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Flocculating and suspending properties of commercial citrus pectin and pectin extracted from pomelo (*Citrus maxima*) peel

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ABSTRACT

The aim of this study was to extend the application of pectin extracted from pomelo peel to pharmaceutical suspensions. Particularly, the influence of pectin on the stability of indomethacin suspension was investigated. The use of cation, type and concentration of pectin, pH and temperature influenced the flocculating activity of pectin in suspension. The extracted pectin has comparable activity to commercial pectins. Low concentration of pectin and ferric ions allowed obtaining indomethacin suspensions with adequate properties, i.e., suitable stability and redispersibility. The use of pectin as a suspending agent was achieved with higher concentration; the flocculated, redispersible and stable indomethacin suspension could be produced. The pharmaceutical suspension containing pectin as a flocculating or suspending agent may be applied as liquid drug delivery system for pediatric and geriatric patients.

1. Introduction

Pectin is a cell wall structural carbohydrate present in all higher plants. Commercially available pectin is obtained from edible plants, e.g. apple, citrus. Though it is a heterogeneous polysaccharide, pectin contains linear chains of (1-4)-linked α-D-galacturonic acid residues. The linear structure of pectin is partly interrupted by (1,2)-linked side-chains consisting of L-rhamnose residues and some others neutral sugars (Rolin, 1993). The galacturonic acids have carboxyl groups, some of which are naturally presented as methyl esters and others which are reacted with ammonia to produce carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are an important means to classify pectin. The DE less than 50% is so-called low methoxy pectin while DE more than 50% is so-called high methoxy pectin (Rolin, 1993).

Pectin is regarded as safe for human consumption and has been used successfully for many years in food and pharmaceutical industries. Traditionally, pectin has been used as thickener, suspending agent, emulsifying agent, binder and film former (Sriamornsak, 2003). In pharmaceutical application, pectin has been used as a matrix for prolonged drug release (e.g. Sungthongjeen, Sriamornsak, Pitaksuteepong, Somsiri, & Puttipipatkhachorn, 2004; Sriamornsak, Thirawong, Weerapol, Nunthanid, & Sungthongjeen, 2007), as a mucoadhesive polymer for controlled drug delivery (e.g. Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007; Thirawong, Thongborisute, Takeuchi, & Sriamornsak, 2008), as a carrier for floating drug delivery (e.g. Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005; Sriamornsak et al., 2008), and as a carrier for colonic drug delivery (e.g. Sriamornsak, 1999; Sriamornsak, Nunthanid, Wanchana, & Luangtana-anan, 2003), etc. Recently, Chaidedgumjorn et al. (2009) extracted and characterized the pectin from fruit peel of *Citrus maxima* (Burm. f.) Merr. or pomelo. It is the aim of this study to further investigate the pharmaceutical application of the extracted pectin and compare with commercial pectins.

Pharmaceutical suspensions are dispersions of an insoluble drug in an aqueous or non-aqueous continuous phase. The problems that arise when a drug is dispersed in a liquid include sedimentation, caking (leading to difficulty in resuspension), flocculation and particle growth (through dissolution and recrystallization) (Florence & Attwood, 2006). Formulation of pharmaceutical suspensions to minimize caking can be achieved by the production of flocculated systems. A flocculated suspension contains the active drug dispersed throughout the liquid medium. Minute particles of the drug associate themselves with one or more excipients to form an agglomerated mass which is referred to as a "floccule". Other excipients in turn act to suspend the snowflake-like floccules in the water (Ansel, Allen, & Popovich, 2005). The viscosity of a suspen-

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Table 1
Designation and properties of pectin examined in the study.

Pectin type and designation	Degree of esterification (%DE)	Degree of amidation (%DA)	Molecular weight (Da)	Viscosity[a] (Pa s) ± S.D.		
				0.5% (w/v)	1% (w/v)	2% (w/v)
Low methoxy pectin						
CU020	29	20	150,000	6.33 ± 0.24	19.50 ± 1.41	116.08 ± 24.40
CU701	38	0	80,000	8.00 ± 2.36	32.33 ± 1.15	340.61 ± 8.99
High methoxy pectin						
CU501	56	0	180,000	7.28 ± 0.10	26.89 ± 80.67	212.94 ± 7.12
CU201	70	0	200,000	9.17 ± 0.00	38.67 ± 4.01	291.33 ± 25.69
Extracted pectin	78	0	45,000	2.50 ± 0.24	5.92 ± 0.59	21.25 ± 0.59

Note: The %DE, %DA and molecular weight are specified and reported by the manufacturer or previous report.
[a] The viscosity of triplicate samples was measured via Brookfield viscometer at 25 °C.

sion is obviously affected by flocculation. Polymers or gums (e.g. tragacanth, bentonite, alginate, xanthan gum, carboxymethylcellulose sodium, etc.) have been widely used, as a suspending agent, to increase the viscosity of suspension and therefore decrease the sedimentation rate of suspended particles (Ansel et al., 2005).

The overall objective of this study was to investigate the influence of pectin on the stability of indomethacin suspension. In particular, the effects of cation, type and concentration of pectin, pH and temperature on the flocculating activity of pectin in suspension was investigated. Finally, the flocculating and/or suspending properties of commercial pectins and pectin extracted from pomelo peel were determined and compared.

2. Materials and methods

2.1. Materials

Four commercial citrus pectins with different DEs and molecular weights (MWs), namely CU201, CU501, CU701 and CU020 (see Table 1), were kindly provided by Herbstreith & Fox KG (Germany). Indomethacin was purchased from P.C. Drug Center (Bangkok, Thailand). All other chemicals were analytical grade and used as received without further purification. Deionized (DI) water was prepared by reverse osmosis throughout all experiments.

2.2. Preparation of pectin from pomelo peel

Pectin extracted from pomelo (*Citrus maxima*, cultivar *Khao-nam-phueng*) peel was prepared using the method modified from Chaidedgumjorn et al. (2009). Briefly, the peels of *Citrus maxima* were extracted with hot water (80 °C, 3–5 h) and then evaporated using rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) at the temperature of about 80 °C. The dried pectin was kept in desiccator until used.

2.3. Measurement of viscosity of pectin solutions

The apparent viscosity of pectin samples (i.e., 0.5, 1 and 2% (w/v)) was determined at 25 °C using a Brookfield viscometer (Brookfield Engineering Corp., Stoughton, USA) equipped with a UL adapter. All measurements were performed in triplicate.

2.4. Measurement of flocculating activity

The measurement of flocculating activity of different pectins in indomethacin suspension was carried out based on the method reported by Yokoi, Obita, Hirose, Hayashi, & Takasaki (2002). Briefly, 0.1 mL of 0.015% (w/v) pectin solution, 0.25 mL of 10 mM cation solution and 4.65 mL of 0.5% (w/v) indomethacin suspension were mixed in a test tube (diameter 8 mm × height 90 mm). The mixture was stirred with a vortex mixer for 10 s and left standing for 5 min. Two milliliters of supernatant was carefully removed from the upper layer in the test tube, and its absorbance at 550 nm (as OD_{550}) was measured using spectrophotometer (model Lambda 2, Perkin-Elmer, Germany). A control experiment without the pectin solution was carried out in the same manner and absorbance at 550 nm ($OD_{550,\ blank}$) was measured. All measurements were conducted in triplicate. Flocculating activity was calculated using the following equation (Toeda & Kurane, 1991)

$$\text{Flocculating activity} = \frac{1}{OD_{550}} - \frac{1}{OD_{550,\ blank}}. \quad (1)$$

2.5. Zeta potential measurements

The zeta potentials of suspension were determined by phase analysis light-scattering (PALS) using a ZetaPALS instrument (Brookhaven Instruments Co., Holtsville, USA) equipped with Palladium electrodes. The measurements were performed with an applied voltage of 4.0 V and a field frequency of 2.0 Hz. Zeta potential analysis was also performed on suspension with pectin and/or cations. All measurements were carried out at 25 °C.

2.6. Effect of cations, pectin, pH and temperature on the flocculating activity

2.6.1. Effect of type and concentration of cations

To explore the effect of the cations on flocculating activity of pectin, the flocculation experiments were performed using 0.5% (w/v) suspended indomethacin supplemented with various cations, including $AlCl_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $FeSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, NaCl and KCl. The mixture consisting of 4.65 mL of 0.5% (w/v) indomethacin suspension, 0.25 mL of various concentration cations (0.01–100 mM) and 0.1 mL of 0.015% (w/v) pectin solution. The flocculation activity was calculated using Eq. (1).

2.6.2. Effect of pectin grade and concentration

To estimate the influence of pectin grade and concentration on flocculating activity, the mixture suspensions were prepared using five different grades of pectin, i.e., CU201, CU501, CU701, CU020 and extracted pectin. Various amounts of pectin (i.e., 0.002–0.05% (w/v)) were added in the suspensions in order to investigate the effect of pectin concentration.

2.6.3. Effect of pH and temperature

To estimate the influence of pH values on flocculating activity, the mixture suspensions were adjusted to desired pH values using 0.1N HCl and 0.1N NaOH. The pH values of the test suspensions ranged from 2.0 to 7.0. The effects of temperature were also examined using indomethacin and pectin at various temperatures, i.e., 25, 40 and 70 °C. The flocculation activity was calculated using Eq. (1).

Table 2
Formulation of indomethacin suspension using pectin as flocculating agent (F1–F6) and suspending agent (F7–F11).

Composition	Formulation										
	F1 (deflocculation)	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Indomethacin (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerin (mL)	1	1	1	1	1	1	1	1	1	1	1
Pectin CU201 (g)	–	–	–	–	–	0.015	0.5	–	–	–	–
Pectin CU701 (g)	–	–	–	–	0.015	–	–	0.5	–	–	–
Extracted pectin (g)	–	0.015	0.03	0.03	–	–	–	–	0.5	2	5
$FeCl_3$ (g)	–	0.0405	0.0405	0.081	0.0405	0.0405	–	–	–	–	–
Methyl cellulose (MC4000) (g)	0.5	0.5	0.5	0.5	0.5	0.5	–	–	–	–	–
Saccharine sodium (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben (g)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified water q.s. to mL	100	100	100	100	100	100	100	100	100	100	100

2.7. Investigation of pectin as flocculating agent or suspending agent in indomethacin suspension

2.7.1. Preparation of indomethacin suspension

To investigate the potential of extracted pectin as a flocculating agent in the pharmaceutical suspension containing indomethacin, different amounts of pectin (at the pectin to ferric ions ratio of 1:2.7) were used (see Table 2). The solution of ferric ions was added into the formulation to allow the coating of cations on to the indomethacin particles, followed by the addition of pectin. The potential of extracted pectin as a suspending agent in pharmaceutical suspension was observed (Table 2). Commercial pectins were also used in order to compare the results.

2.7.2. Evaluation of indomethacin suspension

Stability of the formulated suspension was observed from its physical appearance, color, and viscosity. Redispersibility is the major consideration in assessing the acceptability of a suspension. The sedimentation volume of the suspension was determined by keeping 50 mL of each suspension in measuring cylinder and storing undisturbed at room temperature. The separation of clear liquid was noted at intervals of 1 week up to 3 weeks. The sedimentation volume was calculated using the formula V_u/V_o, where V_u is the volume of sediment and V_o is the original height of the sample. The larger the value, the better is the suspendability.

The degree of flocculation was determined following the equation $\beta = F/F_\alpha$, where F is the ultimate sedimentation volume in the flocculated suspension and F_α is the ultimate sedimentation volume in the deflocculated suspension. The redispersion of the sediment after storage was investigated. Fixed volume of each suspension (50 mL) was kept in calibrated tubes which were stored at room temperature for various time intervals of 7 days; one tube was removed and shaken to redistribute the sediment and the number of shaking was recorded.

2.7.3. In vitro dissolution study

In vitro dissolution studies were performed using United States Pharmacopeia (USP) dissolution apparatus (Erweka, Germany) equipped with paddle (apparatus 2). Tests were performed in 900 mL of 0.01 M phosphate buffer pH 7.2, at 37 ± 0.1 °C at a paddle rotation speed of 50 rpm. After 20 min, 2-mL samples were withdrawn and filtered through polycarbonate membranes. Quantitative determination of indomethacin was performed spectrophotometrically (model Lambda 2, Perkin-Elmer, USA) at the maximum wavelength (λ_{max}) of 320 nm. Each dissolution study was performed in triplicate.

2.8. Morphology study

Morphological examination of the suspensions was carried out using a scanning electron microscope (Model Maxim-2000, Cam-Scan Analytical, Cambridge, England) equipped with secondary electron detector at an accelerating voltage of 15 keV. The different suspension samples were freeze-dried (Model FreeZone 2.5 Liter Benchtop Freeze Dry Systems, Labconco Corporation, USA) and then coated with gold to a thickness of about 30 nm in a vacuum evaporator.

2.9. Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 9.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Results and discussion

3.1. Viscosity of pectin

The apparent viscosity of pectin solutions at different concentrations is shown in Table 1. A non-linear relationship between apparent viscosity and concentration was observed. The pectin solution, like other polymers, exhibits the pseudoplastic behavior. For dilute solutions, polymer coils were widely separated and did not overlap. As concentration was further increased, coils begin to overlap and entanglements that increased viscosity were formed. From the viscosity results, it can be seen that the viscosity of pectin extracted from pomelo peel was the lowest; this may be due to the lowest molecular weight of extracted pectin.

3.2. Flocculating activity of pectin

Flocculation properties of pectin in an indomethacin suspension were investigated. Flocculation activity of pectin was influenced by addition of cations to the indomethacin suspension. Fig. 1a shows the flocculating properties of different pectins in suspension containing indomethacin and various cations. Solutions of $AlCl_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $FeSO_4{\cdot}7H_2O$, $CaCl_2$, $MgCl_2$, NaCl and KCl were used as the source of cations. Although flocculating activity of pectin was not observed in the presence of some cations, flocculation of the indomethacin suspension occurred by addition of Al^{3+} or Fe^{3+} when using any type of pectin, including pectin extracted from pomelo peel. This is in a good agreement with previous report (Yokoi, Obita, Hirose, Hayashi, & Takasaki, 2002) in which Al^{3+} or Fe^{3+} demonstrates good flocculating properties of pectin in inorganic kaolin suspensions. It has long been known that the addition of cations to suspension is necessary to induce the effective flocculating activity of negatively charged polymers. The role of cations, in our cases,

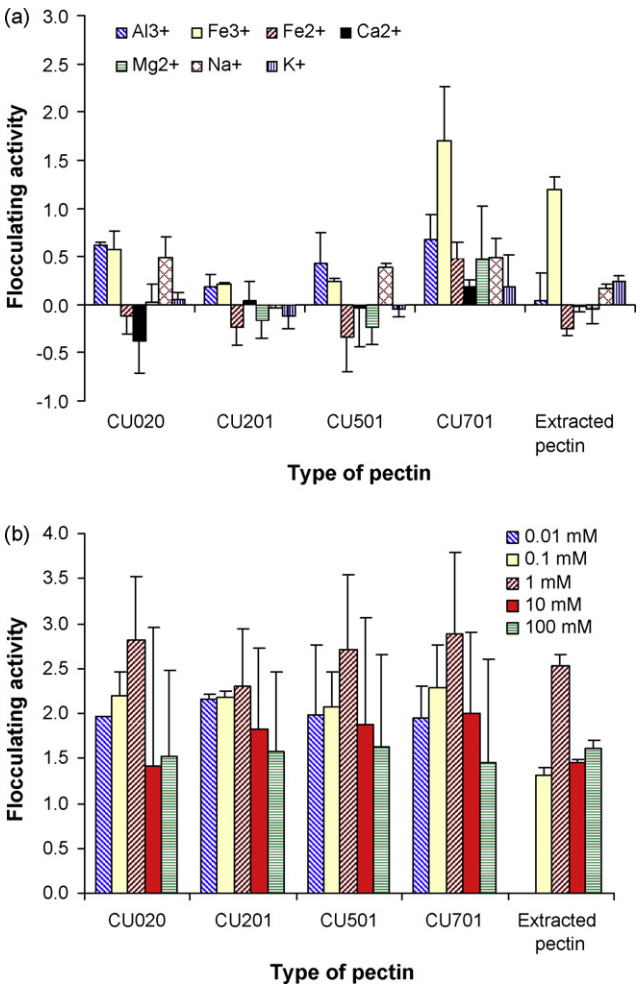

Fig. 1. Flocculating activity of different pectins in suspension containing 0.5% (w/v) indomethacin; (a) effect of various cations (10 mM) and (b) effect of concentration of cation, Fe^{3+}, on flocculating activity ($n = 3$). *Notes*: $Al^{3+} = AlCl_3 \cdot 6H_2O$, $Fe^{3+} = FeCl_3 \cdot 6H_2O$, $Fe^{2+} = FeSO_4 \cdot 7H_2O$, $Ca^{2+} = CaCl_2 \cdot 6H_2O$, $Mg^{2+} = MgCl_2 \cdot 6H_2O$, $Na^+ = NaCl$, and $K^+ = KCl$.

is to increase the adsorption of pectin on the surface of suspended particles by decreasing the negative charge on the pectin molecules (Levin & Friesen, 1987). Flocculation is due to decline of charge density by the added cations, leading to inter-particle bridging between indomethacin particles. Fig. 1b shows the effect of concentration of ferric ions on the flocculating activity of pectin in the indomethacin suspension. It can be seen that the optimum concentration of ferric ions for the flocculating activity of pectins (both commercial and extracted pectins) in the indomethacin suspension was 1 mM. Thus, experiments using ferric ions concentration of 1 mM were conducted to further examine the effect of other factors on flocculating activity.

The flocculating activity of pectins with lower DE is higher than that of higher DE pectin, including extracted pectin. Pectin CU701 demonstrates a better flocculating activity, compared to other pectins. This may be due to the fact that low DE pectins have higher amount of negatively charged carboxyl group (COO–) and could provide more effective sites for ferric cations to form bridge that binds indomethacin particles together (discuss later). The extracted pectin (DE 78%) shows comparable flocculating activity to pectin CU201 (DE 70%) when using ferric ions concentration of 1–100 mM (Fig. 1b).

In general, flocculants cause aggregation of particles by bridging and charge neutralization (Salehizadeh & Shokaosadati, 2001).

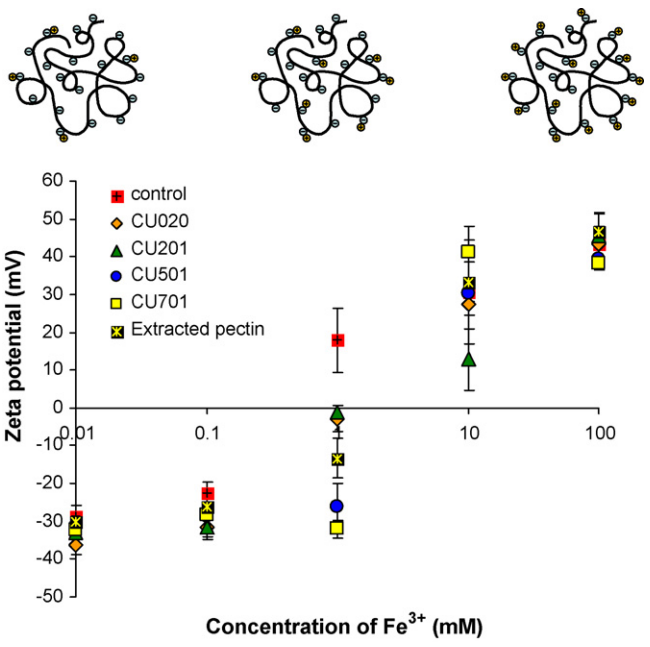

Fig. 2. Dependence of zeta potential on concentration of cation (Fe^{3+}) and pectin type ($n = 3$). The suspension contained 0.5% (w/v) indomethacin.

In this study, it was found that the zeta potential value was negative at a concentration of ferric ions of 0.01–1 mM, and the zeta potential increased with an increased concentration of ferric ions (Fig. 2). From the zeta potential results, the flocculated suspension could be achieved when using 1 mM ferric ions as the zeta potential of particles ranged from −20 to +20 mV as suggested for optimal range to allow the balance between the attractive force and repulsive force. It can be assumed that cations stimulate flocculation by neutralization and stabilization of residual negative charges of the carboxyl group of pectin forming bridge that binds indomethacin particles to each other (Wu & Ye, 2007). After the particles have adsorbed onto the pectin chains, bridging forms. Indomethacin particles could adsorb to or entrap in a long molecular chain, and they could adsorb/entrap simultaneously by the other chains in pectin, leading to the formation of three-dimensional floccules capable of rapid settling. Based on this assumption, the promoting effect of cations on flocculation of pectin is highly dependent on both the concentration and valence of the ions, which has been confirmed by the experimental results shown in Fig. 1. Fig. 3 illustrates the scanning electron micrographs of suspensions containing 0.5% (w/v) indomethacin and pectin in the absence and presence of Fe^{3+}. The SEM images confirmed that the presence of ferric cations promoted the flocculation and bridge formation that binds particles together. In the absence of ferric cations, it is observed that pectin formed films and coated on indomethacin particles.

Fig. 4 shows the effect of pectin concentration on flocculating activity of various pectins in suspension containing 0.5% (w/v) indomethacin and 1 mM ferric ions. The flocculating activity increased with the increased concentration of pectin from 20 to 50 mg/L but decreased at the concentration of pectin exceeded 50 mg/L. The exception is for amidated pectin (CU020) in which the flocculating activity decreased with the increased concentration of pectin. Thus, it could be said that the pectin used at an appropriate amount (i.e., 50 mg/L) could enhance the flocculating activity of pectin. The relationship between pectin concentration and flocculating activity is similar to that of a polysaccharide bioflocculant produced by *Pestalotiopsis* sp. (Kwon et al., 1996) and an extracellular biopolymer produced from a *Bacillus subtilis* DYU1 isolate (Wu & Ye, 2007).

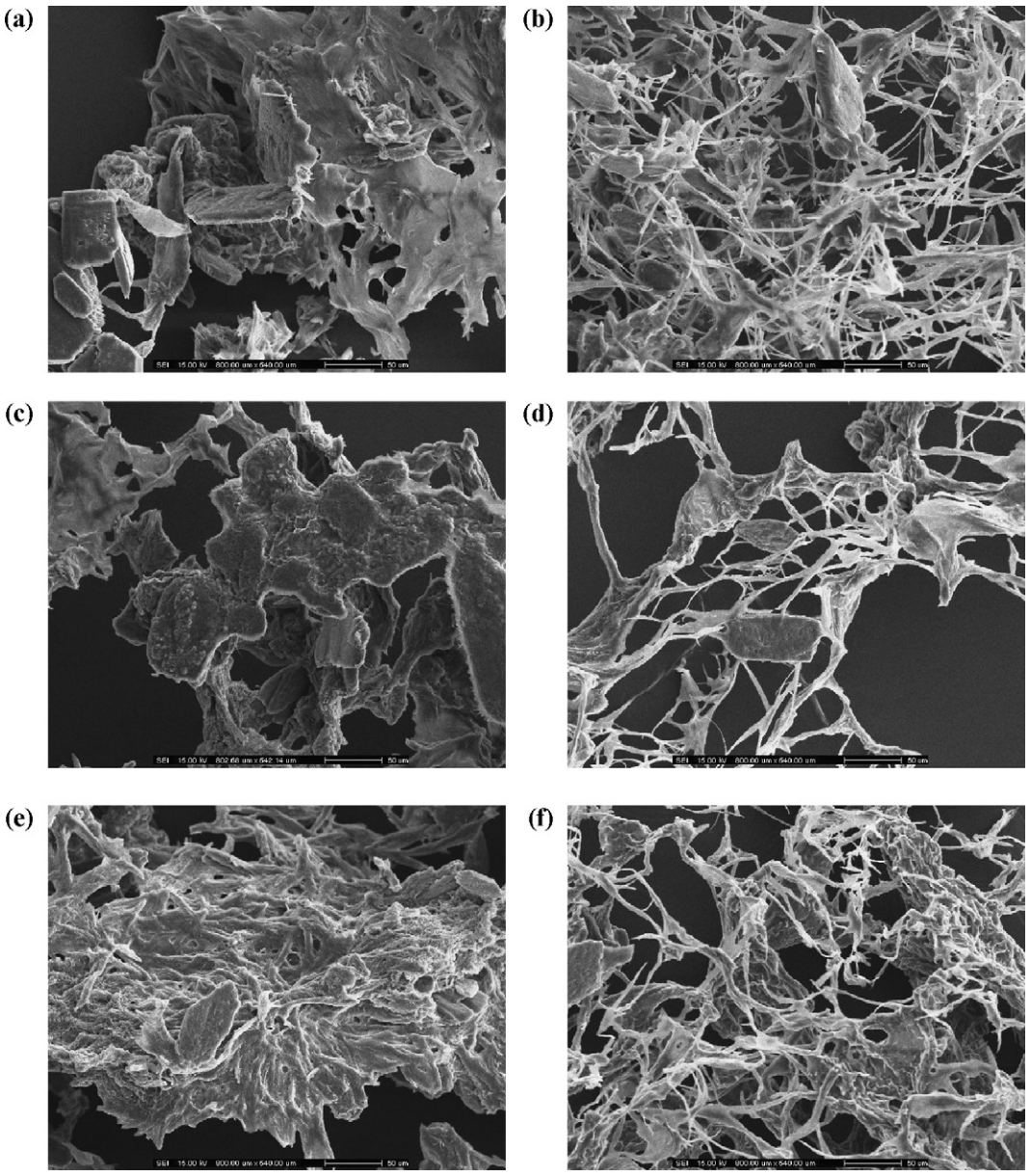

Fig. 3. Scanning electron micrographs of suspensions containing 0.5% (w/v) indomethacin and pectin in the absence and presence of Fe^{3+}; (a) pectin CU201, (b) pectin CU201 + Fe^{3+}, (c) pectin CU701, (d) pectin CU701 + Fe^{3+}, (e) extracted pectin, and (f) extracted pectin + Fe^{3+}.

The effects of temperature and pH on flocculating activity of various pectins in suspension containing indomethacin and ferric ions are shown in Fig. 5. To understand the effect of temperature on flocculating activity of pectin, different pectins were incubated at different temperatures and the flocculating activity of resulting samples was determined. Flocculating activity of pectin was influenced by incubated temperature. The highest flocculating activity was obtained at 70 °C, as shown in Fig. 5a. Therefore, pectin which is a polysaccharide could be classified as thermally stable flocculant. This is similar to Deng, Bai, Hu, & Luo (2003) who reported a thermally stable polysaccharide bioflucculant MBFA9 (produced by *Bacillus mucilaginosus*). If the major component of a bioflocculant is a glycoprotein, its stability will depend on the relative contents of protein and polysaccharide. Flocculants composed of protein or peptide backbone in the structure are usually susceptible to heat. Wu and Ye (2007) reported a temperature dependence of flocculating activity of a glycoprotein bioflocculant produced from bacteria; the flocculating activity and flocculating rate linearly decreased with an increase in incubation temperature. The optimal temperature for their system is 30 °C. Flocculating activity of pectin was also influenced by reaction pH of an indomethacin suspension. Ideally, a suspension should be stable over a wide pH range, thus the study was performed in the pH ranges from 2 to 7. The maximum flocculating activity of pectin was obtained at pH 2 (Fig. 5b). It is possible that, at pH 2, indomethacin was in unionized form (its pK_a is 4.5), therefore the ferric ions were available to associate with the negatively charged pectin, resulting in a higher flocculating activity. The flocculating activity decreased with an increase in pH (Fig. 5b). The decreased flocculating activity at higher pH (e.g. pH above 7) may be due to the lower amount of ferric ions available for association with pectin. Some of ferric ions can interact with the ionized indomethacin (negative charge). Moreover, the decreased activity may also due to the dissolution of indomethacin which makes the measurement of flocculating activity difficult.

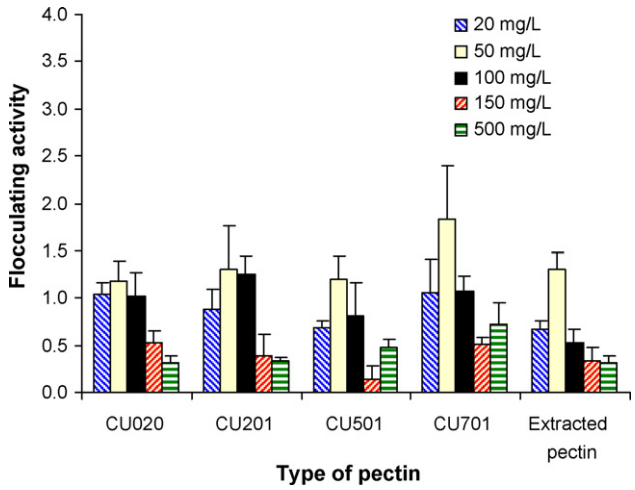

Fig. 4. Effect of pectin concentration on flocculating activity of various pectins in suspension containing 0.5% (w/v) indomethacin and 1 mM Fe^{3+} ($n = 3$).

3.3. Pectin as flocculating agent or suspending agent in indomethacin suspension

In pharmaceutical suspensions, the formulation contains also other ingredients such as wetting agent, viscosity inducing agent, sweetener and preservative. These ingredients may influence the stability of the formulation. Therefore, in this study, the amount of these ingredients (except pectin and ferric ions) was fixed to the same. Different formulations using varied concentration of extracted pectin were comparatively tested on their flocculating ability (F1–F6) and suspending ability (F7–F11), as shown in Table 2.

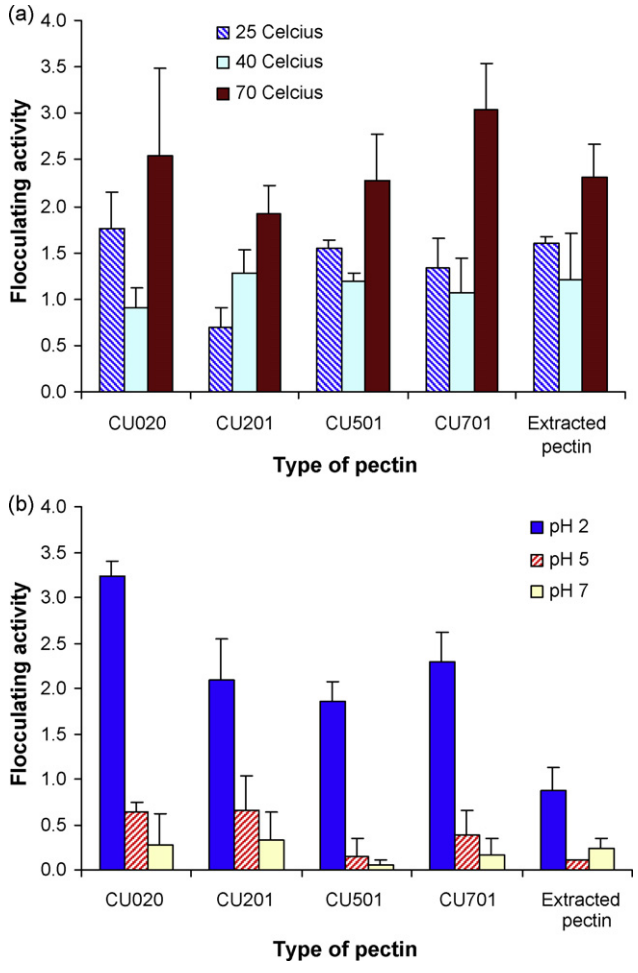

Fig. 5. Flocculating activity of various pectins in suspension containing 0.5% (w/v) indomethacin ($n = 3$); (a) effect of temperature, and (b) effect of pH.

The physical stability of a pharmaceutical suspension is the condition in which the particles do not aggregate and in which they remain uniformly distributed throughout the dispersions. In this study, stability of the formulated suspension was observed from its physical appearance, color, number of shaking (redispersibility), sedimentation volume, and degree of flocculation (Table 3). The formulated suspension was turbid with white or yellow in color. The stability of the indomethacin formulation will be discussed according to the role of pectin in the formulation, flocculating agent or suspending agent.

3.3.1. Use of pectin as flocculating agent in the formulation

Hydrophilic polymers such as alginates, cellulose derivatives, tragacanth, carbomers, etc. have been known to cause flocculation of particles of the dispersed phase (Aulton, 2002). These polymers have a linear branched chain structure and form a gel like network within the system. They get adsorbed on to the surface of the dispersed particles and hold them in a flocculated state. In the present study, pectin has been investigated as a flocculating agent in the indomethacin suspension. The deflocculated formulation (F1) demonstrates a high number of shaking time required for detachment of the sediment (112) and the number was increased to 220 after storage for 3 weeks. In contrast, the flocculated systems using extracted pectin and ferric ions at different ratios (F2–F4) had decreased frequency of adhesion of sedimented particles to the container indicating better redispersibility of the particles. The number of shaking required for detachment of the sediment from the container of the formulations using 15 mg/L of extracted pectin was 16 after storage for 1 week but increased after storage for 3 weeks. The use of extracted pectin to ferric cations of 1:1.35 (F3) showed a decreased adhesion with a lower number of shaking, compared to those used the ratio of 1:2.7 (F2 and F4). The more ferric ions would help to stabilize the particles in the suspension and therefore required lower shaking time. Formulations with commercial pectins (F5–F6) also show a comparable result with extracted pectin.

The sedimentation volume of the suspension was determined by measuring the amount of precipitate over 3 weeks (Table 3). It was found that all formulations those used pectin as flocculating agent (F2–F6) have the sedimentation volume close to 1.0. In general, the floccules form a higher sedimentation volume than non-flocculated particles, and the loose structure permits the aggregates to break up easily and redistribute with agitation. However, in this study, the deflocculated formulation also shows a sedimentation volume close to 1.0 (0.97–0.98). It is likely that the methylcellulose (a viscosity inducing agent) increased the viscosity of the formulation and then reduced the sedimentation of the particles in the suspension over 3 weeks of study.

3.3.2. Use of pectin as suspending agent in the formulation

In general, suspending agent (or thickener) is added in the formulation with the objective to reduce the sedimentation rate of dispersed particles by increasing apparent viscosity of the continuous phase, and thereby slowing down settling in accordance with Stokes Law. The ideal suspending agent should have a high viscosity at negligible shear, i.e., during shelf storage and it should have a low viscosity at high shearing rates, i.e., it should be free flowing during agitation, pouring and spreadibility (Aulton, 2002). The suspending agents include natural polysaccharides, semi-synthetic polysaccharides, clays and synthetic agents. Pseudoplastic substances like

Table 3
Physical characteristics of indomethacin suspension using pectin as a flocculating agent (F1–F6) and suspending agent (F7–F11) after storage for 3 weeks.

Formulation	Color	Number of shaking			Final volume of the sediment[a] (mL)			Sedimentation volume, *F*			Degree of flocculation, β		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
F1	White/turbid	112.0 ± 17.0	239.5 ± 85.5	220.0 ± 127.2	49.0 ± 0.0	49.0 ± 0.0	48.5 ± 0.7	0.98 ± 0.00	0.98 ± 0.00	0.97 ± 0.01	N/A	N/A	N/A
F2	Yellow/turbid	16.0 ± 1.4	20.5 ± 2.1	25.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.03 ± 0.01
F3	Yellow/turbid	8.5 ± 2.1	7.5 ± 0.7	6.5 ± 0.7	50.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.03 ± 0.01
F4	Yellow/turbid	11.0 ± 1.4	16.5 ± 2.1	18.0 ± 2.8	50.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.03 ± 0.01
F5	White/turbid	14.0 ± 1.4	12.0 ± 0.0	12.5 ± 0.7	49.0 ± 1.4	48.5 ± 0.7	49.0 ± 1.4	1.00 ± 0.00	0.99 ± 0.01	1.00 ± 0.00	1.02 ± 0.00	1.01 ± 0.01	1.02 ± 0.00
F6	White/turbid	12.0 ± 0.0	10.0 ± 0.0	17.0 ± 1.4	49.0 ± 0.0	49.0 ± 0.0	49.0 ± 0.0	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
F7	White/turbid	42.5 ± 3.5	43.0 ± 4.2	43.0 ± 4.2	48.0 ± 0.7	50.0 ± 0.0	49.0 ± 0.0	0.97 ± 0.01	1.00 ± 0.00	0.98 ± 0.00	N/A	N/A	N/A
F8	White/turbid	72.0 ± 33.9	103.5 ± 0.7	88.0 ± 31.1	49.0 ± 0.0	48.5 ± 0.7	48.0 ± 1.4	0.98 ± 0.00	0.97 ± 0.01	0.96 ± 0.03	N/A	N/A	N/A
F9	White/turbid	36.5 ± 4.9	23.0 ± 0.0	36.0 ± 16.9	48.5 ± 0.7	50.0 ± 0.0	49.0 ± 0.0	0.97 ± 0.01	1.00 ± 0.00	0.98 ± 0.00	N/A	N/A	N/A
F10	Yellow/turbid	39.5 ± 4.9	30.0 ± 1.4	31.0 ± 5.6	50.0 ± 0.0	50.0 ± 0.0	47.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	0.94 ± 0.00	N/A	N/A	N/A
F11	Brown/turbid	25.0 ± 0.0	19.5 ± 0.7	43.0 ± 2.8	50.0 ± 0.0	50.0 ± 0.0	47.5 ± 0.7	1.00 ± 0.00	1.00 ± 0.00	0.95 ± 0.01	N/A	N/A	N/A

[a] The initial volume of each suspension formulation is 50 mL.

tragacanth, sodium alginate and sodium carboxymethyl cellulose show the desirable qualities.

In this study, the extracted pectin has been used as a suspending agent in the indomethacin suspension (F9–F11) and compared with the commercial pectins (F7 and F8), as shown in Table 2. These formulations contain no other viscosity inducing agent or metal ions. Thus, the influence of pectin as a suspending agent in the formulation can be easily observed. The stability of these formulations (F7–F11) is shown in Table 3. Although the suspensions required higher number of shaking to redisperse the sediment than the flocculated systems, they were much better than the deflocculated formulation. The extracted pectin showed a better redispersibility, compared to the formulations those used commercial pectins, especially pectin with low DE (F8) which showed a high number of shaking times required for detachment of the sediment from the container. It can be seen that the formulation with the highest amount of extracted pectin (F11, 50 g/L) had the lowest number of shaking required for detachment of the sediment. It is probable that the amount of pectin was enough not only forming the floccules but also stabilizing the indomethacin particles by increasing the viscosity of the suspension. However, after 3 weeks, the particles tended to precipitate more, as also evidenced by the sedimentation volume which is decreased at the third week. Overall, the stability of all formulations using extracted pectin or commercial pectins as a suspending agent was excellent with the sedimentation volume exceeded 0.94, compared to other commonly used natural polysaccharides. Mahmud, Oyi, & Allagh (2009) reported that the use of natural gum (acacia or khaya gum) at a concentration of 5% (w/v) (50 g/L) was unable to disperse drug particles in the paracetamol formulation. The values of sedimentation volume (*F*) obtained for the suspensions containing *Acacia senegal*, *Acacia sieberiana* and *Khaya senegalensis* were 0.04, 0.0 and 0.56, respectively.

3.3.3. *In vitro dissolution study*

The dissolution characteristics in pH 7.2 phosphate buffer were performed using USP dissolution test apparatus II (Paddle method). Similar results were obtained among the dissolution of indomethacin from the suspension samples. The dissolution of indomethacin from different formulations of suspension ranged from 80.0% to 97.5% except that from F1 (deflocculation system) in which the percent drug dissolved was 74.5% and 79.1% after storage for 1 and 3 weeks, respectively (Table 4). This is probably due to solid cake forming in the deflocculated system, as also evidenced by the high number of shaking required for detachment of the sediment (Table 3). It seems that the investigated factors do not much influence the drug dissolution. According to the United State Pharmacopeia, not less than 80% of labeled amount of indomethacin should be dissolved within 20 min. Therefore, the dissolution of indomethacin from all formulations studied was passing the dissolution acceptance criteria after both 1-week and 3-week storage.

Table 4
Percent drug dissolved within 20 min (Q_{20}) from indomethacin suspensions after storage for 1 and 3 weeks ($n = 3$).

Formulation	Percent drug dissolved within 20 min (Q_{20})	
	After 1-week storage	After 3-week storage
F1	74.5 ± 0.1	79.1 ± 1.6
F2	81.5 ± 3.3	80.0 ± 1.9
F3	91.9 ± 4.7	87.8 ± 3.0
F4	82.4 ± 3.6	84.1 ± 5.5
F5	83.2 ± 3.9	84.3 ± 9.3
F6	88.6 ± 9.2	83.7 ± 6.1
F7	97.5 ± 2.3	93.8 ± 2.7
F8	93.9 ± 0.7	90.2 ± 1.4
F9	86.2 ± 5.0	90.7 ± 2.7
F10	96.1 ± 7.8	93.5 ± 2.3
F11	87.2 ± 3.8	85.6 ± 1.9

4. Conclusion

The application of pectin extracted from pomelo peel was extended to pharmaceutical suspensions in this study. The use of cation, type and concentration of pectin, pH and temperature influenced the flocculating activity of pectin in suspension. The extracted pectin has comparable activity to commercial pectins. From the results obtained, the use of low concentration of pectin and ferric ions allowed obtaining indomethacin suspensions with adequate properties (suitable stability and redispersibility). The use of pectin as a suspending agent was achieved with higher concentration; the flocculated and redispersible indomethacin suspension, which was stable throughout the storage period of 3 weeks, could be produced. The pharmaceutical suspension obtained from this study shows a technical potential as a delivery system for indomethacin, or other water-poorly soluble drugs, in liquid preparations. The advantages include reproducible drug delivery, easy flavoring and taste masking, and pediatric/geriatric patient-friendly consumption.

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