Study on adsorption properties of persimmon tannin-based gels for acidic and basic compounds

Dissertation

Study on adsorption properties of persimmon tannin-based gels for acidic and basic compounds

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Chapter 1

General Introduction

1. 1. Persimmon tannin

Persimmons are a number of species of trees in the genus *Diospyros*. There are about 400 species widely found in tropical and subtropical regions. Most are distributed in the Asia, Africa, and Central and South America. Several of the species are edible. Persimmon is a functional material that has been used in various applications such making tanning paper, tanning fishing net, and for removing protein during the brewing process of the Japanese rice wine "sake" [1][2].

Persimmon has various benefits for health such as antioxidant activity, anti-inflammatory activity, hypolipidemic activity, enzyme inhibiting, detoxification effects on snake venom and dispelling the effects of alcohol, etc. These properties are associated with Persimmon proanthocyanidin [3].

Persimmon fruits (*Diospyros kaki* L.) are rich in soluble and non-soluble condensed tannins (proanthocyanidins). Low molecular weight soluble tannins are believed to be responsible for the astringency of the persimmon fruit [1].

1. 2. Chemical structures of persimmon tannin

Matsuo and Ito [1] first reported on the composition and structure of persimmon condensed tannin (PT). Their proposed structure consists of coupled flavan-3-ols (Fig. 1.1), catechin, catechin-3-*O*-gallate, gallocatechin, and gallocatechin-3-*O*-gallate residues as the repeating units, with a molar ratio of 1:1:2:2, respectively. They also showed that the PTs are large molecular weight (ca. 13.8 kDa) polymers belonging to the proanthocyanidin B group, with carbon-carbon interflavan linkages between C-4 of one unit and C-8 (or C-6) of another. According to [4], the stereochemistry of the flavan-3-ol unit of the PT is mainly 2,3-cis, which corresponds to epi-type catechins as shown in Fig. 1.2. Proposed structure of PT is shown in Fig. 1.3.

1. 3. The purpose of this study

Tannins are polyphenolic compounds, widely distributed in many plant species, where they serve as defense mechanisms against predators. They form complexes and precipitates with macromolecules such as proteins, lipid, polysaccharides, and heavy metals

[5][6][7]. This properties associated with acidic character of the hydroxyl groups and nucleophilic moiety of the phenolic ring of tannin. It makes PT as promising adsorbent.

PTs are water-soluble compounds, which restricts their practical application as adsorbents in aqueous systems. Hence, insolubilization of PT such as crosslinking gelation or immobilization to water-insoluble matrices are required in order to overcome this issue. Various methods have been reported for tannin gelation. Most of them involve formaldehyde or other aldehydes in basic or acidic media [8][9][10]. Other researchers have reported acidic gelation ([11] and autoxidation processes [12].

In this study, PT gel was prepared by three methods such as autoxidation using oxygen, modification using formaldehyde and amine, and immobilization of PT on cellulose. In chapter 3, we prepared PT gel by autoxidation. PT was gelated by applying oxygen gas and natural light to the aqueous PT solution without using any harmful reagents and catalysts. Adsorption behaviors of the PT gel were tested for caffeine. In chapter 4, gelation was performed by modification of PT. In this method, two types of PT gels were prepared by modification of PT using formaldehyde and amine compound. Their adsorption to dyes in aqueous solution was studied. Last, in the chapter 5, PT was immobilized on cellulose Viscopearls and employed to adsorb caffeine in aqueous solution.

However, before preparation of PT gel, characterization of PT was carried out in chapter 2.

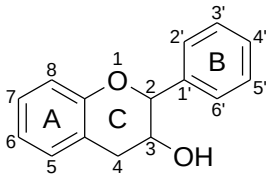


Fig. 1.1 Structure of flavan-3-ol.

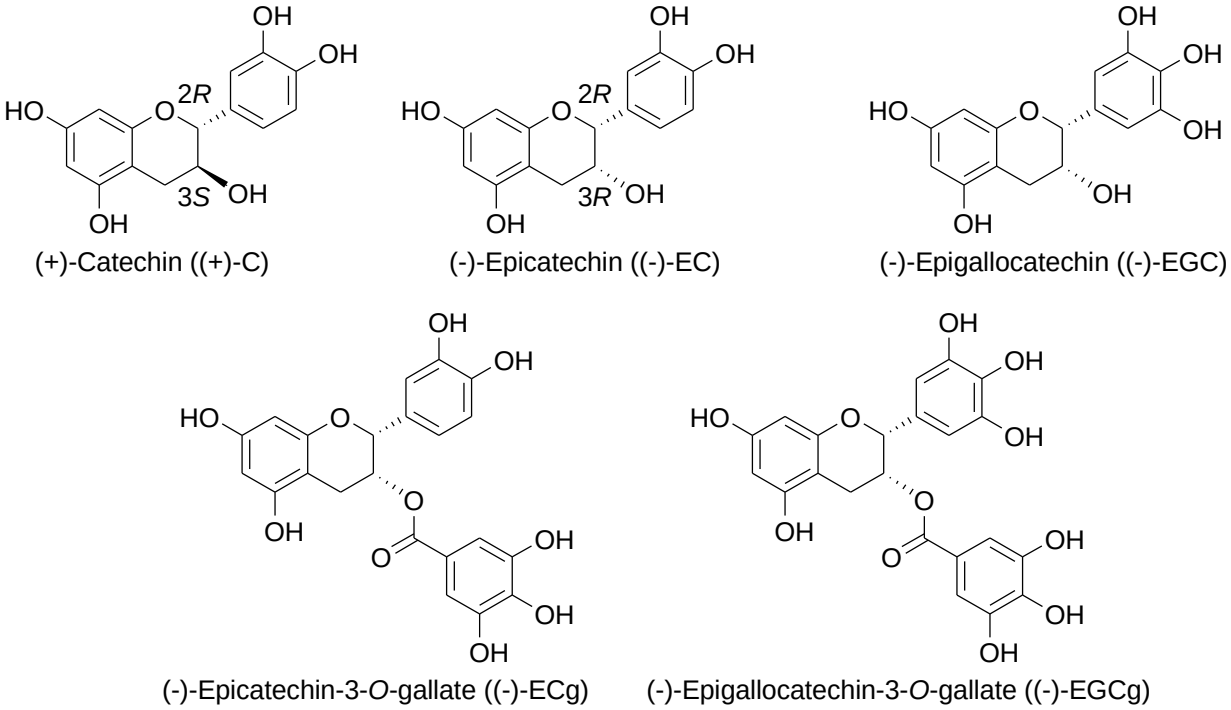
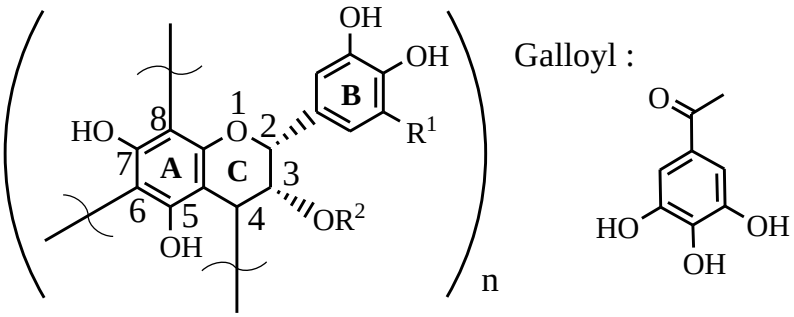


Fig. 1.2 Structure of catechins



R^1 = H or OH, R^2 = H or galloyl

Fig. 1.3 Propose structure of PT

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Chapter 2

Characterization of Persimmon Tannin

2.1. Introduction

Chemical characterization of PT has long been hampered because of its heterogeneous character and high molecular weight. Matsuo and Ito [1] first reported on the composition and structure of persimmon condensed tannin. Their proposed structure consists of coupled flavan-3-diols, catechin, catechin-3-*O*-gallate, gallocatechin, and gallocatechin-3-*O*-gallate residues as the repeating units, with a molar ratio of 1:1:2:2, respectively. They also showed that the PTs are large molecular weight (ca. 13.8 kDa) polymers belonging to the proanthocyanidin B group, with carbon-carbon interflavan linkages between C-4 of one unit and C-8 (or C-6) of another (Fig. 1.3). According to Xu *et al*. [2], the stereochemistry of the flavan-3-ol unit of the PT is mainly 2,3-cis, which corresponds to epy-type catechins as shown in Fig. 1.2. Composition of proanthocyanidins contained in the fruit of the persimmon varies depending on the variety and seeds [3], ripening [4] persimmon juice squeezed fruit astringent, physical properties of these proanthocyanidins through steps such as fermentation and filtration it is contemplated that the denatured.

Structural analysis of proanthocyanidins is difficult due to the heterogeneity of the subunit and molecular weight. Method to analyze the proanthocyanidins are divided into two types. First is analysis of proanthocyanidins as it is such as analysis using size exclusion chromatography. It will give information of average molecular weight or molecular weight distribution. The second is a method of analyzing the degradation product such as phloroglucinol decomposition to identify and quantify constituent subunit. In this chapter, we analyze the structure of persimmon proanthocyanidin derived using various techniques to evaluate the functionality.

2.2. Experimental

2.2.1. Materials and reagents

(-)-epicatechin (EC), (-)-epicatechin-3-*O*-gallate (ECg), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-*O*- gallate (EGCg), were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Soluble PT (*kaki-shibu*) was a generous gift from Kakitafu Co. Ltd. (Osaka, Japan). Aqueous ammonia was purchased from Wako Pure Chemical Industries, Ltd.

(Osaka, Japan). Ammonium chloride was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Dihydrate phloroglucinol, ascorbic acid, sodium acetate, and methanol were purchased from Kanto Chemical Co., Inc. Concentrated sulfuric acid were purchased from Wako Pure Chemical Industries, Ltd. (+)-C were purchased from Aldrich. Acetic acid was purchased from Wako Pure Chemical Industries, Ltd., lithium chloride, DMF, and N, N-dimethylformamide were purchased from Kanto Chemical. Folin-Ciocalteu reagents were purchased from Sigma-Aldrich. Sodium carbonate were purchased from Kanto Chemical Co., Inc. Other chemicals are commercial products of analytical grade. Ultra-pure water (Arium 611UV system, Sartorius, Germany) was used throughout the study.

2.2.2. Measurement of the UV absorption spectrum of PT

PT solution was freeze-dried to get freeze-dried PT (FDPT). A 50.0 mg of FDPT was dissolved in 250 mL ultra-pure water to prepare FDPT aqueous solution of 200 ppm as a stock solution. The stock solution was diluted with ultra-pure water to obtain a desired concentration ranging from 20 to 100 mg/L. In addition, each catechin ((-)-EC, (-)-EGC, (-)-ECg, (-)-EGCg) was prepared in 15 mL of ultra-pure water to be 0.25 mM. The absorbance of PT and catechins were recorded using a Shimadzu UV-2500 spectrophotometer (Shimadzu Corp., Kyoto, Japan), using quartz cuvettes with 10 mm path lengths. Extinction coefficients ε ' (L g^{-1} cm^{-1}) was calculated using the Lambert-Beer law.

2.2.3. pH dependence of UV absorption

The pH was adjusted using 2 M aqueous ammonia and 2 M hydrochloric acid aqueous solution to be 7.8, 8.9, 10.0 (ammonia / ammonium chloride buffer). The pH of the solution was measured by HORIBA pH METER F-13. FDPT were adjusted with ultra-pure water to be 1200 ppm, catechins (EC, EGC, ECg, EGCg) is 1500 ppm, respectively. The UV absorption spectra were measured by mixing 2.9 mL buffer solution or ultra-pure water and 0.1 mL aqueous solution of FDPT or catechins.

2.2.4. Phloroglucinol decomposition method

A 5 g of phloroglucinol and 1 g of ascorbic acid dissolved in 60 mL of methanol, then was added 0.5 mL of concentrated hydrochloric acid. The total amount to 100 mL by adding more methanol, to obtain a phloroglucinol solution. Sodium acetate of 0.33g was dissolved in 100 mL ultrapure water to prepare sodium acetate buffer 40 mM.

Decomposition was based on the method of the Kennedy *et al*. [5]. As much as 1 mL of phloroglucinol solution was added to 5.00 mg FDPT. Then, it was heated in water bath at 50 º C for 20 min. After then, it was removed from water bath and returned to room

temperature. Next, it was filtrate (pore size 0.45 μm, Advantech Co., Ltd.) and added 1 mL of sodium acetate buffer solution. Hydrolyzate solution of 20 μL was subjected to high performance liquid chromatography (HPLC). As shown in Table 2-1, elution was carried out at 280nm, column Inertsil ODS-3 (φ 4.6 × 250mm, 5 μm, GL Science Co., Ltd.), a column temperature of 40 ºC, 1.0 mL/ min flow rate, 1% (v / v) acetic acid (A solution), 5% at 10 min methanol (B solution), 20% in 30 minutes, 40% at 55 minutes, 90% at 65 minutes as the final was subjected to linear gradient elution to keep in 10 minutes.

Peaks was identified by LC / MS. The condition is follows: eluted with 0.341 mL / min flow rate, YMC-Triart C18 1.9 μm column (100 × 2.1 mm, 1.9 μm), column temperature 30 º C, elution at a flow rate of 0.341 mL / min, 2% (v /v) acetic acid (A solution), 5% in 2.32 minutes methanol (B solution), 40% in 11.42 minutes, 90% in 13.45 minutes, was a linear gradient elution to keep 1.01 minutes at the end using Waters Synapt™HDMS QTOF/MS (Waters Corp, Ltd., Milford, MA, USA.), Capillary voltage 2.8 kV, temperature 120 ºC, analysis range m / z 100 - 1500 Da, ionized by ESI, in the negative mode..

(+)-C, (-) from the concentration versus the detection area is measured using an HPLC solution was prepared as a standard solution of 100 mg / L methanol solution-EGCg, obtained at this time, the response of each standard sample the determined coefficient at this time was set to 1 the response factors of (+)-C and (+)-C.. (-) was the reference papers 5) of the Kennedy et al. response factor of the degradation products of-EGCg other than (Table 2-2).

2.2.5. Gel permeation chromatography

The molecular weight of the freeze dried sample, obtained from the soluble PT, was determined using a gel permeation chromatography (GPC) instrument (GL-7400 Series, GL Sciences Inc., Japan) with a UV detector. The column used was an Inertsil WP300 Diol (ϕ 4.6 × 250 mm, 5 μm; GL Sciences Inc.), protected with a precolumn of the same material. The isocratic method used a mobile phase consisting of *N*, *N*-dimethylformamide (DMF) containing 0.3% (v/v) glacial acetic acid, 1.7% (v/v) water and 0.05 M lithium chloride. The flow-rate was maintained at 0.3 mL/min, and the column temperature was 35℃. The elution was monitored at 280 nm. Calibration curves were constructed using polystyrene standards with molecular weights ranging from 2,000 to 30,000.

2.2.6. Measurement of IR spectrum

For infra-red (IR) analysis, the PT gel was dried by vacuum drying. The IR spectrum of the dried gel was recorded on a Horiba FT-720 Fourier transform infrared (FT-IR)

spectrometer (Horiba, Kyoto, Japan). FT-IR measurements were carried out by the KBr method at 20 scans per spectrum with 4 cm^{-1} resolution.

2.2.7. Folin-Ciocalteu test

The polyphenol content was quantified in accordance with the method Kimura et al. [6]. As much as 1 mL of FDPT (100 ppm) was stirred with 1 mL Folin-Ciocalteu reagent diluted two-fold with ultra-pure water. After 3 minutes, the mixtures was added with 5 mL 0.4 mol / L aqueous sodium carbonate solution, and held for 5 minutes at 50 º C, then water-cooled for 1 hour. It was measured the absorbance at 765 nm in a spectrophotometer.

2.2.8. Elemental analysis

About 2.0544 mg sample was analyzed using Micro Corder JM10 (J-Science Lab Co., Ltd., Kyoto, Japan).

2.3. Results and discussion

2.3.1. Lyophilization

Weight change before and after freeze-drying of persimmon tannin solution was obtained. Calculated concentration of persimmon tannin solution is shown in Table 2.1. The concentration is approximately 5.0%.

2.3.2. Ultraviolet-visible absorption spectrum

Fig. 2.1 shows the absorption spectra of soluble PT and 4 catechins such as (-)-EC, (-)-EGC, (-)-ECg, and (-)-EGCg) in water. When comparing the four catechins, it were observed 2 horns differences. First, absorption intensity of non-gallated catechins such as EC and EGC and gallated catechin such as ECg and EGCg was greatly different. The intensity was greatly different because of galloyl group of gallated catechins [6]. Second, B ring of EC has two hydroxyl groups while EGC has three hydroxyl groups so that the intensity differ. However, both has absorption maximum around 275 nm. Also,when absorption spectra of PT compared to these catechin, the shape of the absorption spectrum of PT is coincident with those of catechins. The transition band is not affected by polymerization, indicating that each subunit has separate conjugate system [7].

2.3.3. pH dependence of the UV absorption spectrum

To examine the UV spectra change due to pH change in the PT solution, PT solution was prepared in pH buffer 7.8-10. The result is shown in Fig. 2.2. In ultra-pure water (pH ≈6), absorption maximum was observed at 275 nm. If the pH is increased to 8.9, intensity peak at 275 nm is reduced. A new absorption appears and intensity is gradually increased to near 320 nm. Also, isosbestic point is observed in the 286 nm. Furthermore, when raising the pH

to 10.0, 322 nm absorption of further increases, the spectrum deviates from the isosbestic point.

Fig. 2.3 compares absorption of PT and catechins in pH 8.9. Gallated catechins such as (-)-ECg, and (-)-EGCg showed a new absorption band at 320 nm as while non-gallated catechin such as (-)-EC and (-)-EGC) not shown. PT showed little absorption at around 320 nm. So, the absorption spectrum changes due to pH change is a specific to gallated catechins . The result was consistent with Okumura et al. [8].

PT subunits has a conjugated system in which isolated as mentioned in section 2.3. Thus the conjugated system of each unit is assumed to be independent of gallated catechins subunits contained in the sample ratio, that is gallate rate of proanthocyanidins it is possible to estimate in a simple manner.

When it is adjusted to pH 8.9 with ammonia / ammonium chloride buffer, shows a relationship between absorbance at a concentration of catechins and 322 nm in Fig 2.4. (-) - ECg and (-) - data of EGCg is were riding on a straight line passing through the same origin of the slope. on the other hand, it is a linear relationship is obtained through the origin in the free catechins, the slope is small, reflecting the small extinction coefficient at 322 nm.

Here, assuming that the conjugated system of each subunit are independent , and therefore expressed by the sum of the absorbance of catechin subunits constituting absorbance FDPT, gallate catechins contained in FDPT of the following formula was calculated (weight) percentage .

$$m_{gallated} \cdot x + m_{non\ gallated} \cdot (1\text{-}x) = m_{FDPT} \tag{2.1}$$

$$0.02999\, x + 0.00217(1\text{-}x) = 0.01277$$

$$x = 0.38$$

where $m_{gallated}$ is the slope of the calibration curve of gallated catechin, $m_{non\ gallated}$ is the slope of the calibration curve of non gallated catechin, m_{FDPT} is the slope of the calibration curve of FDPT, x is mass fraction of gallated catechin.

Therefore, the weight ratio of gallate catechins contained in the PT was estimated to be 38%.

2.3.4. Phloroglucinol decomposition method

Ascorbic acid was analyzed by reversed-phase HPLC of the solution obtained in the reaction of phloroglucinol excessive presence, in acidic solution, as shown in. Fig. 2.5 chromatogram obtained is added to the phloroglucinol, was performed by LC / MS retention time and standard identification of peaks confirmed the main peak of nine new. as a result,

EC-Ph, EGC-Ph, ECg as a decomposition product of proanthocyanidin extension units derived from -Ph I considered, EGCg-Ph was confirmed. on the other hand, the degradation product peak of terminal subunit origin was not confirmed most. than this, the average degree of polymerization of proanthocyanidins contained in FDPT sample is very large .

For three peaks EC-Ph, EGC-Ph, the ECg-Ph, UV response factor obtained when the 1 (+)-C is shown in Table 2.2, the report is for EGCg-Ph past no. was estimated by the following calculation the response factor of EGCg-Ph in the same way than the additive property of the absorption intensity that there was shown in Section 2.3.2.

$$\begin{aligned} A_{EGCGg\text{-}Ph} &= A_{EGC\text{-}Ph} + A_{gallate} \\ RMR_{EGCGg\text{-}Ph} &= RMR_{EGC\text{-}Ph} + RMR_{gallate} \\ &= RMR_{EGC} + (RMR_{ECg\text{-}Ph} - RMR_{EC\text{-}Ph}) \\ &= 0.34 + (3.70 - 1.06) \\ &= 2.98 \end{aligned} \qquad (2.2)$$

$A_{EGC\text{-}Ph}$, $A_{EGCg\text{-}Ph}$, $A_{gallate}$: Absorbance of EGC-Ph, EGCg-Ph, and galloyl moiety respectively.

$RMR_{EC\text{-}Ph}$, $RMR_{EGC\text{-}Ph}$, $RMR_{ECg\text{-}Ph}$, $RMR_{EGCg\text{-}Ph}$, $RMR_{gallate}$: Relative molar response of EC-Ph, EGC-Ph, ECg-Ph, EGCg-Ph and galloyl moiety respectively.

Detection area of each peak, from mass conversion relative response factor CRMR calculated from it and the relative molar response factor RMR, was calculated subunit composition ratio in the sample from (Table 2.3) this, EC:. EGC: ECg: EGCg = 1: 62: 6:. 31 also (amount-of-substance ratio equivalent), charged the amount of the sample and from the detected sample volume, conversion to four compounds these 80%, and the average molecular weight of the subunit 360 was calculated.

2.3.5. Gel permeation chromatography

The GPC chromatogram of PT and calibration curve of polystyrene and polyphenols are shown in Fig. 2.6 and Fig. 2.7, respectively.

Although the determination of molecular weight by GPC measurement is carried out by comparing the retention time and the molecular weight is known sample components, the retention time affect factors such interactions with molecular size and filler sample components. It is desirable that such electronic distribution and functionality of the correlation or molecules of molecular size and molecular weight are similar in thus the sample components and the standard components. Now, polyphenols calibration curve of molecular weight 290.27 (+) - C and theaflavin digallate of molecular weight 868.7 has been created in the standard sample, Meanwhile polystyrene is created based on the four types of the standard sample from the mean molecular weight of 2000 to 30000. In order to calculate

a more accurate molecular weight, from that proanthocyanidins is a polyphenol flavonoids system, Also polyphenol flavonoids system it is desirable to be used as a standard sample. However, availability of known molecular weight of the polymer polyphenols is difficult, In this experiment using a low-molecular-weight polyphenol standard, Molecular weight estimation is large uncertainty in the polymer area. On the other hand, it can be applied to a broader molecular weight range polystyrene, differences in the molecular structure is also necessary to pay attention.

GPC was used to assess PT size distribution. The chromatogram shows a pronounced peak at approximately 5.31 min (Fig. 2.7). This peak corresponds to ultra high MWs that exceed the GPC column size exclusion limit of approximately 600,000. Thus, the sample contains very high molecular weight gel components.

2.3.6. Measurement of IR spectrum

. Fig. 2.8 shows the IR spectra of the PT gel and the two catechin standards, EGCg and C. The PT gel and EGCg show similar spectral patterns to each other. Thus, the C = O stretching of the gallate group is observed in the 1690 cm^{-1} region. In the 1400 − 1600 cm^{-1} region, the aromatic C = C stretching modes are dominantly observed. The mixed C-O stretching and OH bending vibrations are observed in the 1150 − 1350 cm-1 region.

2.3.7. Folin-Ciocalteu test

Gradually the addition of yellow Folin-Ciocalteu reagent to the sample solution was changed to dark green, holding to 50 ºC after addition of Na_2CO_3 solution into which the solution was colored blue indigo. The absorption spectrum of the blue solution was measured by an ultraviolet-visible spectrophotometer. Fig 2.9 show absorption spectrum of (-)-EGCg ranging from 0 - 0.40 mM. There is a broad absorption band near 750 nm. Absorbance increases as the concentration of the sample solution is high and the intensity is proportional to the sample concentration.

Fig. 2.10 compare calibration curves for Folin-Ciocalteus's assay with EC, ethyl gallate EGCg standards and PT sample. Folin-Ciocalteu test is based on the reduction of the phenolic OH groups of polyphenols. The horizontal axis of the graph was assumed to be obtained by multiplying the number and sample concentration of OH groups that represents the normality of the OH groups in the solution.

In contrast, sample 50, 100 mg / L when using PT aqueous solution, the calibration curve is greatly deviated from a group of standards, the slope was 0.62 times that of EGCg. PT subunit composition ratio and the unit the number of OH groups is based on values obtained

by phloroglucinol degradation.

2.3.8. Elemental analysis

The results of elemental analysis of FDPT is shown in Table 2.4. Calculated value determined by phloroglucinol degradation compared with the measured values.

2.4. Conclusion

PT is composed of catechin units (EC, EGC, ECg, EGCg). The PT contained very high molecular weight components where gallated catechins content was estimated to be about 40%. gallate catechins content phloroglucinol decomposition method and GPC measurement also. It suggested that the oxidative polymerization of the PT is in progress after the production and manufacturing process of persimmon. In addition, it is supported by Folin-Ciocalteu test and elemental analysis where autoxidation occurred in PT.

Table 2.1 Yields of freeze-dried persimmon tannin from the PT solution.

	Solution (g)	Yield (g)	Concentration (wt%)
1	155.607	7.828	5.03
2	96.594	4.750	4.92
3	252.201	12.578	4.99
		Average	4.98

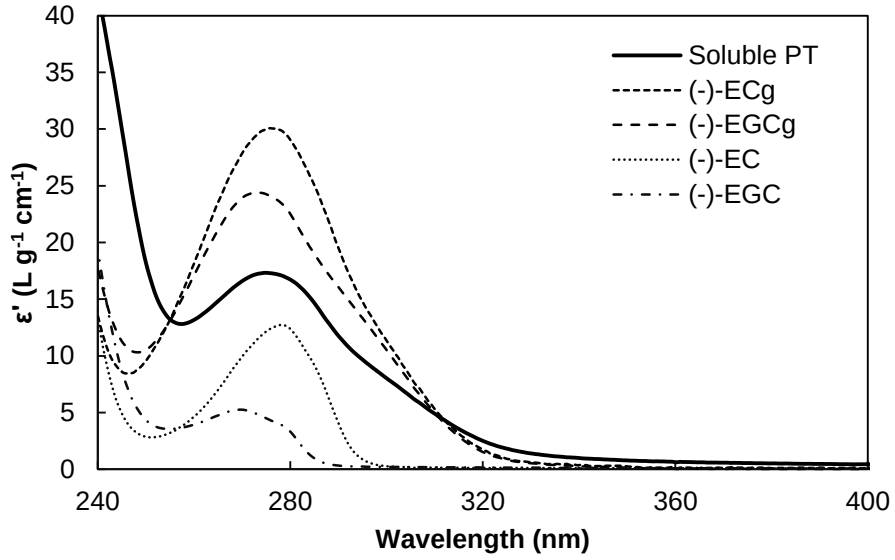

Fig. 2.1 Comparative UV-Vis spectra and absorption maximum absorption wavelengths of soluble PT and catechins in water.

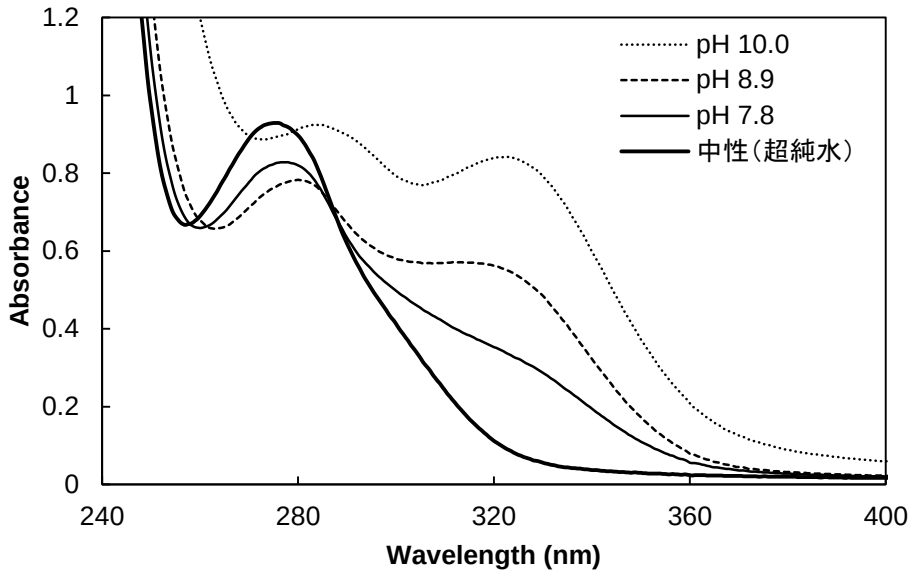

Fig. 2.2 UV absorption spectra of soluble PT (50 μg/L) at different pHs.

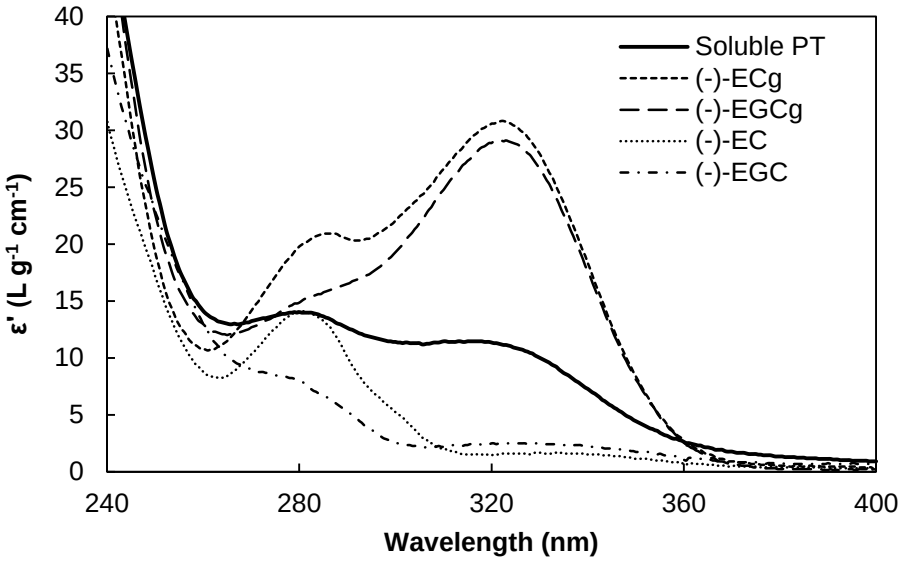

Fig. 2.3 UV spectra of soluble PT and catechins in pH 8.9 NH_3-NH_4Cl buffer.

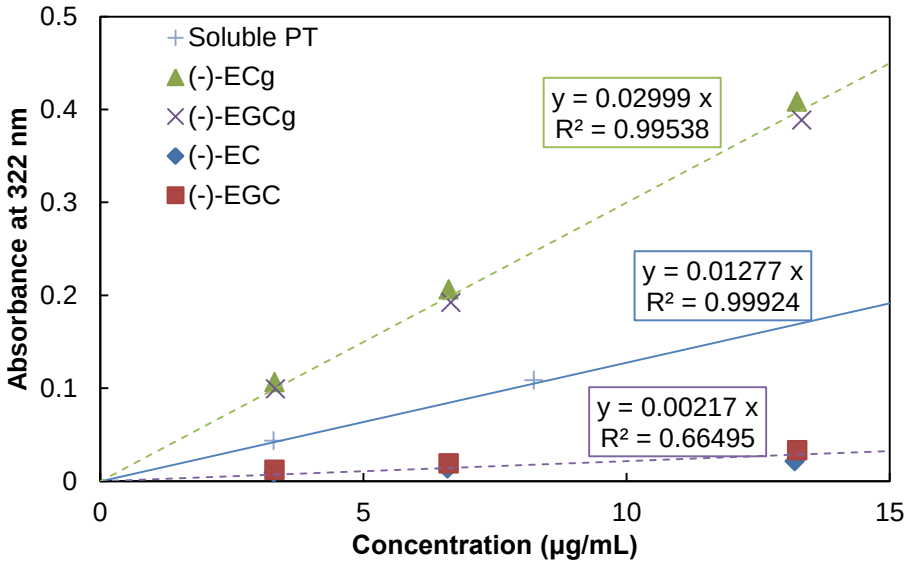

Fig. 2.4 Correlation between catechin concentrations and absorbances at 322 nm.

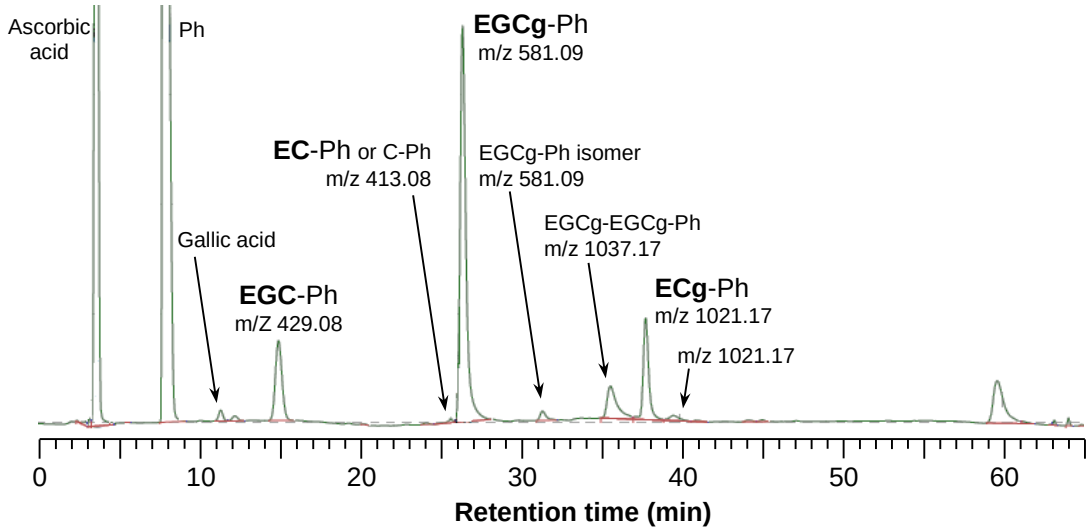

Fig. 2.5 HPLC chromatogram of PT cleavage products following acid-catalyzed cleavage in the presence of phloroglucinol (Ph).

Table 2.2 Absorptivity properties for common proanthocyanidin cleavege products. [a)]

Compound	Molar absorptivity (ε280) [b)]	Relative molar response (RMS) [c)]
C	3988	1.00
EC-Ph	4218	1.06
EGC-Ph	1344	0.34
ECg-Ph	14766	3.70
EGCg-Ph	-	2.98 [d)]

[a)] Quoted from Kennedy and Jones [9]

[b)] In methanol. [c)] Relative to (+)-catechin.

Table 2.3 Estimation of subunit composition for FDPT by phloroglucinol assay

Compound	Area (Observed value)	Relative molar response [b)]	Corrected relative mass response [b,c)]	Mol%	Wt%
EC	-	1 [a)]	1		
EGC	-	-	-		
ECg	-	3.16 [a)]	2.44		
EGCg	91416	2.39	1.60		
EC(or C)-Ph	138005	1.06 [a)]	1.06	1%	1%
EGC-Ph	4167262	0.34 [a)]	0.32	62%	52%
ECg-Ph	4463656	3.70 [a)]	2.43	6%	8%
EGCg(or GCg)-Ph	18819251	2.98 [d)]	1.89	31%	39%

[a)] Quoted from Kennedy and Jones, [9] [b)] ε_{280} (UV absorption) relative to EC.

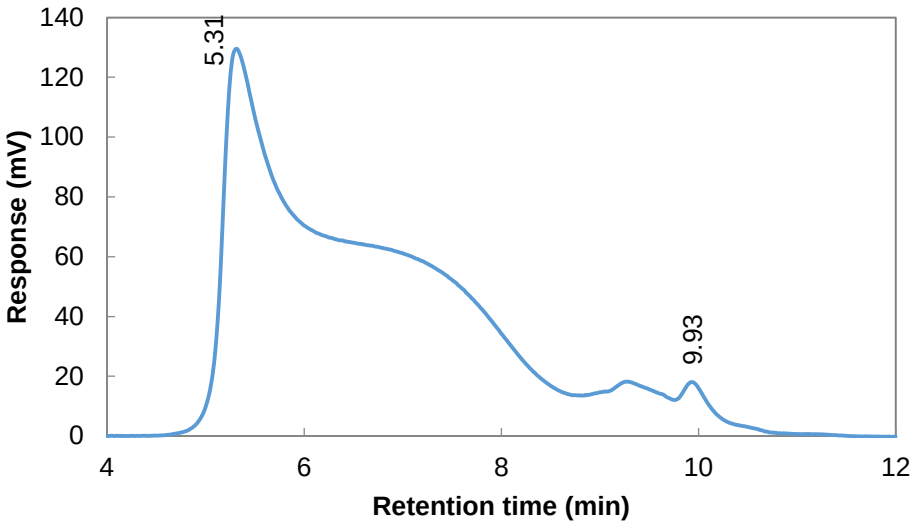

Fig. 2.6 GPC chromatogram of soluble PT dissolved in DMF.

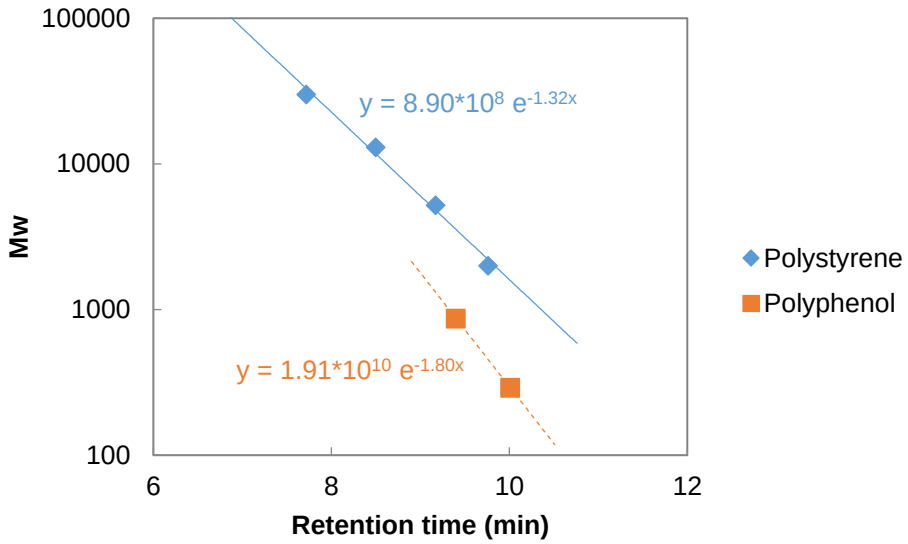

Fig. 2.7 Calibration curves based on polystyrene and polyphenols**.**

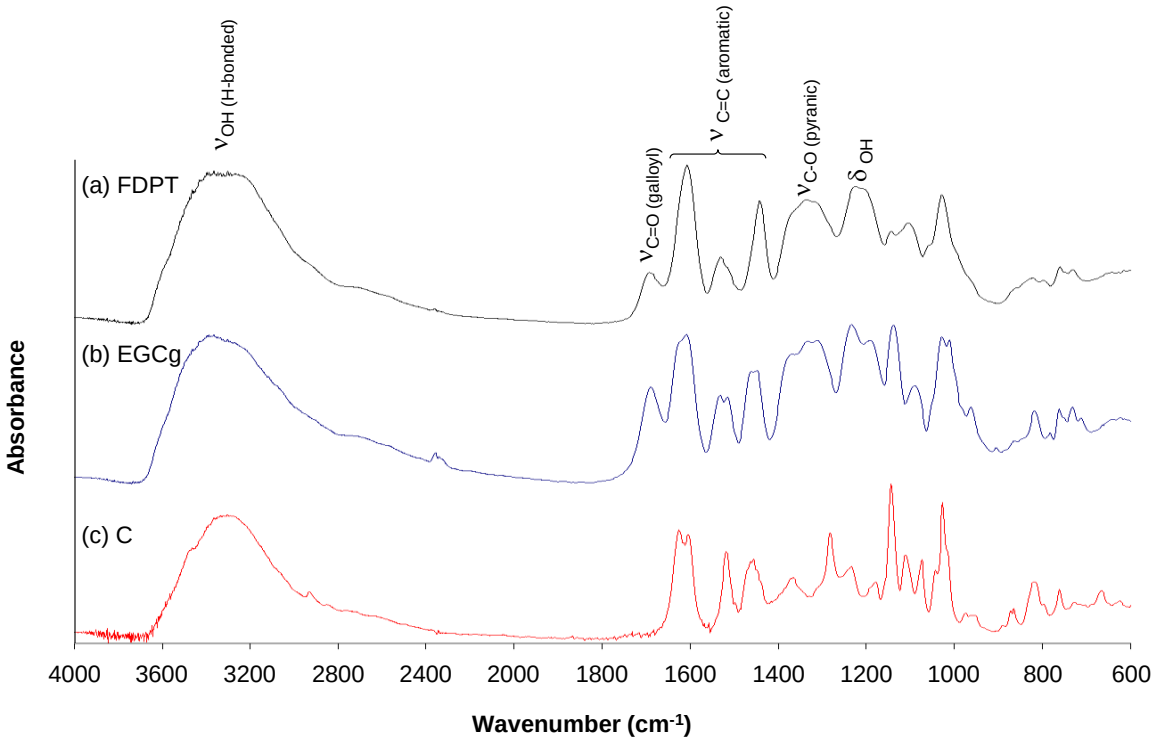

Fig. 2.8 IR spectra of FDPT and catechins

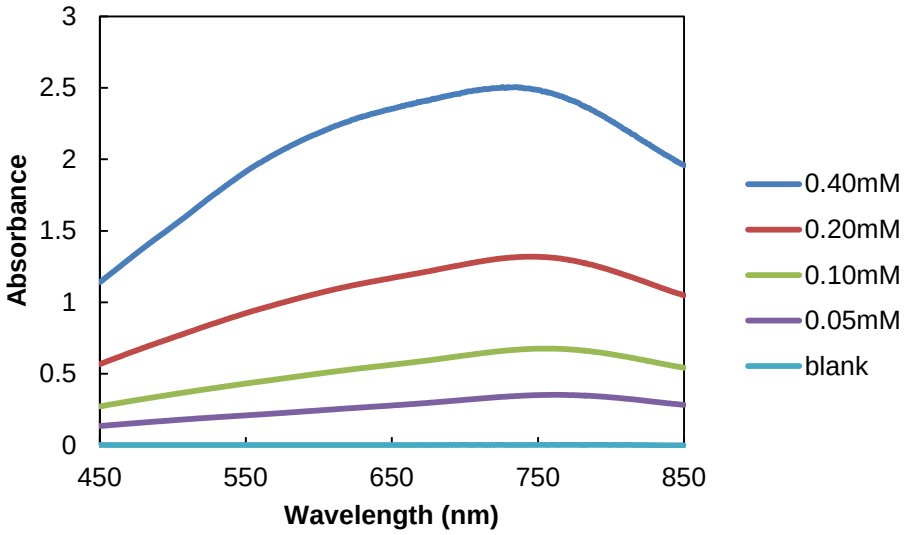

Fig. 2.9 Visible adsorption spectra of the reaction mixtures of (-)-EGCg Folin-Ciocalteu's phenol reagent on Folin-Ciocalteu's assay

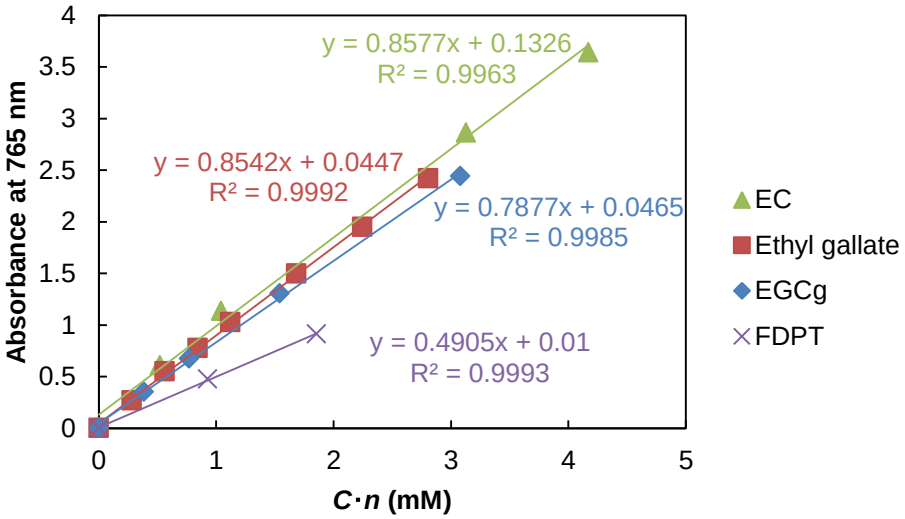

Fig. 2.10 Comparative calibration curves for Folin-Ciocalteus's assay with EC, ethyl gallate and EGCg standards and PT sample. Subunit composition of PT was estimated based on phloroglucinol decomposition.

C : Concentration of phenolic compound (mM) *n* : The number of phenolic hydroxyl group of compound

Table 2.4 Element analysis data of FDPT.

Element	Composition (wt%)	
	Calculated	Observed
C	58.77	53.05
H	3.78	4.12
O	37.45	42.65
N	-	0.18

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Chapter 3

Autoxidation of Persimmon Tannin and Caffeine Adsorption Properties

3. 1. Introduction

Kaki-shibu, a fermented product made from the juice of the immature astringent persimmons, has been traditionally used as an important source of condensed tannins for making tanning paper, for tanning fishing net, and for removing protein during the brewing process of the rice wine "*sake*" and soy sauce in Japan [1]. Various experimental methods have been described for the tannin gelation. Most of them involve the use of formaldehyde or other aldehyde in a basic or acidic medium [2][3][4] and other workers also reported on the acid gelation [5][6]. Recently, we have developed a simple and environmentally friendly method for preparing water-insoluble persimmon tannin gels based on commercial *kaki-shibu* without using any harmful reagents and catalysts [7]. Oxygen gas and the natural light are applied to the persimmon tannin solution in the gelation process. In the adsorption experiment for green tea components, the gel was found to be selective for caffeine. Green tea contains caffeine and low molecular weight polyphenols, catechins as the principal constituents. Caffeine is responsible for the stimulating effect of tea and the health benefits are mainly due to catechins. In recent years, there is an increasing interest in developing an environmentally friendly method of removing caffeine from food products.

In this chapter, we reported on the characterization and preparation of PT gel. Then, the PT gel was employed to adsorb caffeine from solution. The adsorption properties of the PT gel for caffeine are also studied using the equilibrium isotherm.

3. 2. Experimental

3.2.1. Materials and reagents

Caffeine was purchased from Nacalai Tesque (Kyoto, Japan). Green tea catechin standards, (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-*O*-gallate (ECg), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-*O*- gallate (EGCg), were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Soluble PT (*kaki-shibu*) was a generous gift from Kakitafu Co. Ltd. (Osaka, Japan). The chemical structure of caffeine and

these catechins are shown in Fig. 3.1. Other chemicals are commercial products of analytical grade. Ultra-pure water (Arium 611UV system, Sartorius, Germany) was used throughout the study.

3.2.2. Preparation of the PT gel and adsorbates

The PT gel was prepared by autoxidation process where the gelation reaction was carried out at room temperature on exposure to natural light and O_2 gas. The soluble PT (500 mL) was put in 1,000 mL Erlenmeyer flask equipped with a three-way stopcock which was attached a rubber balloon. The flask was kept under a positive pressure of oxygen by the use of an oxygen-filled balloon. The start of gelation was noticed from an increase in the viscosity of the solution. After the gelation was completed in 3 weeks, the residue gelled was crushed and washed with water several times until the supernatant becomes clear. The obtained gel was kept in brown bottle under wet condition, which was termed as the PT gel. This PT gel was used throughout all adsorption experiment.

A stock solution of caffeine (1,000 mg/L) was prepared by dissolving a required amount of caffeine in ultrapure water. The stock solution was diluted with ultrapure water to obtain a desired concentration ranging from 25 to 100 mg/L.

3.2.3. Characterization of the PT and the PT gel

The molecular weight of the freeze dry sample obtained from the soluble PT was measured by a gel permeation chromatography (GPC) instrument (GL-7400 Series, GL Science Inc., Japan) with a ultra violet (UV) detector. The column used was an Inertsil WP300 Diol GL Sciences Inc., Japan, φ4.6×250 mm, 5μm, protected with a precolumn of the same material. The isocratic method used a mobile phase consisting of *N*, *N*-dimethylformamide (DMF) containing 0.3% (v/v) glacial acetic acid, 1.7% (v/v) water and 0.05 M lithium chloride. The flow-rate was maintained at 0.3 mL/min and a column temperature of 35 °C. The elution was monitored at 280 nm. Calibration curves were constructed using polystyrene standards with molecular weights ranging from 2,000 to 30,000.

The UV–vis absorption spectra were recorded on a Shimadzu UV-2500 spectrophotometer (Shimadzu Corp., Kyoto, Japan) with a 2 nm resolution, using quartz cuvettes of 10 mm path length. The freeze dry PT sample was used for this measurement.

For infra-red (IR) analysis, the PT gel was dried by vacuum drying methods. The IR spectra of the dried gel was recorded on a Horiba FT-720 Fourier transform infrared (FT-

IR) spectrometer (Horiba, Kyoto, Japan). FT-IR measurements were carried out by the KBr method at 20 scans per spectrum with 4 cm^{-1} resolution.

3.2.4. Adsorption experiments

Batch method was used to study the adsorption behavior of the PT gel. Adsorption experiments were conducted to study the effect of contact time, caffeine concentration, gel dose, and temperature. The experiments were carried out in screw cap tube 20 mL by varying the PT gel dose from 0.1 to 2 g/20 mL and initial caffeine concentration from 25 to 100 mg/L. The mixture was agitated at 250 rpm at ranging temperature from 30 to 60 °C. The contact time was varied from 0 to 30 minutes. Then the mixture was centrifuged and filtered and the absorbance of the filtrate was determined by HPLC machine with column temperature of 40 °C, sample volume of 10 µL, mobile phase of methanol/water including 0.2% phosphoric acid 20 : 80 (v/v), flow rate of 0.8 mL/min, and measurement wavelength of 280 nm. The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10ATvp pump, a Shimadzu SPD-M10Avp UV detector and a Shimadzu CTO-10Avp column oven, and a CAPCELLPAK C18 column (4.6 × 100 mm, 3 µm, Shiseido Co., Ltd, Tokyo, Japan).

All of experiments were performed in duplicate. Adsorb ratio (%) and the amount of caffeine adsorbed on the PT gel, q_e (mg/g), was calculated by the following equations:

$$\text{Adsorbed ratio} = ((C_0 - C_t)/C_0) \times 100 \quad (3.1)$$

$$q_e = (C_0 - C_e)V/M \quad (3.2)$$

where C_0, C_t and C_e are the initial, at time t, and equilibrium MB concentrations in solution (mg/L), respectively. V is the volume of solution (L) and M is the mass of adsorbent (g). Equilibrium data were analyzed using the Langmuir and Freundlich isotherm equations, and characteristics parameters for each isotherm were determined.

3. 3. Results and Discussion

3.3.1. IR spectral characterization of PT gel

Fig. 3.2 shows IR spectra of the PT gel and those of two catechin standards, EGCg and C. The PT gel and EGCg show similar spectral patterns to each other. Thus, the C=O stretching of the gallate group is observed in the 1690 cm^{-1} region. In the 1400-1600 cm^{-1} region, the aromatic C=C stretching modes are dominantly observed. The mixed C-O stretching and OH bending vibrations are observed in the 1150-1350 cm^{-1} region.

3.3.2. Adsorption of green tea components

In a preliminary experiment, we have tested adsorption of the PT gel for green tea components. As shown in Fig. 3.3, caffeine was adsorbed about 56%, whereas four kinds of catechins were adsorbed only 10-20%. This result indicates that adsorption of caffeine on the PT gel is much stronger than that of catechins. Caffeine is a typical purine base carrying four basic nitrogens in the heterocyclic ring (Fig. 3.1). These electron-rich nitrogens may play an important role in the interaction with the PT gel. For catechins, the gallate-type catechins such as EGCg and ECg showed stronger adsorption than the non-gallate type catechins, EC and EGC [8].

3.3.3. Effect of contact time and initial concentration on adsorbed ratio of caffeine

Contact time is an important parameter because it determines the rate of the adsorbates removal and the results are shown in Fig. 3.4. As shown in Fig. 3.4, the fast rate of adsorption is found at the first 5 minutes and the equilibrium was attained in about 15 minutes. The adsorption is higher in the beginning due to the large surface area of adsorbents available for adsorption of the adsorbates and along with the gradual occupancy of these sites, the adsorption became less efficient [9].

Fig. 3.5 shows that increase of initial concentration of caffeine will decrease the adsorbed ratio slightly. It is due to the increase in the number of caffeine molecule competing for the available binding sites on the PT gel. Thus, available active sites of the PT gel are saturated at higher concentration of caffeine [10][11].

3.3.4. Effect of adsorbent dose

It was noted from Fig. 3.6 that the percentage of caffeine removal is varied with varying adsorbent mass and and increased with increase in adsorbent dose. The percentage of caffeine removal increase from 15% to 72% for an increase in adsorbent dose from 0.1 g to 2 g. It is due to the increase in number of active adsorption sites avalaible for the adsorption.

3.3.5. Effect of temperature on adsorbed ratio of caffeine and thermodynamic parameter

Experiments were performed at different temperature ranging from 30 to 60^{o}C for. The adsorbed ratio decreased with increase of temperature from 30 to 60 ^{o}C for 25-100 mg/L caffeine concentrations (Fig. 3.7). This observation indicates that the adsorption is exothermic process.

Thermodynamic parameters, such as change in Gibbs free energy (ΔG^o) (kJ/mol), enthalpy (ΔH^o) (kJ/mol) and entropy (ΔS^o) (J/K.mol) were determined using the following equations

$$\ln K_0 = -\Delta G^o / RT = \Delta S^o / (R) - \Delta H^o / (RT) \quad (3.3)$$

where K_o is the equilibrium constant, T is the temperature in Kelvin, R is the gas constant. The value of ΔH^o and ΔS^o was obtained from the slope and intercept of Eq. 2. Thermodynamic parameters of adsorption were shown in Table 3.1.

The ΔG^o for physisorption ranges from −20 kJ/mol to 0 kJ/mol and for chemisorption from −80 kJ/mol to −400 kJ/mol [12][13]. The values of ΔG^o shown in Table 3.1 indicated that the adsorption is spontaneous physisorption. In addition, the values of ΔG^o reveal that the adsorption is feasible at low temperatures. The ΔG^o for hydrogen bonding and dipole force are 2-40 kJ/mol and 2-29 kJ/mol, respectively [14][15][16].

The result shown in Table 3.1 suggested that the interaction between the adsorbent and the adsorbate is hydrogen bonding and weak attractive force. An acidic character of the hydroxyl groups and the nucleophilic properties of the A phenolic ring of persimmon tannin gel are responsible to these kind of interactions to caffeine [17][18][19]. The negative value of ΔH^o indicates the exothermic nature of adsorption. Therefore, adsorbed ratio declined when temperature rose (Fig. 3.7). The negative values of ΔS^o indicated the decrease in caffeine concentration in solid-liquid interface revealing as well the increase in ^{O}caffeine concentration on the solid phase. It also confirmed the increase of the uniformity at the solid-liquid interface during adsorption [12][20].

3.3.6. Adsorption isotherms

Plotting q_e vs C_e can be used as a qualitative approach to identify a suitable isotherm model. As shown in Fig. 3.8, curves were approximately linear. It indicated a constant affinity for a wide range of concentrations of adsorbates along with cooperative adsorption. Langmuir and Freundlich equations are the appropriate model for this adsorption [21].

Langmuir and Freundlich isotherms were tested for adsorption of caffeine on the PT gel. Langmuir's isotherm model for linear form is given by the following equation:

$$1/q_e = 1/(q_m K_L C_e) + 1/q_m \quad (3.4)$$

where q_m is Langmuir constant related to the complete coverage (mg/g), K_L is the Langmuir constant which is an energy constant, indicating adsorptivity of the solute.

The linear form of Freundlich isotherm is given by the following equation:

$$\log q_e = (1/n) \log C_e + \log K_F \quad (3.5)$$

where n is Freundlich isotherm constant related to adsorption intensity, K_F is Freundlich isotherm constant related to adsorption capacity (L/g).

The value of n indicates the degree of non-linearity between solution concentration and adsorption as follows: if the value of n is equal to unity, the adsorption process is linear; if the value is below to unity, this implies that adsorption process is chemical; if the value is above unity, adsorption is a favorable physical process [11].

The data obtained for Langmuir and Freundlich equation conform best to both with high correlation coefficient varying from 0.991 to 0.999. It indicates that the PT gel surface is homogeneous and coverage of caffeine at the outer surface of the PT gel is monolayer [11][12]. The isotherm parameters of adsorption were listed in Table 3.2. Values of n suggest that adsorption is physisorption.

The maximum adsorption capacity of a monolayer was found to be 65.8 mg/g for the PT gel. This result is lower than adsorption of activated carbon of 275 mg/g [22] but higher than adsorption of NIPAAm-based hydrogels of 10.2-18.7 mg/g [23] and natural clay adsorbent sepiolite of 48.7 mg/g [24]. In addition, they do not compare with adsorption of another component of green tea such as catechins. The PT gel not only have high adsorption to caffeine but also adsorb selectively to caffeine as shown in Fig. 3.3). This is very important because caffeine can be removed and at the same time retaining catechins as an essential compounds in a food product.

Langmuir constant, K_L, can be used to predict the affinity between adsorbate and adsorbent using separation factor or dimensionless equilibrium parameter, R_L,

$$R_L = 1/(1 + K_L C_0) \quad (3.6)$$

The value of R_L suggested the type of Langmuir isotherm to be irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$), or unfavorable ($R_L > 1$) . The R_L was found to be 0.517 to 0.853 for concentration of 25-100 mg/L and temperature of 30-60 ºC [11]. It indicates the favorable adsorption.

3. 4. Conclusion

In this experiment, the PT gel has been prepared by autoxidation process. The PT gel employed to adsorb caffeine. The PT gel effectively adsorb caffeine from aqueous solution.

Adsorption of caffeine on the PT gel was influenced by contact time, initial concentration of caffeine, adsorbent dose, and temperature. Equilibrium was reach for 15

min. Adsorbed ratio increased with decreasing caffeine concentration and temperature. Otherwise, adsorbed ratio increased with increasing adsorbent dose.

The adsorption isotherms follow both Langmuir and Freundlich model. The maximum adsorption capacity was found to be of 65.8 mg/g. Adsorption of caffeine on the PT gel is favorable physisorption.

Value of ΔG^{o} and ΔH^{o} indicated the spontaneous and exothermic nature of adsorption. In addition, interaction between the adsorbent and the adsorbate is hydrogen bonding and weak attractive force.

These data suggest that the PT gel can be used as an effective adsorbent to remove caffeine from green tea drinks.

Table 3.1 Thermodynamic parameters for the adsorption of caffeine on the PT gel

C_0 (mg/L)	K_0				ΔG^o (kJ/mol)				ΔH^o (kJ/mol)	ΔS^o (J/K.mol)
	30 ºC	40 ºC	50 ºC	60 ºC	30 ºC	40 ºC	50 ºC	60 ºC		
25	115.2	92.6	67.4	53.8	-12.0	-11.8	-11.3	-11.0	-21.8	-32.4
50	109.0	76.3	55.4	39.9	-11.8	-11.3	-10.8	-10.2	-28.0	-53.3
75	109.5	75.1	56.8	46.5	-11.8	-11.2	-10.8	-10.6	-23.8	-39.9
100	100.9	74.9	52.2	42.0	-11.6	-11.2	-10.6	-10.4	-25.0	-44.3

Table 3.2. Isotherm parameters for the adsorption of caffeine on the PT gel

Isotherm model	Parameter	Temperature (ºC)			
		30	40	50	60
Freundlich	n	1.09	1.17	1.19	1.19
	K_F (L/g)	0.865	0.839	0.750	0.690
Langmuir	q_m (mg/g)	65.8	56.7	47.3	43.5
	K_L (10^3 L/mg)	9.36	9.21	8.06	6.92

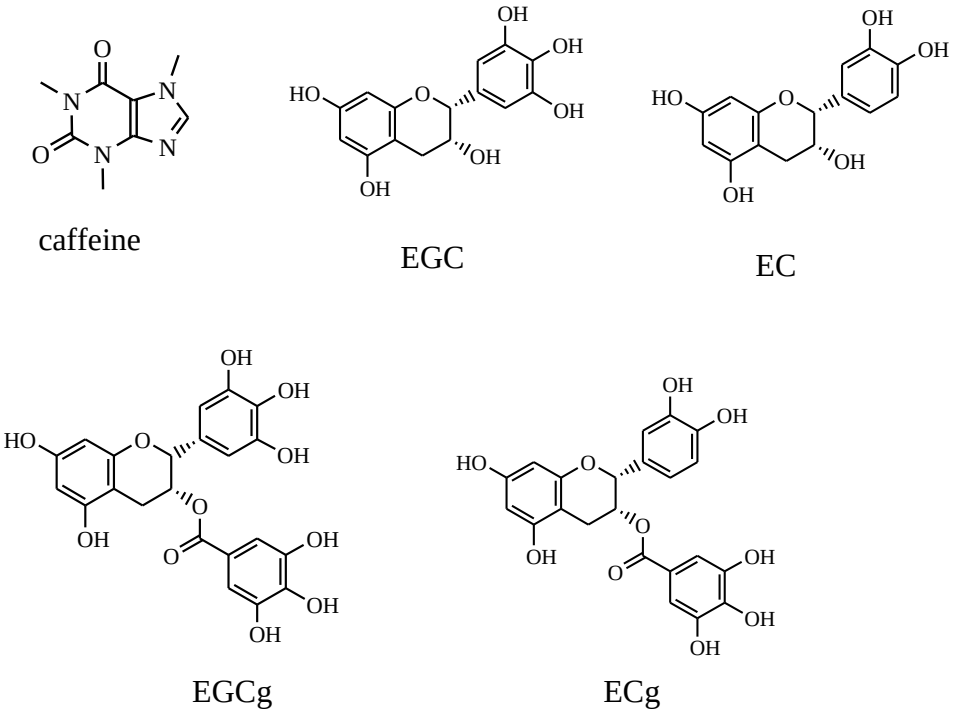

Fig. 3.1 Chemical structure of green tea components

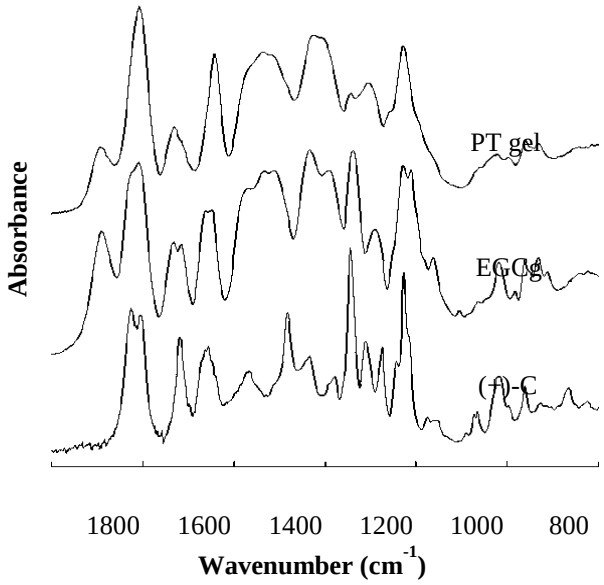

Fig. 3.2 IR spectra of the PT gel and catechin

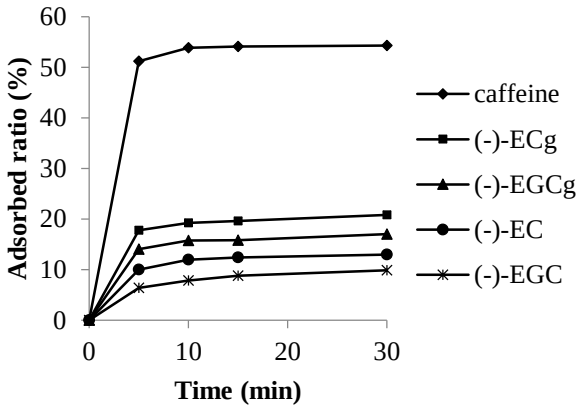

Fig. 3.3 Adsorption of caffeine and catechins on the PT gel: initial concentration of caffeine and catechins of 100 ppm, gel dose of 1 g/20 mL, contact time of 30 minutes, temperature of 30 ^{o}C

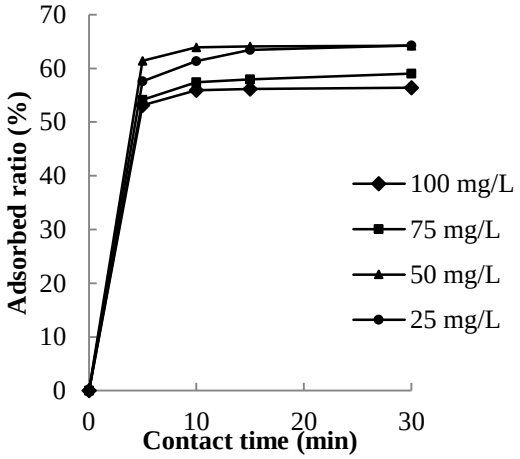

Fig. 3.4 Effect of contact time on the adsorption of caffeine for different initial concentration at 30 ^{o}C, the PT gel dose of 1 g/20 mL

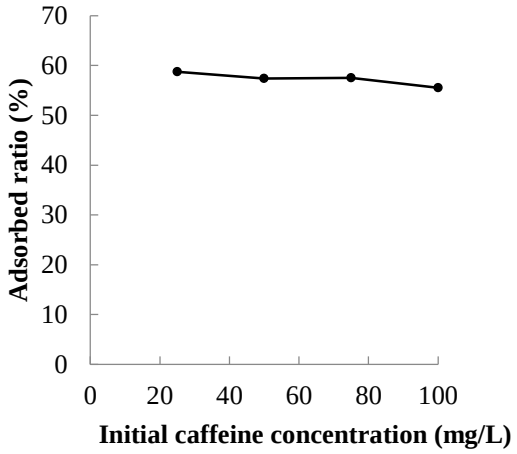

Fig. 3.5 Effect of initial concentration on the adsorption of caffeine at 30 °C, the PT gel dose of 1 g/20 mL, contact time of 30 min

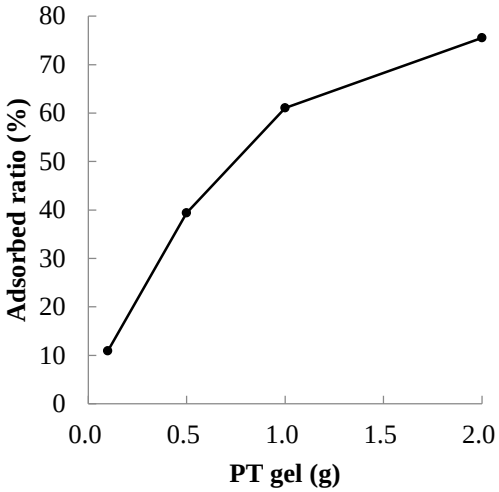

Fig. 3.6 Effect of the PT gel dose on the adsorption of caffeine for initial caffeine concentration of 100 mg/L, contact time of 30 min, and temperature of 30 °C

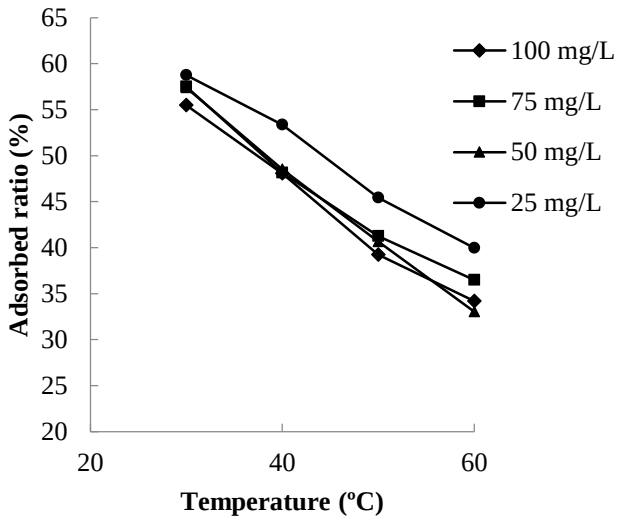

Fig. 3.7 Effect of temperature on the adsorption of caffeine for the initial caffeine concentration of 100 mg/L, the PT gel dose of 1 g/20 mL, and contact time of 30 min

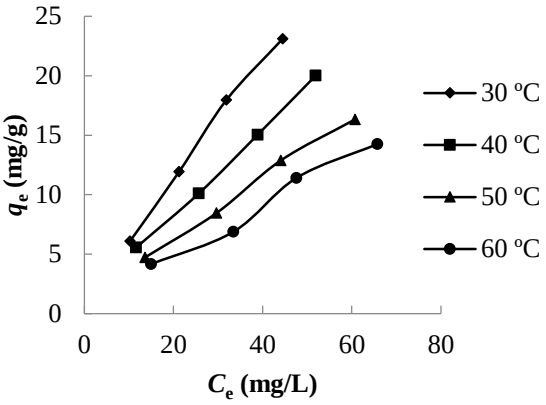

Fig. 3.8 Adsorption isotherm of caffeine on the PT gel

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Chapter 4

Formaldehyde- and Amine-modified Persimmon Tannin and Dyes Adsorption Properties

4.1. Introduction

Organic dyes are widely used as pigments in various industries such as textiles, cosmetics, foods, printing and plastics. Because of their strong color, hazards to human health, high chemical oxygen demands and low biodegradability, the effluents of industrial dyestuffs are the major source of environmental pollution. Therefore, the importance of dye removal is critical during wastewater treatment.

Many techniques have been developed for removing dyes from aquatic environments, including biological, chemical and physical methods. Adsorption techniques have been found to be effective because of their low cost and simplicity. Various activated carbon substrates have been used because of their excellent adsorption properties. However, they are rather expensive. Thus, researchers have studied alternative low-cost adsorbents with high adsorption properties [1].

Natural materials are potential low-cost and eco-friendly adsorbents [2]. A promising class of adsorbents are those prepared from tannins. Tannins are polyphenolic compounds, widely distributed in many plant species, where they serve as defense mechanisms against predators. They form complexes and precipitates with macromolecules such as proteins, lipid, polysaccharides, and heavy metals [3][4][5].

Tannins are water-soluble compounds, which restrict their practical application as adsorbents in aqueous systems. This can generally be overcome through tannin gelation by crosslinking, or immobilizing tannins into water-insoluble matrices [6]. Various methods have been reported for tannin gelation. Most of them involve formaldehyde or other aldehydes in basic or acidic media [7][8][9]. Other researchers have reported acidic gelation ([10] and autoxidation processes [11].

In Chapter 3, we demonstrated the removal of caffeine, a typical purine base, from green tea infusion using PT gels. They were prepared by autoxidation process of a soluble

PT solution [12]. The gel showed a selective adsorption for basic compounds, but it required considerable time for its preparation. Herein, we extended our work by reporting the preparation of a PT gel formed with formaldehyde in acidic conditions. The adsorption properties of the gel for basic dyes such as methylene blue (MB), a basic/cationic dye, are studied. To enhance adsorption properties of the PT gel for an acidic/anionic dye, such as remazol brilliant orange 3R (RBO), PT gel was also modified by the aminomethylation reaction [13].

4.2. Experimental

4.2.1. Materials and reagents

MB and RBO were obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan and DyStar Singapore Pte. Ltd., respectively. Chemical structures are shown in Fig. 4.1. Soluble PT (*kaki-shibu*) was given by Kakitafu Co. Ltd. (Osaka, Japan). Formaldehyde (37%) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ammonium chloride was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other chemicals used were commercial products of analytical reagent grade. Ultra-pure water (Arium 611UV system, Sartorius, Göttingen, Germany) was used throughout this study.

4.2.2. Reaction of model compound with formaldehyde

Various concentration of formadehyde (0.5-25 eq) were mixed with 0.5 mmol compound model (phloroglucinol, pyrogallol, ethyl gallate). The mixture was added with 0.06 N HCl. The mixed solution was stirred at 250 rpm for 2 hours, and left overnight. The resulted gel was crushed and washed with 20-30 mL ultra-pure water.

4.2.3. Electron density calculation

Electronic state of phloroglucinol, pyrogallol, and ethyl gallate was calculated using semi-empirical molecular orbital method (PM5, CAChe MOPAC 2002).

4.2.4. PT gel preparation

4.2.4.1 Preparation of formaldehyde-modified PT gel

PT solution was gelled by the crosslinking reaction with formaldehyde at room temperature. Various concentrations of formaldehyde (0.02-20%) were reacted with 20 mL of 5% PT solution. The mixture was diluted to 40 mL of ultra-pure water and then added 0.4 mL of HCl solution. The mixed solution was stirred at 250 rpm for 2 hours, and left overnight. The obtained gel was crushed and washed with ultra-pure water until the supernatant was clear. The gel was stored in a brown bottle in a wet state, and was termed formaldehyde-

modified persimmon tannin (FPT) gel. The same batch of FPT gel was used in all adsorption experiments.

4.2.4.2. Preparation of amine-modified PT gel

2 % of formaldehyde solution was mixed with 0.66 g of ammonium chloride. Another mixture was prepared with a reagent amount of 100 times less than the previous mixture. The mixture was diluted with 10 mL and ultra-pure water. The mixture was stirred at 250 rpm for 1 hour and then 10 mL were added to 5% PT solution. Then, the mixture was stirred at 250 rpm for 24 hours. The obtained gel was crushed and washed with ultra-pure water until the supernatant was clear. The gel was stored in a brown bottle in a wet state, and named amine-modified persimmon tannin (APT) gel.

4.2.5. Characterization of gels

Infrared (IR) spectra of dried FPT and APT gels were recorded in a Horiba FT-720 Fourier transform infrared (FT-IR) spectrometer (Horiba, Kyoto, Japan). FT-IR measurements were recorded with KBr discs, and the spectra averaged was over 20 scans at a 4 cm^{-1} resolution. Elemental analysis was carried out for the FPT gel and the APT gel with the highest adsorbed ratio using Micro Corder JM10 (J-Science Lab. Co., Ltd., Japan).

4.2.6. Adsorption experiments

Batch method was used to study the adsorption behavior of FPT gel. Adsorption experiments were conducted to study the effect of initial pH, FPT gel dose, contact time, initial dye concentration and temperature. Experiments were carried out in a 20 mL screw cap tube. This contained 0.03 g of FPT gel dose per 20 mL solution, an initial dye concentration of 75-175 mg/L, and pH values of 3-11. The pH was adjusted using 0.1 M HCl and 0.1 M NaOH solutions. The mixture was agitated at 250 rpm at temperatures of 30 °C. The contact time varied over a range of 0-90 minutes. After that, the mixture was centrifuged, the absorbance of the supernatant was recorded using a Shimadzu UV-2500 spectrophotometer (Shimadzu Corp., Kyoto, Japan), using quartz cuvettes with 10 mm path lengths.

The same experimental set up was carried out for obtaining the adsorption behavior of RBO in APT gel. However, initial dye concentration varied over a range of 100-200 mg/L.

The adsorbed ratio (%) and the amount of dye adsorbed on the PT gel, q_e (mg/g), were calculated as Eqn. (3.1) and Eqn. (3.2). Equilibrium data were analyzed using the Langmuir and Freundlich isotherm equations, and characteristics parameters for each isotherm were determined.

4.3. Results and Discussion

4.3.1. Reactivity of catechin unit

Reactivity of A, B, and G ring of catechin unit with formaldehyde was verified using model compound which similar with A, B, and G ring. They are phloroglucinol, pyrogallol, and ethyl galate, respectively. The results are demonstrated in Fig. 4.2.

As shown in Fig. 4.2, phloroglucinol form precipitation with formaldehyde while ethyl gallate is not. Whereas pyrogallol was precipitated by 23 eq. formaldehyde. It indicated that A ring of catechin unit is the most reactive towards formaldehyde.

4.3.2. Electron density

Reactivity can be predicted by electron density. In this experiment, electron density was calculated using semi-empirical molecular orbital method (PM5, CAChe MOPAC 2002). The results show that electron density of carbon in phloroglucinol is the highest, as shown in Fig. 4.3. It indicated that A ring of catechin unit is the most susceptible to electrophilic attack.

4.3.3. Characterization of gels

Fig. 4.4 compares IR spectra of freeze-dried PT with those of FPT gels prepared from different concentration of formaldehyde solutions. Several IR bands characteristic of polyphenols can be distinguished. The hydrogen-bonded O-H stretching vibration of the phenolic group is seen as a broad intense band at ca. 3300 cm-1. The C=O stretching vibration of galloyl group is observed at ca. 1700 cm-1. Aromatic C=C stretching modes dominate the 1400-1600 cm-1 region. The mixed C-O stretching and O-H bending vibrations are observed at 1150-1350 cm-1. The aromatic C-H bending vibration at 1030 cm-1 (denoted by an arrow) in the spectrum of freeze-dried PT exhibits a decreasing intensity in the spectra of FPT gels, as the formaldehyde content in the gels increases. This finding indicates that the cross-linkings by formaldehyde occur at the aromatic rings. This is supported by result in sub-section 4.3.1 and 4.3.2.

Fig. 4.4 also shows IR spectra of APT gels ((f)-(g)). In the APT gel spectra, shoulders are newly observed around 2300 cm^{-1} and 1070 cm^{-1} which are assigned to the N-H stretching and C-N stretching bands, respectively. This observation indicates the existence of the aminomethyl group in the APT gels. Intensities of the shoulders increase for the APT gels with the increasing amount of ammonium chloride. This finding also supports the aminomethylation in the APT gels. Decrease in the intensity of the aromatic C-H bending

vibration at 1030 cm^{-1} is also observed in APT gel spectra, suggesting the cross-linkings at the aromatics rings similar to the FPT gels.

The results of the elemental analysis showed a higher nitrogen concentration in the APT gel. (Anal. Found for the FPT gel: H, 4.53; C, 50.84; N, 0.07 compared to Anal. Found for the APT gel: H, 4.93; C, 47.68; N,2.93). This result support the aminomethylation of the phenolic OH groups in the APT gel.

4.3.4. Comparison of adsorption capacity of FPT and APT gel

The observed adsorbed ratio of MB is higher for the FPT gel (58.4 %*) than the APT gel (2.3 %**) (Initial MB concentration: 100 mg/L; contact time: 60 min; temperature: 30 ^{0}C; gel dose: *0.1 g, **0.05g; volume of MB solution: 20 mL; pH: natural). MB has a larger adsorbed ratio for the FPT gel than RBO. This is due to the positive charges of MB that can interact with the electronegative phenolic OH moieties of FPT gel, while RBO, an anionic dye, has repulsion with those moieties. On the contrary, the adsorbed ratio of RBO is higher in APT gel (96.6 %) than in FPT gel (3.3 %) (Initial RBO concentration: 100 mg/L; contact time: 60 min; temperature: 30 ^{0}C; gel dose: *0.1 g, **0.05g; volume of RBO solution: 20 mL; pH: natural). Electrostatic attraction works between the APT gel and RBO, while MB experiences repulsion with APT gel. Thus, the aminomethylation of the PT gel enhanced its adsorption to acidic dyes. These results showed that the FPT gel is an effective adsorbent for cationic dyes, whereas the APT gel is an effective adsorbent for anionic dyes.

We also examined the adsorbed ratio of MB on the FPT gels prepared using various formaldehyde concentrations. We found that the higher concentration of formaldehyde, the smaller the adsorbed ratio. When the higher concentration of formaldehyde is used, the more cross-linkages are formed, which leads to a decrease in the nucleophilicity of the phenolic rings and so the adsorption capability of the gel to MB. This is supported by the FT-IR spectra shown in the Fig. 4.4 ((b)-(e)).

For APT gel, the degree of the aminomethylation on the PT gel is higher when the higher concentration of ammonium chloride is used. These results are also consistent with FTIR analysis as shown in Fig. 4.4 ((f)-(g)). Thus, the higher concentration of ammonium chloride, the higher adsorbed ratio of RBO on APT gel.

4.3.5. Effect of pH on dyes adsorption

Fig. 4.5 shows the effect of pH in MB adsorption in FPT gel and as well as in RBO adsorption in APT gel. Adsorption of MB in FPT gel increases slightly as pH solution increases, and increases dramatically at pH > 10. Fig. 4.1 shows that PTs are composed of

various catechin units carrying the A, B and gallate phenolic rings. The pK_a (acid dissociation constant) values of these phenolic groups are reported within a range of 7.6 to 11.2 [14]. Thus, the slight increase in adsorption at pH < 10 corresponds to the gradual dissociation of catechin protons. At higher pH, the number of negatively charged OH^- sites increases, and enhances adsorption of positively charged dye cations through electrostatic attraction [15].

At low pH values, H^+ ions occupy most of the adsorption sites on the APT gel surface and, consequently, more RBO dye was adsorbed because of the electrostatic attraction in the positively charged surface. At a higher pH, the number of negatively charged sites increased, restricting the adsorption of negatively charged dye anions through electrostatic repulsion.

4.3.6. Effect of contact time and MB initial concentration on MB adsorption

Contact time is an important parameter because it determines the rate of adsorbate removal. Fig. 4.6 shows that MB adsorption occurs rapidly in the first 15 minutes, and equilibrium is attained in ca. 30 minutes. Initially, adsorption is faster, because larger surface area is available for adsorption. The gradual occupancy of these sites leads a slower adsorption [16]. Fig. 4.6 also shows that an increase of initial MB concentration decreases the adsorbed ratio. This is due to the increasing number of MB molecules competing for available binding sites on the FPT gel. Active sites in FPT gel become saturated at higher MB concentrations [17][18]. The same result was also obtained in adsorption of RBO in APT gel, as shown in Fig. 4.7.

4.3.7. The Gibbs free energy change

The Gibbs free energy change of dyes adsorption in PT gel is determined by the classic Van't Hoff equation:

$$\Delta G° = -RT \ln K \tag{4.1}$$

where ΔG^0 is the standard free energy change (kJ/mol), T is the absolute temperature (K), R is gas constant (J/mol.K), and K (L/g) is an equilibrium constant obtained by multiplying the Langmuir constants q_m and K_L [19]. The value of ΔG^0 is used to determine the nature of the adsorption process. The process is spontaneous at a given temperature if the value of ΔG^0 has a negative value.

The value of $\Delta G°$ for physisorption ranges from −20 to 0 kJ/mol, and for chemisorption from −80 to −400 kJ/mol [20][21]. The value of $\Delta G°$ for hydrogen bonding and dipole forces are 2-40 and 2-29 kJ/mol, respectively [22][23]. The determined $\Delta G°$ is -19.5 kJ/mol and -13.2 kJ/mol for MB in FPT gel and RBO in APT gel, respectively. It

indicates that adsorption could be designated as spontaneous physisorption. In addition, it suggests that interaction between the adsorbent and adsorbate is hydrogen bonding with a weakly attractive force. Acidic hydroxyl groups and the nucleophilic phenolic A ring of FPT gel constituent are responsible for these interactions with MB [24][25]. For APT gel, the amine group is responsible for the interaction with RBO.

4.3.8. Adsorption isotherms

Langmuir and Freundlich isotherms were used to evaluate dyes adsorption on the PT gel. Equation of the Langmuir isotherm is given by:

$$q_e = (q_m K_L C_e)/(1 + K_L C_e) \quad (4.2)$$

where q_m is the Langmuir constant relating to complete coverage (mg/g) and K_L is the Langmuir energy constant which indicates adsorptivity of the solute.

Equation of Freundlich isotherm is given as follows

$$q_e = K_F C_e^{1/n} \quad (4.3)$$

where n is the Freundlich isotherm constant related to adsorption intensity and K_F is the Freundlich isotherm constant related to adsorption capacity $(mg/g)(L/mg)^{1/n}$.

The value of n indicates the degree of non-linearity between solution concentration and adsorption. If n is equal to or near unity, adsorption is linear. If n is significantly below unity, the adsorption process is chemical. If n is above unity, adsorption is a favorable physical process [17].

The experimental data of MB adsorption on FPT gel and RBO adsorption on APT gel fit the Langmuir model best, as shown in Fig. 4.8 and Fig. 4.9, respectively. The adsorption isotherm parameters are listed in Table 4.1. Values of n suggest that adsorption occurred via physisorption. It agreed with the thermodynamics analysis. The maximum adsorption capacity was 1102.4 mg/g and 743.3 at 30 °C for MB in FPT gel and RBO in APT gel, respectively. The adsorption capacity of FPT gel to MB was comparable with activated carbon and other reported adsorbents [26][27][28][15]; the same for the adsorption capacity of APT gel to RBO [29][30].

Langmuir constant, K_L, can be used to predict the affinity between adsorbate and adsorbent using a separation factor or dimensionless equilibrium parameter, R_L, as Eq. (3.6).

The R_L values for MB and RBO at a different initial concentration are within the ranges 0.002-0.005 and 0.018-0.036, respectively. These values indicated that adsorption is

favorable. In addition, adsorption of MB in FPT gel is more favorable than adsorption of RBO in APT gel.

4.4. Conclusion

PT was cross-linked with formaldehyde in acidic conditions and the obtained FPT gel was employed in adsorption experiments of MB from aqueous solution. Likewise, PT was modified with amine and employed to adsorb RBO from aqueous solution.

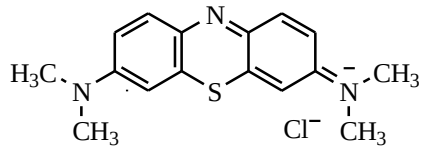
Adsorption of dyes in PT gel was influenced by pH, contact time, and initial dyes concentration. Adsorption of MB in FPT gel is higher at a higher pH, while adsorption of RBO in APT gel is higher at a lower pH. The adsorbed ratio of both, MB in FPT gel and RBO in APT gel increased overtime and attained equilibrium at 30 minutes. The adsorbed ratio for both increased as the initial concentration decreased.

The measured adsorption isotherm for MB adsorption on FPT gel and RBO adsorption on APT gel fitted better to the Langmuir model. The maximum adsorption capacity was 1102.4 mg/g and 743.3 at 30 °C for MB in FPT gel and for RBO in APT gel, respectively. Adsorption of both occurred via favorable physisorption.

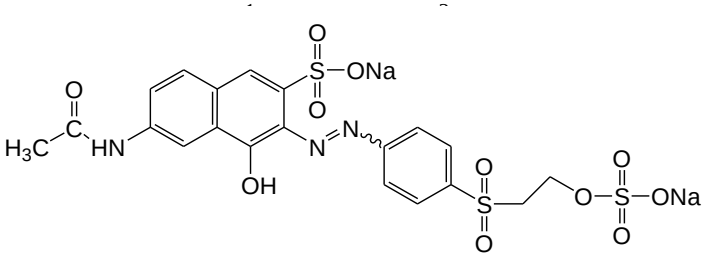
The $\Delta G°$ value indicated that adsorption of dyes in PT gel was a spontaneous physisorption. The interaction between adsorbent and adsorbate is hydrogen bonding with a weakly attractive force. FPT gel is an effective adsorbent for basic/cationic dyes, whereas APT gel is an effective adsorbent for acidic/anionic dyes from aqueous solution.

Table 4.1. Isotherm parameters for dyes on PT gel

Isotherm model	Parameter	Dyes adsorption on PT gel	
		MB on FPT gel	RBO on APT gel
Freundlich	n	36.6	10.8
	K_F $(mg/g)(L/mg)^{1/n}$	964.5	475.0
Langmuir	q_m (mg/g)	1102.4	743.3
	K_L (L/mg)	2.6	0.27



(a)



(b)

Fig. 4.1 Chemical structure of (a) MB and (b) RBO

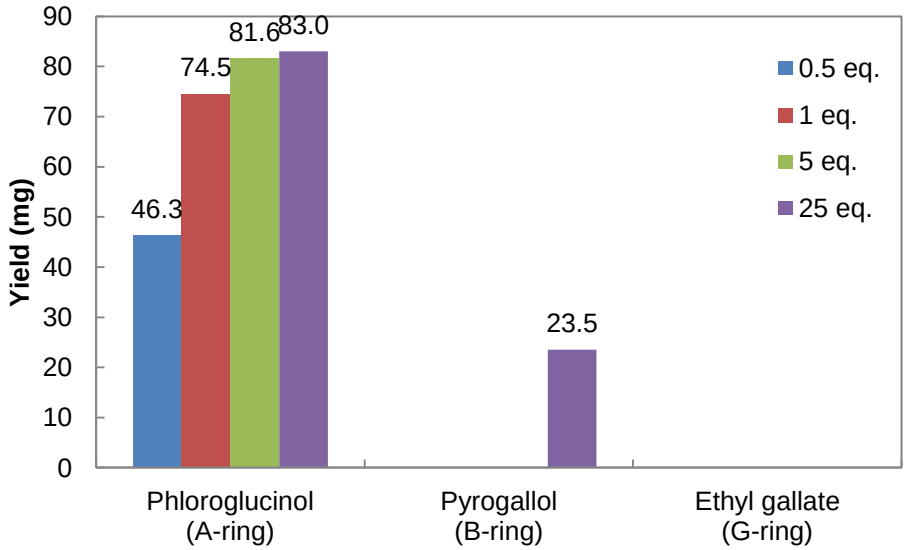


Fig. 4.2 Yields of the reaction of model compounds with formaldehyde.

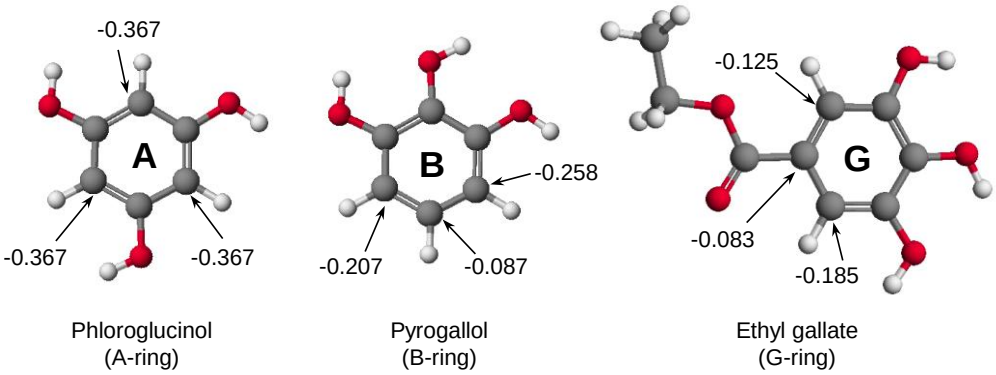


Fig. 4.3. Electron density of model compounds

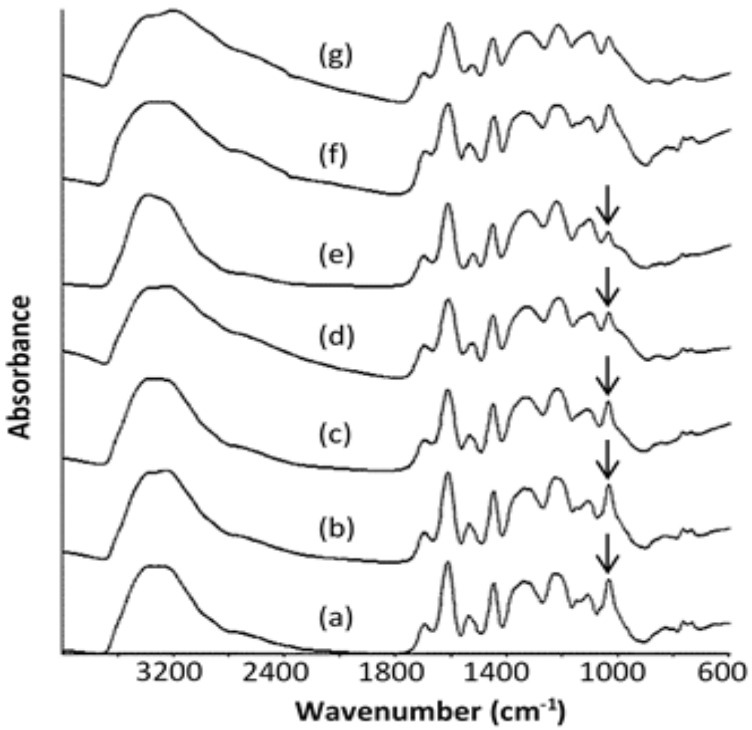


Fig. 4.4 IR spectra of (a) freeze-dried PT, (b)-(e) FPT gels prepared from 0.02, 0.2, 2.0 and 20% concentration formaldehyde solutions, respectively, (f) APT gels prepared from 0.02% formaldehyde solution and 6.6 mg ammonium chloride, and (g) APT gels prepared from 2.0% formaldehyde solution and 0.66 g ammonium chloride

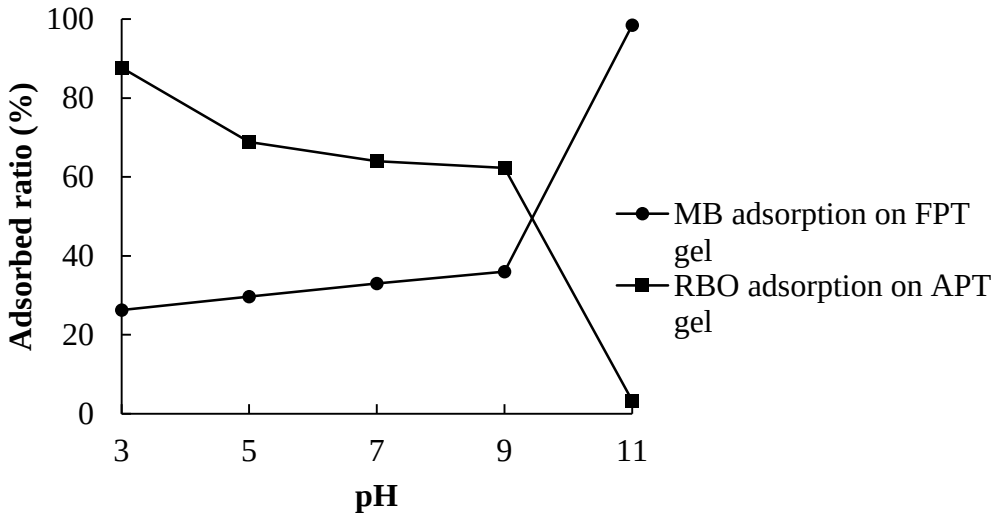


Fig. 4.5 Effect of pH on FPT adsorption for MB and APT adsorption for RBO. Initial dyes concentration: 100 mg/L; FPT and APT gel dose: 0.05 g/20 mL and 0.03 g/20 mL solution, respectively; contact time: 60 min, temperature: 30 °C

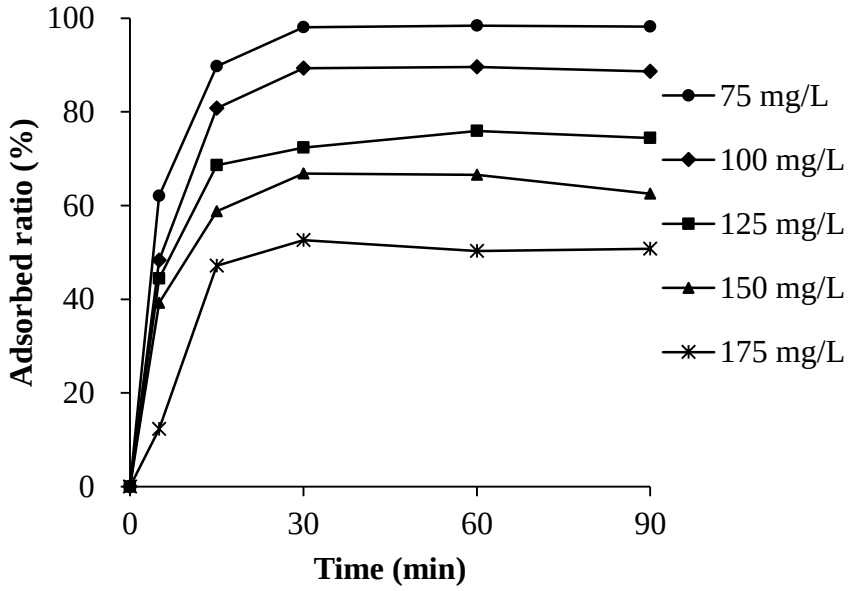


Fig. 4.6 Effect of contact time and initial MB concentration on MB adsorption, for differing initial MB concentrations. pH: 11; FPT gel dose: 0.03 g/20 mL solution; temperature: 30 °C

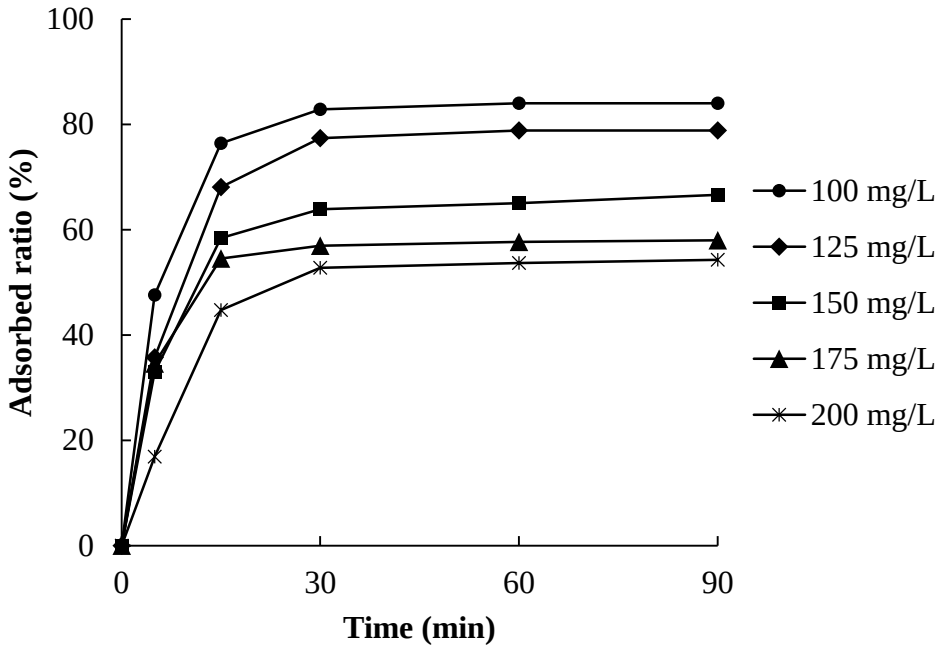


Fig. 4.7 Effect of contact time and initial RBO concentration on RBO adsorption, for differing initial RBO concentrations. pH: 3; APT gel dose: 0.03 g/20 mL solution; temperature: 30 °C

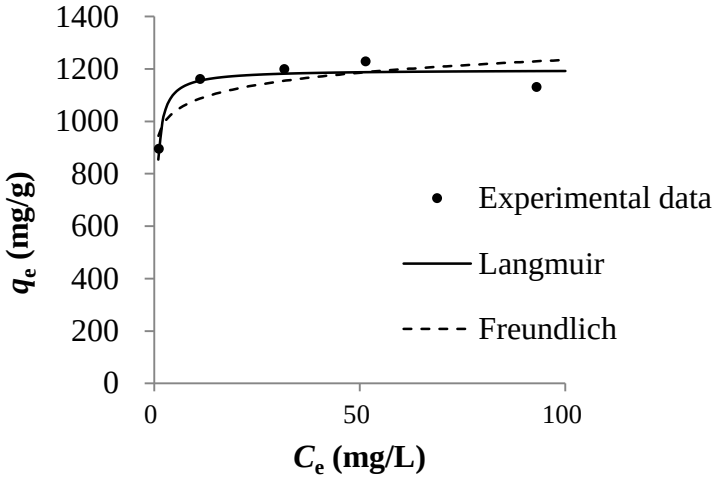


Fig. 4.8 The fit of the experimental data of MB adsorption on FPT gel to the Langmuir and Freundlich models. pH: 11; FPT gel dose: 0.03 g/20 mL solution; contact time: 60 min; temperature: 30 °C

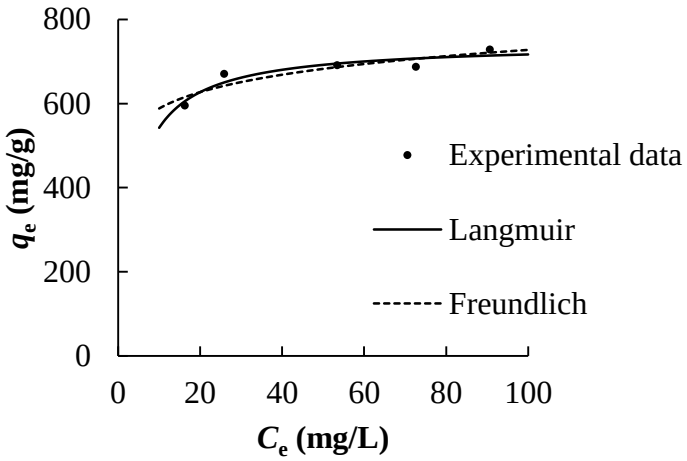


Fig. 4.9 The fit of the experimental data of RBO adsorption on APT gel to the Langmuir and Freundlich models. pH: 3; APT gel dose: 0.03 g/20 mL solution; contact time: 60 min; temperature: 30 °C

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Chapter 5

Immobilization of Persimmon Tannin on Cellulose and Caffeine Adsorption Properties

5.1. Introduction

Tannin adsorbent can be prepared by immobilization of tannins into a water insoluble matrix [1] [2]. In recent years, great efforts have been made in immobilizing tannins onto various water –insoluble matrices such as agarose, viscose rayon fiber [3], cellulose [2], and other matrices [4]. However, none of them have reported adsorbents made of an organic, eco-friendly material without crosslinkers, a non-soluble core, and simple elaboration.

The preparation and characterization of persimmon tannin gel by immobilization on cellulose viscopearls is reported in this work. The adsorption properties of persimmon tannin immobilized on cellulose viscopearls (PTIC) for caffeine were also studied. The effect of different parameters such as different PTIC gel samples (day 1, day 7, day 14, day 28, and day 42), gel dosage, initial caffeine concentration, and contact time were investigated. The equilibrium data on batch adsorption studies were carried out to understand adsorption process. In order to improve immobilization of PT gel on cellulose viscopearls and the adsorption capacity.

5.2. Experimental

5.2.1. Materials and reagents

Caffeine was purchased from Nacalai Tesque (Kyoto, Japan). Soluble PT (*kaki-shibu*) was obtained from Flium Kakitafu Co. Ltd. (Osaka, Japan). Cellulose beads (Viscopearl-A) were obtained from Rengo. Ultra-pure water (Aurium 611UV system, Sartorius, Germany) was used in this study.

5.2.2. Preparation of persimmon tannin immobilized on cellulose viscopearls

As much as 600 mL of 5% PT solution was added with 200 ml (w/v) of cellulose spheres Viscopearls-A into the flask at room temperature. After, the resulting mixture was stirred slowly during all experimental set up, and aged for 42 days to compare PT immobilization. Then, 100 ml of sample was taken up during this experiment (day 1, day 7, day 14, day 28, day 42 samples) and washed with water several times until the solution was

clear. After that, an amount of PTIC was stored under a wet state for carrying out batch adsorption experiments, while the rest was dried in a dessicator for their characterization.

5.2.3. Spectroscopic measurements

Infra-red (IR) analysis of dried PTIC were recorded on a Horiba FT-720 Fourier transform infrared (FT-IR) spectrometer (Horiba, Kyoto, Japan). FT-IR measurements were carried out by KBr method at 20 scans per spectrum with 4 cm^{-1} resolution.

5.2.4. Adsorption experiments

Batch adsorption studies were conducted to investigate the adsorption behavior of the PTIC gels. Adsorption experiments were performed to study the effect of various parameters such as different PTIC gel samples (day 1, day 7, day 14, day 28, day 42), gel dosage, initial caffeine concentration, and contact time. Experiments were carried out in a 20 mL screw cap tube container with caffeine solution containing different PTIC gel samples. The different PTIC gels were tested using 0.25 mg of day 1, day 7, day 14, day 28, and day 42 sample with 50 mg/L of caffeine solution. PTIC gel dosage was varied with 0.25 g and 1 g of day 42 sample with a caffeine solution concentration of 50 mg/L. To evaluate the effect of initial caffeine solution concentration of 25 to 150 mg/L, different PTIC gel samples (day 1, day 7, day 14, day 28, day 42) were used. All mixtures were agitated manually a temperature of 30 °C where contact time varied over a range of 0-60 minutes. Then, the mixture was centrifuged, the absorbance of the supernatant was recorded using a Shimadzu UV-2500 spectrophotometer (Shimadzu Corp., Kyoto, Japan), using quartz cuvettes with 10 mm path lengths.

All the experiments were performed in duplicate. After the equilibrium, the final concentration C_t was measured. The percentage removals of caffeine solution adsorbed on the PTIC or adsorbed ratio (%) was calculated using the Eqn. (3.1). Equilibrium adsorption capacity q_e(mg/g) was calculated using the Eqn. (3.2). The equilibrium data were analyzed using the Langmuir and Freundlich isotherms, and characteristic parameters for the isotherm were determined.

5.3. Results and Discussion

5.3.1. IR spectral characterization of FPT gel

Fig. 5.1 compares IR spectra of PTIC gel with those of cellulose viscopearl and freeze-dried PT. Subtraction of cellulose viscopearls spectrum from day 42 PTIC spectrum gives Fig. 5.1 (c). Overall spectral pattern Fig. 5.1 (c) is similar to that of freeze-dried PT and IR bands

characteristics of persimmon tannin are distinguished; a broad hydrogen-bound O-H stretching band of the phenolic group around 3300 cm^{-1}, the C=O stretching band of the gallate group around 1700 cm^{-1} and the aromatic C=C stretching bands in the 1400-1600 cm^{-1} region. These findings indicate that PTs are successfully immobilized onto cellulose viscopearl in PTIC gels. However, some differences are also observed between spectra Fig. 5.1 (c) and (d) in the C-O stretching region around 1050 cm^{-1} which suggests interactions between the PT and the cellulose components.

5.3.2. Effect of different PTIC gels and adsorbent dosage in caffeine adsorption

In order to verify the effect of different PTIC gels on caffeine adsorption, batch adsorption experiments with 0.25 g dosage of sample, and initial caffeine concentration at 50(mg/L) were carried out for control and all samples (day 1, day 7, day 14, day 28, day 28). Fig. 5.2 showed the highest adsorbed ratio at day 28 of PTIC gel, and the minimum value at day 1, the rest exhibited values in between. Under the same experimental condition, the adsorbed ratios of day 28 sample is greater almost by 2.5 times. The adsorbed ratio of day 14, day 21, and day 28 PTIC did not differ significantly. Immobilization of PT on cellulose viscopearl started to saturate in day 14, so that an increase of immobilization time will not increase the number of PT that were immobilized on cellulose viscopearls.

Gel dosage data for day 28 was the most representative and the highest value observed. Adsorption ratio values in between (day 7, day 14, day 21) followed a regular trend for caffeine adsorption. Therefore, the adsorption ratios for day 28 sample at 50 (mg/L) and different amount of samples (0.25-1 g) were determined (Fig.5.3). When dosage of sample was increased (1 g) adsorption ratio increased, as well. It can be attributed to the active sites available for adsorption. Due to the adsorption ratio increasing, the amount of sample was also increasing. As expected, for an adsorbent dosage of 1g of day 28 PTIC sample and initial caffeine concentration at 50 (mg/L) the highest adsorbed ratio of 43.3 % was exhibited (Fig. 5.3). Therefore, it represented the best and most suitable experimental condition for caffeine adsorption on PTIC gel.

5.3.3. Effect of contact time and initial concentration on adsorbed ratio of caffeine

Contact time is a parameter that determines the rate of adsorbate removal. The rate of removal was higher in the beginning but it gradually decreased with time until it reached the equilibrium [5]. Fig. 5.4 shows that the rate of removal is higher the first 5 minutes, while equilibrium was attained at 15 min. Adsorption is higher in the beginning due to a larger

surface area of adsorbents available for adsorption of the adsorbates. As these sites become occupied, adsorption became less efficient [6].

Fig. 5.4 also shows that an increase in initial caffeine concentration decreases the adsorbed ratio. This is due to the increase in the number of caffeine molecules competing for available binding sites on the PTIC gel. Thus, the available active sites of the PTIC gel become saturated at higher concentration of caffeine [7].

5.3.4. Estimation of change in Gibbs free energy

Thermodynamic parameters such as change in Gibbs free energy was determined using the classic Van't Hoff equation, Eqn. (4.1). The value of ΔG^0 is used to determine the nature of the adsorption process. The process occurs spontaneously at a given temperature if the value of ΔG^0 has a negative value. The determined $\Delta G°$ is -4.1 kJ/mol.

The $\Delta G°$ for physisorption ranges from −20 kJ/mol to 0 kJ/mol and for chemisorption, it ranges from −80 kJ/mol to −400 kJ/mol [8][9]. The values of $\Delta G°$ indicated that the adsorption can be designated as spontaneous physisorption. The $\Delta G°$ for hydrogen bonding and dipole force are 2 − 40 kJ/mol and 2 − 29 kJ/mol, respectively ([10][11][12]. The results suggest that the interaction between the adsorbent and the adsorbate is hydrogen bonding with a weak attractive force.

5.3.5. Adsorption isotherms

Equilibrium data, known as adsorption istherms, are basic parameters for the design of adsorption systems. In order to calculate the adsorption capacity of PTIC gels, the experimental data were fitted to the Linearized Langmuir isotherm and Linearized Freundlich isotherm, Eqn. (3.4) and Eqn. (3.5), respectively.

Fitting the data with the Langmuir and Freundlich equations resulted in high correlation coefficients, varying from 0.99 to 1.00. This indicates that the PTIC gel surface is homogeneous and coverage of caffeine on the outer surface of the PTIC gel is a monolayer [13][12]. The maximum adsorption capacity of a monolayer was 48.3 mg/g for the PTIC gel. The isotherm parameters of adsorption are listed in Table 5.1.

5.4. Conclusion

In this study, PT was successfully immobilized on cellulose viscopearls in an aqueous condition having adsorbed caffeine from aqueous solution. Adsorption was influenced by various parameters such as different PTIC gel sample (day 1, day 7, day 14, day 28, and day 42), gel dosage, initial caffeine concentration, and contact time. Adsorbed ratio increased, as

gel dosage increased. Otherwise, adsorbed ratio increased as initial caffeine concentration decreased.

The Linearized Langmuir and Freundlich adsorption isotherm models were used to describe the adsorption equilibrium of caffeine solution onto the PTIC gel. The adsorption isotherms follow both the Langmuir and Freundlich models. The maximum adsorption capacity was found to be 48.3 mg/g at 30°C for the PTIC gel. Adsorption of caffeine on the PTIC is a favorable physisorption process. According to these results, day 28 PTIC sample, with 1g of adsorbent dosage, and an initial caffeine concentration of 50 (mg/L) , demonstrates that the adsorption capacity is improving.

FTIR analysis confirmed that PT was successfully immobilized onto cellulose viscopearl. Since Persimmon Tannin gel is an important source of condensed tannins, adsorption ratio experiments confirmed that this method provides an eco-friendly way for removing caffeine from the food industry without crosslinkers and carrying out a simple preparation.

Very good caffeine adsorption resulted in the PTIC gel samples relative to the reference control viscopearls. It was proved that the cellulose viscopearls acted as a useful and suitable insoluble matrix to immobilized PT gels, instead of using crosslinkers.

These results indicated that PTIC gels can become leading and environment-friendly organic adsorbed candidates in food industry. The adsorbent is expected to be an effective alternative for removing caffeine from tea production.

The determination of $\Delta G°$ value indicated that adsorption of caffeine in the PTIC gel was a spontaneous physisorption process. The interaction between adsorbent and adsorbate is hydrogen bonding with a weakly attractive force.

Table 5.1. Isotherm parameter for caffeine adsorption on the PTIC gel

Isotherm model	Parameter	Value
Freundlich	n	0.6
	K_F (mg/g)(L/mg)$^{1/n}$	1.8
	r^2	1.00
Langmuir	q_m (mg/g)	48.3
	K_L (L/mg)	0.1
	r^2	0.99

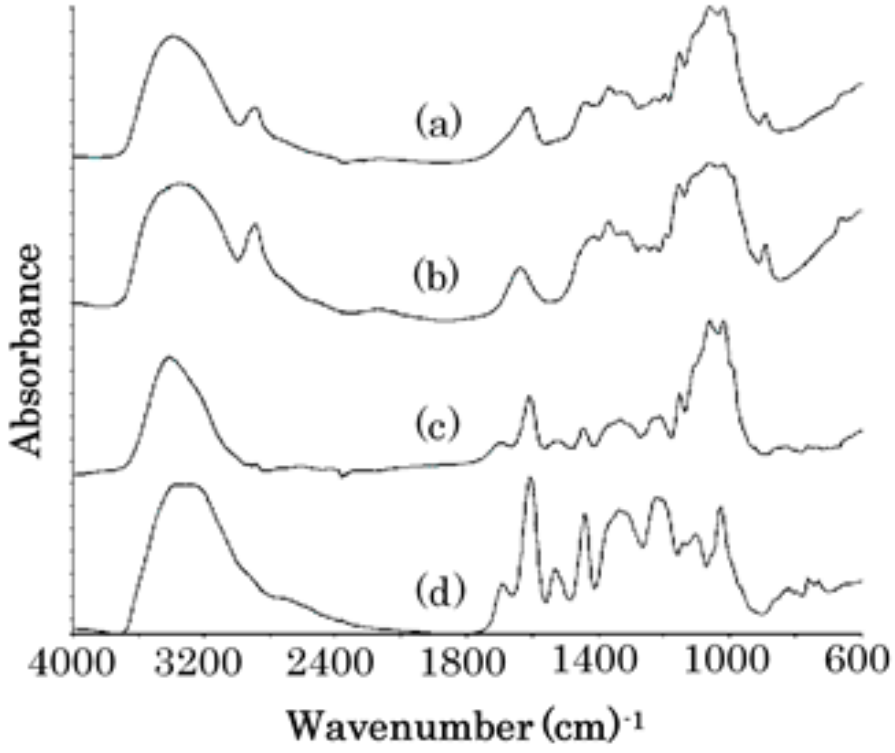

Fig. 5.1 IR spectra of (a) day 42 PTIC gel, (b) cellulose viscopearl, (c) subtraction of (b) from (a), (d) freeze-dried PT

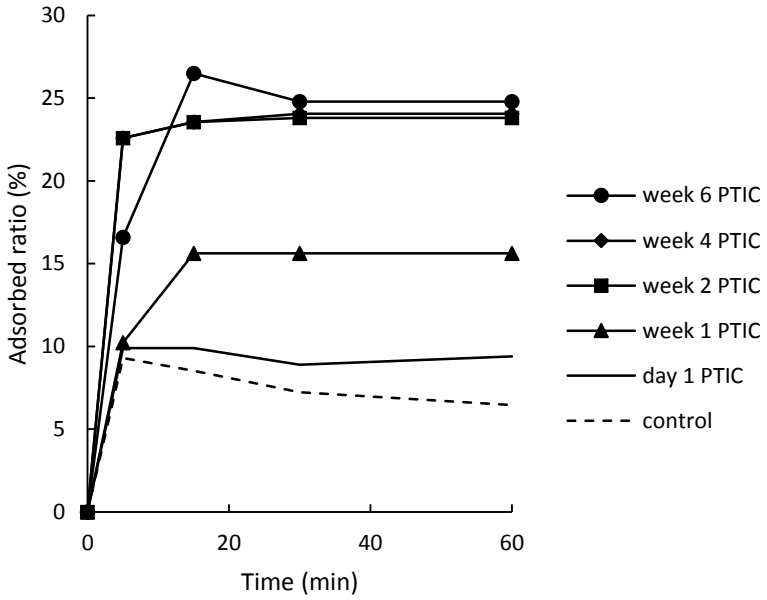

Fig. 5.2 Adsorbed ratio of day 1, week 1, week 2, week 4, and week 6 PTIC gel and cellulose viscopearl (control) at 30 °C, 50 mg/L of caffeine solution, and 0.25 g of adsorbent dosage

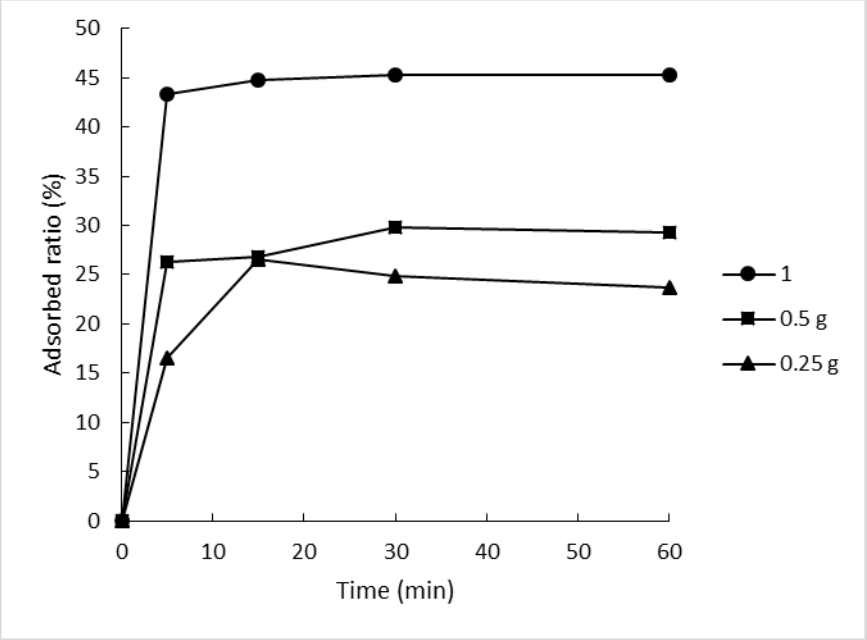

Fig. 5.3 Effect of day 42 week PTIC gel dose on adsorption of caffeine at 30° C and initial concentration of 50 mg/L.

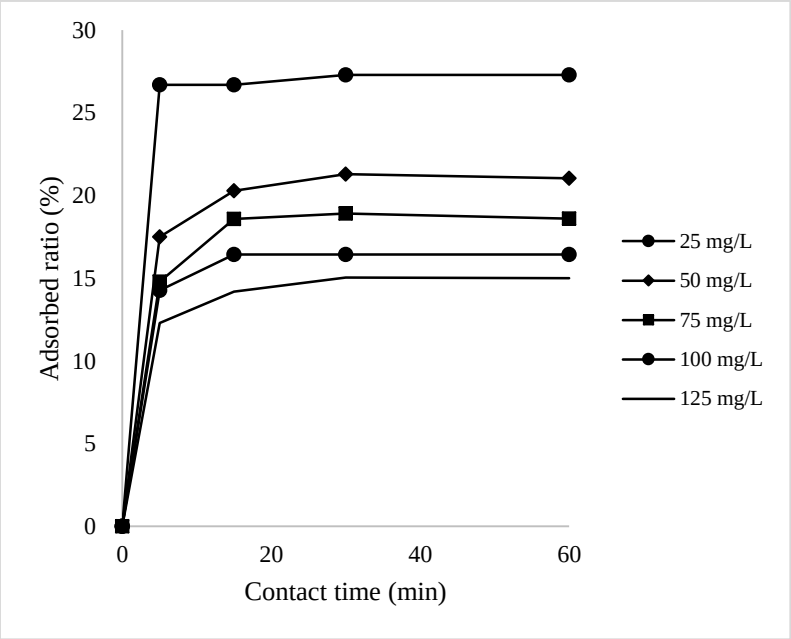

Fig. 5.4 Effect of contact time on the adsorption for different initial concentrations of caffeine at 30°C and day 42 PTIC gel dose of 0.25g/ 20 mL

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Chapter 6
General Conclusion

In this study, PT gel was prepared by three methods such as autoxidation using oxygen, modification using formaldehyde and amine, and immobilization of PT on cellulose.

In chapter 3, we prepared PT gel by autoxidation. PT was gelated by applying oxygen gas and natural light to the aqueous PT solution without using any harmful reagents and catalysts. Adsorption behaviors of the PT gel were tested for caffeine. In this experiment, the PT gel has been prepared by autoxidation process. The PT gel employed to adsorb caffeine. The PT gel effectively adsorb caffeine from aqueous solution. Adsorption of caffeine on the PT gel was influenced by contact time, initial concentration of caffeine, adsorbent dose, and temperature. Equilibrium was reach for 15 min. Adsorbed ratio increased with decreasing caffeine concentration and temperature. Otherwise, adsorbed ratio increased with increasing adsorbent dose. The adsorption isotherms follow both Langmuir and Freundlich model. The maximum adsorption capacity was found to be of 65.8 mg/g. Adsorption of caffeine on the PT gel is favorable physisorption. Value of ΔG^{o} and ΔH^{o} indicated the spontaneous and exothermic nature of adsorption. In addition, interaction between the adsorbent and the adsorbate is hydrogen bonding and weak attractive force. These data suggest that the PT gel can be used as an effective adsorbent to remove caffeine from green tea drinks.

In chapter 4, gelation was performed by modification of PT. In this method, two types of PT gels were prepared by modification of PT using formaldehyde and amine compound. Their adsorption to dyes in aqueous solution was studied. PT was cross-linked with formaldehyde in acidic conditions and the obtained FPT gel was employed in adsorption experiments of MB from aqueous solution. Likewise, PT was modified with amine and employed to adsorb RBO from aqueous solution. Adsorption of dyes in PT gel was influenced by pH, contact time, and initial dyes concentration. Adsorption of MB in FPT gel is higher at a higher pH, while adsorption of RBO in APT gel is higher at a lower pH. The adsorbed ratio of both, MB in FPT gel and RBO in APT gel increased overtime and attained equilibrium at 30 minutes. The adsorbed ratio for both increased as the initial concentration decreased. The measured adsorption isotherm for MB adsorption on FPT gel and RBO adsorption on APT gel fitted better to the Langmuir model. The

maximum adsorption capacity was 1102.4 mg/g and 743.3 at 30 °C for MB in FPT gel and for RBO in APT gel, respectively. Adsorption of both occurred via favorable physisorption. The $\Delta G°$ value indicated that adsorption of dyes in PT gel was a spontaneous physisorption. The interaction between adsorbent and adsorbate is hydrogen bonding with a weakly attractive force. FPT gel is an effective adsorbent for basic/cationic dyes, whereas APT gel is an effective adsorbent for acidic/anionic dyes from aqueous solution.

Last, in the chapter 5, PT was immobilized on cellulose Viscopearls and employed to adsorb caffeine in aqueous solution. In this study, PT was successfully immobilized on cellulose viscopearls in an aqueous condition having adsorbed caffeine from aqueous solution. Adsorption was influenced by various parameters such as different PTIC gel sample (day 1, day 7, day 14, day 28, and day 42), gel dosage, initial caffeine concentration, and contact time. Adsorbed ratio increased, as gel dosage increased. Otherwise, adsorbed ratio increased as initial caffeine concentration decreased. The Linearized Langmuir and Freundlich adsorption isotherm models were used to describe the adsorption equilibrium of caffeine solution onto the PTIC gel. The adsorption isotherms follow both the Langmuir and Freundlich models. The maximum adsorption capacity was found to be 48.3 mg/g at 30°C for the PTIC gel. Adsorption of caffeine on the PTIC is a favorable physisorption process. According to these results, day 28 PTIC sample, with 1g of adsorbent dosage, and an initial caffeine concentration of 50 (mg/L) , demonstrates that the adsorption capacity is improving. FTIR analysis confirmed that PT was successfully immobilized onto cellulose viscopearl. Since Persimmon Tannin gel is an important source of condensed tannins, adsorption ratio experiments confirmed that this method provides an eco-friendly way for removing caffeine from the food industry without crosslinkers and carrying out a simple preparation. Very good caffeine adsorption resulted in the PTIC gel samples relative to the reference control viscopearls. It was proved that the cellulose viscopearls acted as a useful and suitable insoluble matrix to immobilized PT gels, instead of using crosslinkers. These results indicated that PTIC gels can become leading and environment-friendly organic adsorbed candidates in food industry. The adsorbent is expected to be an effective alternative for removing caffeine from tea production. The determination of $\Delta G°$ value indicated that adsorption of caffeine in the PTIC gel was a spontaneous physisorption process. The interaction between adsorbent and adsorbate is hydrogen bonding with a weakly attractive force.

However, before preparation of PT gel, characterization of PT was carried out in chapter 2. PT is composed of catechin units (EC, EGC, ECg, EGCg). The PT contained very high molecular weight components where gallated catechins content was estimated to be about 40%. gallate catechins content phloroglucinol decomposition method and GPC measurement also. It suggested that the oxidative polymerization of the PT is in progress after the production and manufacturing process of persimmon. In addition, it is supported by Folin-Ciocalteu test and elemental analysis where autoxidation occurred in PT.

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