$

where  $C$  is the curcumin concentration and  $C_{eq}$  is the equilibrium solubility of curcumin. Based on the area under the curve (AUC) of the DS-time profiles, the conclusion data have been illustrated (Figs. 4-6).

The degree of supersaturation as a function of time in various conditions was calculated by using Graphpad Prism software (Graphpad software Inc.) and divided by the  $AUC_{240min}$  for a saturated solution (DS: 1) [17], [19].

#### D. Statistical Analysis

All results were expressed as means ± standard deviations (S.D.). Statistical comparisons were performed by Student's T-test or one-way ANOVA. Differences were considered significant at p-value ( $P < 0.05$ ).

### IV. RESULT AND DISCUSSION

#### A. Solubility Measurement

The equilibrium solubilities were determined for curcumin with and without different type and amount of natural gum in FaSSGF, SGF, and SIF. The mean values for the solubility data in three media are shown in Figs. 1-3. When the amount of each gum was increased (from 0.5-3% w/w) in SGF, the solubility of curcumin were significantly increased by about 1-3-fold compared to the absence of gum except for gelatin (Fig. 1). In the FaSSGF, a similar behavior was observed in which the increase of the solubility of curcumin was about 1.2-3 fold (Fig. 2). In the case of SIF, all natural gum enhanced curcumin solubility compared to the absence of gum, but the concentration of the gums had no effect on drug solubility except for gelatin (at high concentration) and pectin. The presence of pectin at 3% w/w gave the highest value for curcumin solubility both in the SGF and SIF. The pH of the testing media was considered to be one of the most crucial parameters affecting curcumin solubility. It was possible that the interactions between the drug and the polymer (via hydrogen bonding, hydrophobic interactions, or ionic interactions) were altered at different pH values of the gastrointestinal medium.

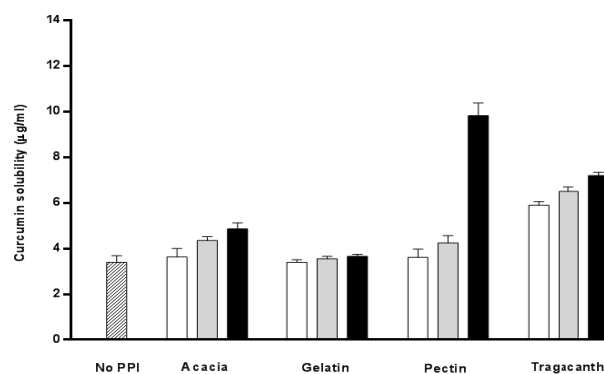


Fig. 1 Solubility (mean ± SD, n = 5) of curcumin in simulated gastric fluid (SGF) without polymer (NO PPI; striped white bar) or with polymer at concentration of 0.5 % (white bar), 1 % (gray bar), and 3 (black bar) % (w/w)

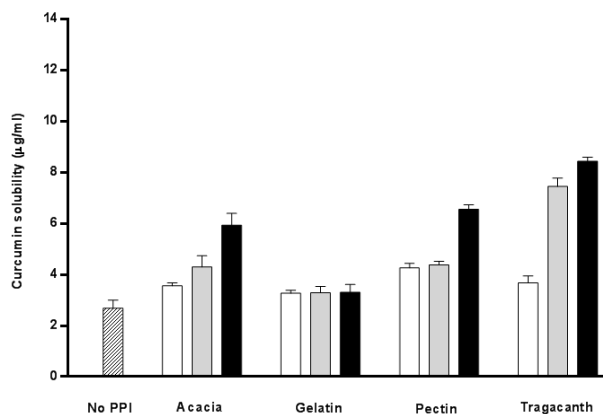


Fig. 2 Solubility (mean  $\pm$  SD, n = 5) of curcumin in fasted state simulated gastric fluid (FaSSGF) without polymer (NO PPI; striped white bar) or with polymer at concentrations of 0.5 % (white bar), 1 % (gray bar), and 3 (black bar) % (w/w)

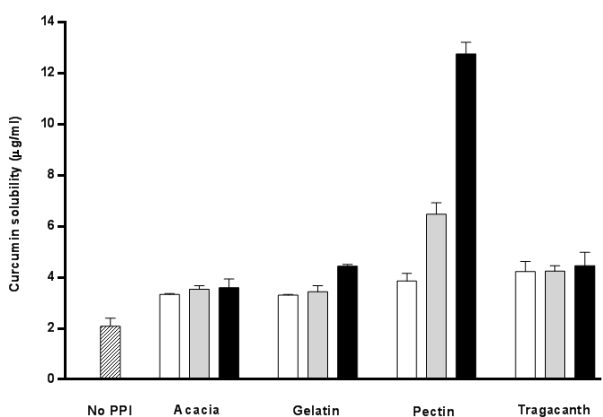


Fig. 3 Solubility (mean  $\pm$  SD, n = 5) of curcumin in simulated intestinal fluid (SIF) without polymer (NO PPI; striped white bar) or with polymer at concentration of 0.5% (white bar), 1% (gray bar), and 3 (black bar) % (w/w)

### B. Supersaturation Assay

Supersaturation of curcumin was performed in FaSSGF, SGF and SIF. To measure the degree of supersaturation, the obtained concentrations needed to be compared to the equilibrium solubility in exactly the same test medium. The quantification of supersaturation allows for the identification of the extent of saturation ( $DS < 1$ : subsaturated,  $DS = 1$ : saturated,  $DS > 1$ : supersaturated) as a measure of the thermodynamic tendency for precipitation [20]. The initial degree of supersaturation was always adjusted to 30 for the precipitation tendency level. The DS-time profiles are shown in Figs. 4-6. They are presented as an area under the curve which indicates the fold increase in the  $AUC_{240min}$  in the presence of natural gum as opposed to the DS-time profile in the absence of natural gum. The effects of the gum on supersaturation stabilization were compound dependent. Among the tested gums, pectin performed the best with significant increases in  $AUC_{240min}$  for all of the gastrointestinal media by about 3-fold in SGF, 2.4-fold in FaSSGF and 2-fold in SIF when compared to the absence of gum. The DS data obtained in all media showed a similar pattern with added

pectin. Gelatin, acacia and tragacanth showed a degree of supersaturation in the same way as for both the SGF and FaSSGF. However, the presence of acacia gave the lowest DS in the SIF. A supersaturation process with the presence of natural gum can maintain curcumin solubilization above its equilibrium solubility without precipitation. The increased drug concentration was readily available for absorption. It has been shown by previous studies that the transepithelial permeation was enhanced by supersaturated solutions of drugs [17], [21], [22]. From the curcumin DS data, the results indicated that pectin was a better precipitation inhibitor than acacia, gelatin and tragacanth. However, the mechanism of natural gums in inhibiting precipitation of curcumin in gastrointestinal media is yet a challenging area for further research.

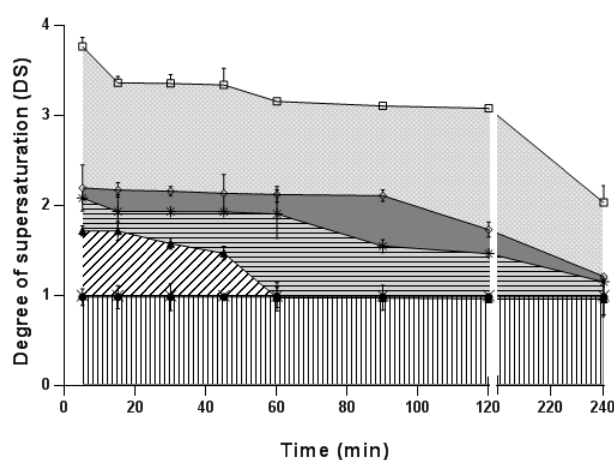


Fig. 4 Degree of supersaturation-time profiles (mean  $\pm$  SD, n = 3) of curcumin in simulated gastric fluid (SGF), and SGF with a 1% polymer precipitation inhibitor. In addition, the saturation profile (DS = 1) of curcumin in SGF is presented. The equilibrium solubility ( $\times$ ), No PPI (●), Acacia (\*), gelatin (◇), Pectin (□), tragacanth (▲)

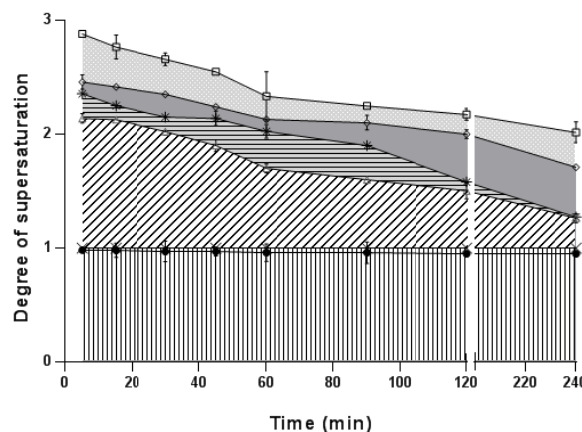


Fig. 5 Degree of supersaturation-time profiles (mean  $\pm$  SD, n = 3) of curcumin in fasted state simulated gastric fluid (FaSSGF), and FaSSGF with a 1% polymer precipitation inhibitor. In addition, the saturation profile (DS = 1) of curcumin in FaSSGF is presented. The equilibrium solubility ( $\times$ ), No PPI (●), Acacia (\*), gelatin (◇), Pectin (□), tragacanth (▲)

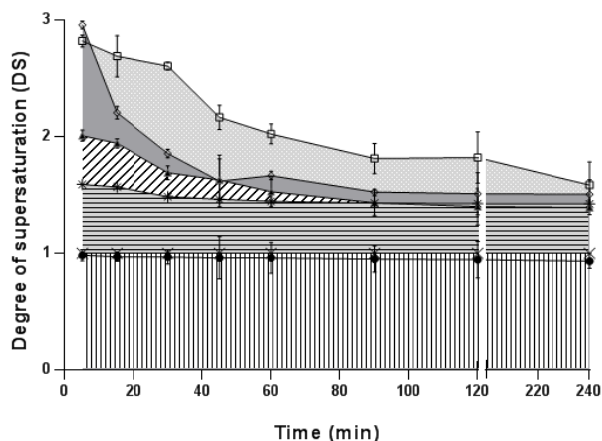


Fig. 6 Degree of supersaturation-time profiles (mean  $\pm$  SD, n = 3) of curcumin in simulated intestinal fluid (SIF), and SIF with a 1% polymer precipitation inhibitor. In addition, the saturation profile (DS = 1) of curcumin in SIF is presented. The equilibrium solubility ( $\times$ ), No PPI ( $\bullet$ ), Acacia ( $*$ ), gelatin ( $\diamond$ ), Pectin ( $\square$ ), tragacanth ( $\blacktriangle$ )

#### V. CONCLUSION

In summary, a supersaturation process obtained in the presence of a small amount of natural gum used as a precipitation inhibitor maintained drug solubility above its equilibrium solubility without precipitation. To measure the degree of supersaturation, the obtained concentrations needed to be compared to the equilibrium solubility in exactly the same test medium. The results from this study indicated that various kinds and amounts of natural gum were useful for enhancing the solubility and degree of supersaturation of curcumin. The degree of supersaturation in the gastrointestinal media with added pectin produced the highest solubility (about 3-fold) over the control when compared to acacia, pectin and tragacanth.

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