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Phenylcarbamate-Modified Paper for Paper Chromatographic Analysis of Hydrophobic Compounds

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Abstract: Paper chromatography is a low-cost and facile analytical method traditionally used to analyze hydrophilic substances. For the application to substances with lower polarity, we prepared a stationary phase based on filter paper modified with phenyl isocyanate (PI-FP), bearing phenyl carbamate moieties for hydrophobic, π - π , and electrostatic interactions. The preparation and chromatographic methods were established by selecting papers, comparing different chemical structures, optimizing the modification procedure, investigating eluents, and quantitatively parameterizing the separation behavior based on the character of the analytes. PI-FP exhibited better separation performance than esterified FPs and enabled chromatographic analysis of various dyes with both positive and negative $\log P$ (calculated water-octanol partition coefficient). We also demonstrated an application of this system for a preparative separation of dyes using thread-like paper modified with PI.

Keywords: paper chromatography; separation; carbamate; urethane; hydrophobic; modified paper



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1. Introduction

Paper chromatography is a low-cost and very facile analytical method traditionally used. No expensive instruments are necessary for chromatographic separation and analysis, and paper chromatography was used in the dawn of chromatography [1]. However, the low separation efficiency and limited range of analytes are the critical disadvantages, and the position of paper chromatography has been almost superseded by other chromatographic analyses like thin-layer chromatography (TLC).

Developments of microfluidic paper-based analytical devices (μ PADs) and combinations of paper chromatography with other analytical methods are recalling the importance of paper chromatography [2–4]. The importance of rapid and accurate point-of-care testing using μ PADs has rapidly increased due to the recent COVID-19 epidemic requiring mass testing. However, the available analytes are hydrophilic substances due to the high polarity of cellulose, and strategies to expand the analytes are in a high demand.

In TLC and high-performance liquid chromatography (HPLC) using microcrystalline cellulose particles as the stationary phase, chemically modified cellulose supports are developed to enhance the separation abilities and expand the range of analytes. Okamoto et al. developed cellulose stationary phases for enantiomeric separation [5–8] by modification of cellulose with aromatic isocyanates [9]. Various practical stationary phases of modified cellulose have been developed after that [8,10] and are commercialized for HPLC.

The aromatic urethane moieties interact with analytes by the Lewis acidic NH groups, Lewis basic carbonyl groups, aromatic rings for π - π and hydrophobic interactions, and other substituents controlling the electronic character of the aromatic rings and adding further interactions. The modification of cellulose with isocyanates is also effective in other applications requiring affinity with other molecules. Filters for oil-water separation

were developed by modification with hydrophobic polymers through the urethanization of filter papers (FPs) [11,12]. Silanized and urethanized cellulose FPs were applied as extraction phases for synthetic and natural hormones bearing hydrophobic structures [13]. Urethanized cellulose was also applied for reinforcing polymers by the improved affinity with the polymeric matrices [14,15].

Despite the functionalization ability of paper, only a few examples were reported for stationary phases of chemically modified papers for separation, while modifications to enhance detectability were investigated using metals for surface plasmon [16–18]. Non-covalent modifications of paper were carried out with humic acid [19], lignin [20], and a dendrimer [21] to prepare stationary phases for paper-chromatographic separation of ions. Plant oils can be applied for non-covalent preparation of reverse-phase stationary phases for the separation of plant extracts [22]. A paper-based device for the detection of environmental hormones by electrochemical luminescence was developed using paper modified by electrostatic modification with chitosan [23]. These modifications are easy, but the non-covalent nature limits the long-term stability and use of eluents having affinities with the modifiers.

We accordingly focused on covalently modified hydrophobic papers for paper chromatography as applied in HPLC. Stationary phases of modified papers require sufficient modification without collapsing their shape. In this study, we established the preparation and chromatographic methods by selecting papers, comparing different chemical structures, optimizing the modification procedure, investigating eluents, and quantitatively parameterizing the separation behavior based on the character of the analytes. FP modified with phenyl isocyanate (PI-FP) was the best among examined and enabled chromatographic analysis of various dyes. We also demonstrated an application of this system for preparative separation of dyes using thread-like paper modified with PI.

2. Materials and Methods

2.1. Materials

The following papers were used: filter paper No. 4 for vacuum filtration (Kiriyama Glass Works Co., Tokyo, Japan), Advantec No. 50 and No. 51B filter papers for chromatography (Toyo Roshi Kaisya Ltd., Tokyo, Japan), and Kimwipes S-200 wiping paper (Nippon Paper Crexia Co. Ltd., Tokyo, Japan). Phenyl isocyanate (PI) (>98.0%), acetic anhydride (>99.0%), Basic Red 2 (purity not disclosed, lot number GB01), Basic Red 5 (purity not disclosed, lot number UYC5F), Basic Blue 17 (purity not disclosed, lot number XYP7J) were purchased from Tokyo Chemical Industry (Tokyo, Japan). *N,N*-dimethylformamide (DMF) (>99.5%), isopropanol (>99.7%), methanol (>99.8%), acetone (>99.5%), iodine (>99.8%), sodium thiosulfate pentahydrate (>99.0%), Methylene Blue (>98.5%), and benzoyl chloride (>99.0%) were purchased from Kanto Chemical (Tokyo, Japan). Tertrazine (14.0%, mixture with 86.0% dextrin), New Coccine (15.0%, mixture with 85.0% dextrin), and Brilliant Blue fcf (8.0%, mixture with 92.0% dextrin) were purchased from Kyoritsu Foods (Tokyo, Japan). Methyl Orange (>98.0%) was purchased from Fuji Film Wako Pure Chemical (Osaka, Japan). Calculated water-octanol partition coefficient ($\log P$), calculated indices of water solubility ($\log S$), and polar surface area (PSA) were computed using Property Explorer at chemexper.com. Water was purified using an EYELA (Tokyo, Japan) Still Ace SA2100-A water purification machine.

2.2. Measurements

Fourier-transfer infrared (FTIR) spectra were measured on a JASCO (Tokyo, Japan) FT/IR-460 Plus spectrometer. Scanning electron microscopy (SEM) images were recorded with a Hitachi (Tokyo, Japan) SU-8000 scanning electron microscope. UV-vis spectra were recorded on a HACH (Loveland, CO, USA) DR-5000 spectrometer.

2.3. Modification of FP with PI

Paper slips (4 cm × 1 cm) were washed with acetone before the reaction. The paper and PI were added in DMF, and the mixture was gently stirred. After the reaction, the paper was washed sequentially with DMF and methanol, and dried under reduced pressure.

2.4. Modification of FP with Acetic Anhydride

Paper slips (4 cm × 1 cm) were washed with acetone before the reaction. The paper and iodine (8 mg) were added to acetic anhydride (10 mL) and reacted at 60 °C for 3 h. Then, a saturated aqueous solution of sodium thiosulfate (0.2 mL) and ethanol (30 mL) were added, and the mixture was stirred for 30 min. The paper was washed sequentially with water and ethanol, and dried under reduced pressure.

2.5. Modification of FP with Benzoyl Chloride

Paper slips (4 cm × 1 cm) were washed with acetone before the reaction. The paper and benzoyl chloride (0.5 mL) were added to a THF (12 mL) solution of pyridine (3 mL), and the mixture was reacted at 50 °C for 7 h. Then, the paper was washed twice with ethanol, and dried under reduced pressure.

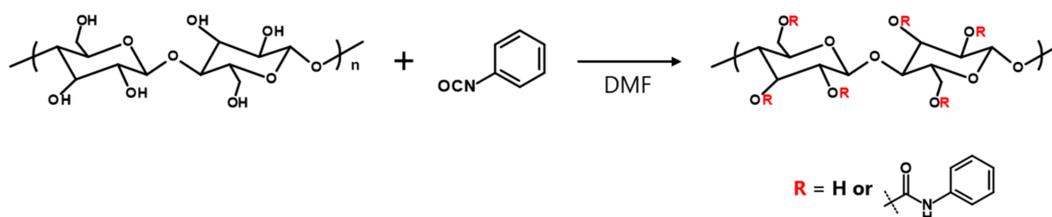
2.6. Paper Chromatography Analysis

Samples were plotted on an FP stationary phase and soaked in 0.8 mL of eluent in a glass vial. The stationary phase was taken out from the container after a development of 3 cm and then dried at room temperature. The image of the dried stationary phase was captured and analyzed using the analysis software ImageJ [24,25]. Color images were converted to gray-scale images. Relationships between color depths and the development direction were made from plot profiles of gray values. An R_f value was calculated by averaging positions with relative concentrations higher than 90% in an image. Triplicate experiments were carried out, and the averaged R_f values are indicated.

3. Results

3.1. Modification of FP

The reaction of FP for chromatography (No. 50 or No. 51B, 4 cm × 1 cm) with an excess amount of PI was performed in DMF (Scheme 1, Tables 1 and 2). PI-FP was obtained by washing with DMF and subsequently with methanol and then drying under reduced pressure at room temperature.



Scheme 1. Modification of cellulose filter paper with phenyl isocyanate.

FTIR spectra of PI-FP obtained from FPs No. 50 and No. 51B show a peak at 1710 cm^{-1} originating from the C=O stretching vibration of the carbamate group (Figure 1), which supports the modification of FP by PI. The degrees of substitution (DS) of the modified FPs were approximately 5%, and the relative intensities of the peak derived from the carbamate group were lower than those of the O-H and C-O stretching vibrations.

Table 1. Modification of chromatographic filter paper (No. 50) with PI (conditions: filter paper size = 4 cm × 1 cm, DMF = 9 mL).

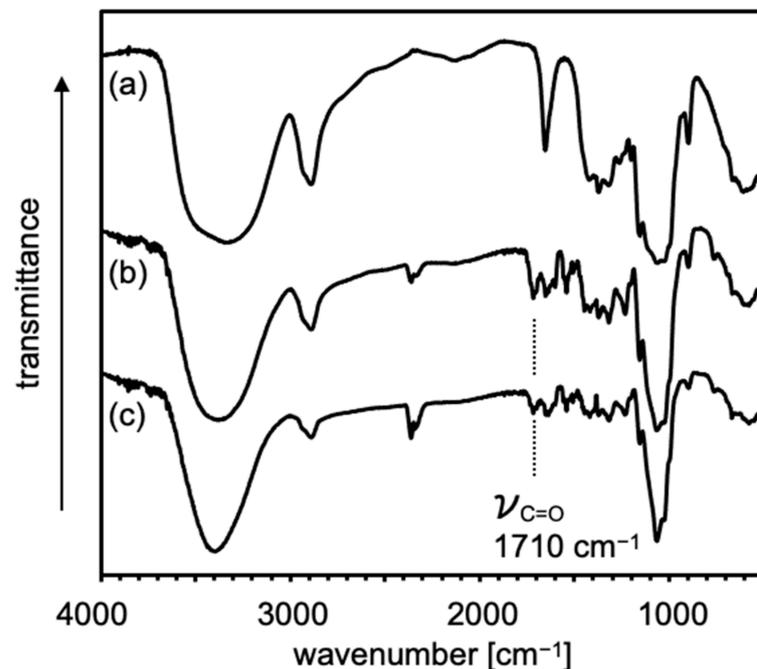
Run	Temp. (°C)	PI (mL)	Time (min)	DS (%) ¹
1	100	1	60	Dissolved
2	100	1	30	Dissolved
3	80	1	60	Dissolved
4	80	1	30	3–5
5	80	1.5	30	Partially dissolved
6	80	1.5	20	3–4
7	80	2	20	3–4
8	40	1	30	1
9	40	1	20	1

¹ Determined by gravimetric increase.

Table 2. Modification of chromatographic filter paper (No. 51B) with PI (Conditions: filter paper size = 4 cm × 1 cm, DMF = 9 mL).

Run	Temp. (°C)	PI (mL)	Time (min)	DS (%) ¹
1	80	1	30	Partially dissolved
2	80	1	20	Partially dissolved
3	80	1.5	20	Partially dissolved
4	60	1	30	Partially dissolved
5	60	1	20	2

¹ Determined by gravimetric increase.

**Figure 1.** FT-IR spectra of (a) chromatographic filter paper (No. 50), (b) PI-modified filter paper (No. 50), and (c) PI-modified filter paper (No. 51B).

The effect of the conditions for modifications of FPs No. 50 and No. 51B are shown in Tables 1 and 2, respectively. The DSs of modified FP were calculated by the weight changes. DS increased as the reaction temperature increased, while the morphology was not retained under higher temperatures and PI feeds. Therefore, the reaction time and temperature should be controlled to retain the shape of FP, because FPs with higher DS become soluble in DMF. The optimum conditions for FP No. 50 were those in Run 4, 6, and 7, which give PI-FP with DS of 3–5%. Figure 2 shows the SEM images of FP No. 50 and PI-FP obtained

in Run 4 retaining the fibrous structure. The morphology of FP No. 51B collapsed even under the conditions that the filter shape of FP No. 50 was retained. A probable factor is the higher density and thickness of No. 50 than those of No. 51B. PI-FP from FP No. 51 required 5 and 8 min for 3-cm-developments of water and isopropanol/water = 3/2, respectively. FP No. 4 may also retain the shape by the modification with DS of 5%, but the time for developments exceeded 20 min for both of the eluents. We accordingly selected FP No. 50 as the FP for modification and separation.

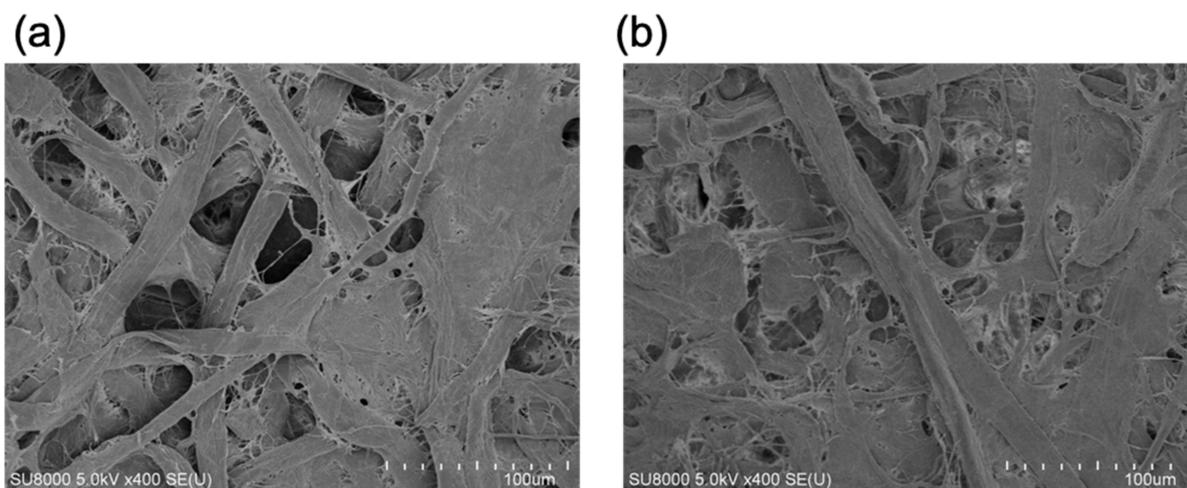


Figure 2. SEM images of (a) chromatographic filter paper (No. 50) and (b) PI-modified filter paper (No. 50).

3.2. Development of Dyes on FP and Modified FPs

We compared the separation behaviors of stationary phases of FP and modified FPs (PI-FP, acetylated FP, and benzoylated FP) with DS of $5 \pm 1\%$. The analytes were five dyes with calculated octanol/water partition coefficients ($\log P$) ranging from -2.8 to 3.6 shown in Figure 3. The hydrophilicity and lipophilicity of the analytes are indicated by smaller and larger $\log P$ values, respectively.

The dyes were developed with mixed eluents of isopropanol/water = 3/2 and 9/1. The R_f values of the development were plotted toward $\log P$ values (Figures 4 and 5).

In the development with isopropanol/water = 3/2, the separation behaviors using non-modified and acetylated FP were almost identical, and weakly positive correlations between $\log P$ and R_f values were observed. On the other hand, development with benzoylated FP resulted in lower R_f values with slight differences, namely, a very weak correlation between $\log P$ and R_f values. By contrast, for PI-FP, a strongly negative correlation was found between R_f and $\log P$ values. This change agrees with the increased hydrophobicity and various interacting groups in PI-FP.

When isopropanol/water = 9/1 was used as the eluent (Figure 5), FP, acetylated FP, and benzoylated FPs showed similar separation behaviors. Only PI-FP showed a different separation behavior, but the lower R_f values resulted in a weak correlation between $\log P$ and R_f values. In the development with isopropanol/water = 3/2, the separation behaviors using non-modified and acetylated FP were almost identical, and weakly positive correlations between $\log P$ and R_f values were observed. On the other hand, development with benzoylated FP resulted in lower R_f values with slight differences, namely, a very weak correlation between $\log P$ and R_f values. By contrast, for PI-FP, a strongly negative correlation was found between R_f and $\log P$ values. This change agrees with the increased hydrophobicity and various interacting groups in PI-FP.

When isopropanol/water = 9/1 was used as the eluent (Figure 5), FP, acetylated FP, and benzoylated FPs showed similar separation behaviors. Only PI-FP showed a different

separation behavior, but the lower R_f values resulted in a weak correlation between $\text{clog}P$ and R_f values.

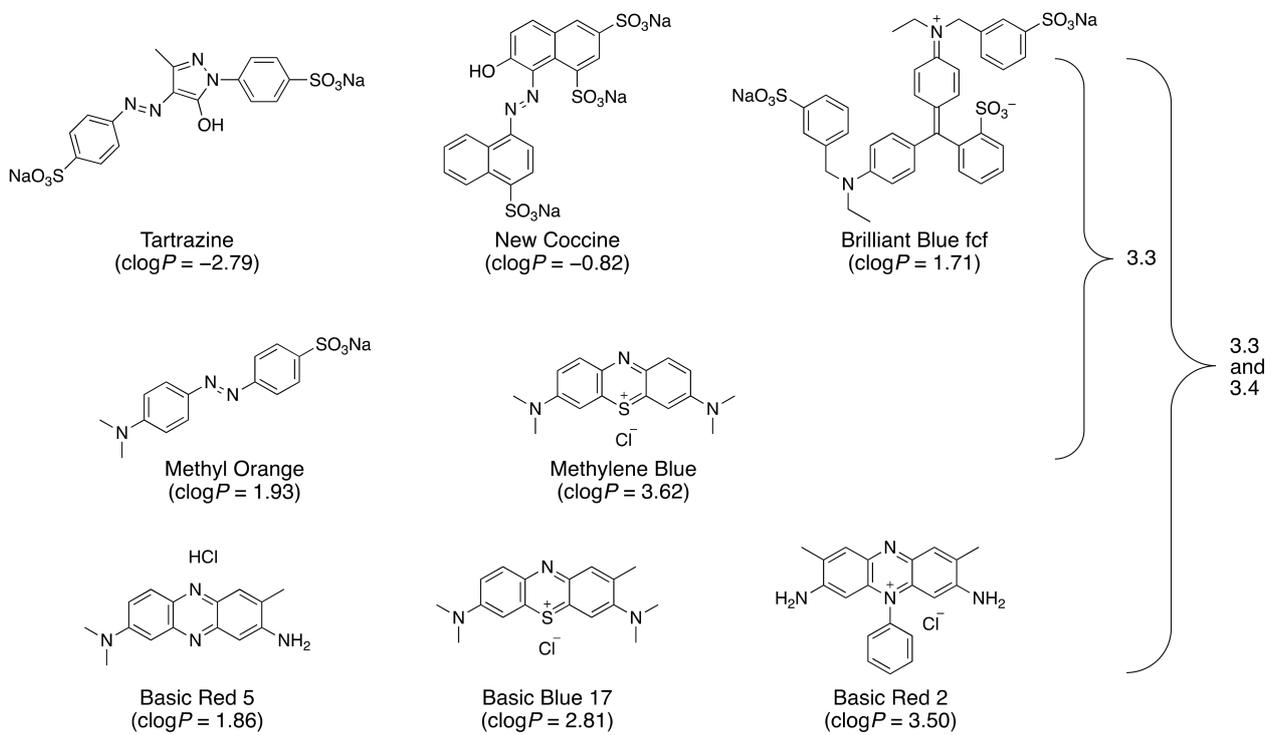


Figure 3. Analyte dyes used in the studies in Sections 3.3 and 3.4 with their $\text{clog}P$ values.

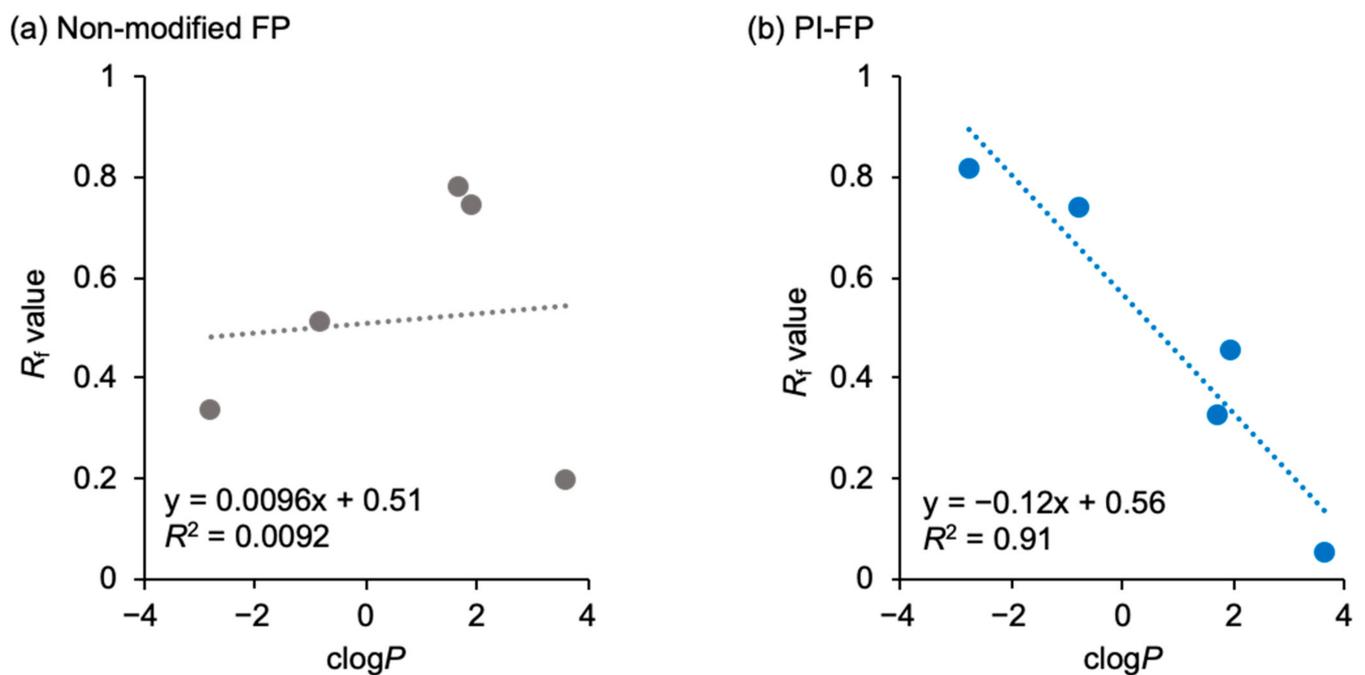


Figure 4. Cont.

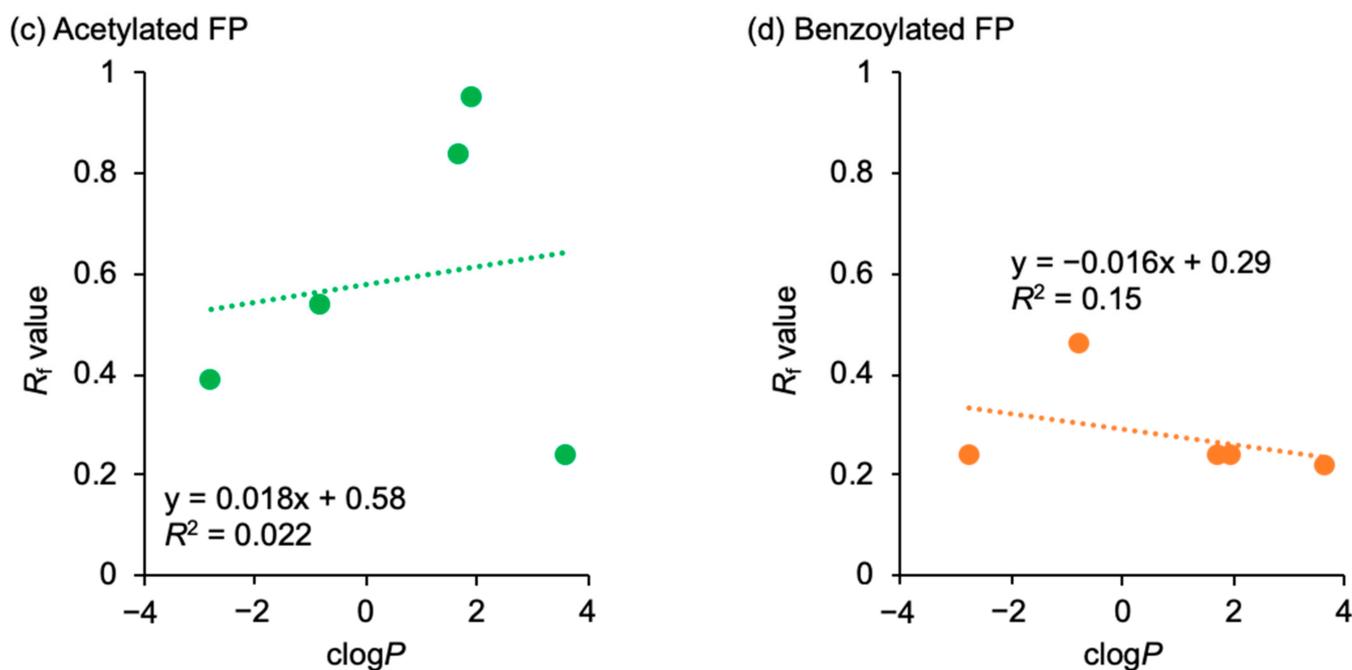


Figure 4. Correlation between $\log P$ and R_f values in elution with isopropanol/water = 3/2 ((a): non-modified, (b): PI-modified, (c): acetylated, and (d): benzoylated filter paper as stationary phase).

This dependence of the separation behaviors on the chemical structures can be explained by the intermolecular interactions between the stationary phase, analytes, and eluents (Figure 6). A plausible reason for the similar separation behaviors of the non-modified and acetylated FP is the low hydrophobicity of the acetyl group with a DS of 5%, affecting negligibly on the separation. Benzoylated FP, having a more hydrophobic phenyl group, exhibited a difference that Brilliant Blue fcf ($\log P = 1.71$) and Methyl Orange ($\log P = 1.93$) had smaller R_f values. However, this change was observed only in the narrow range of $\log P$ from 1.7–1.9. The PI modification resulted in weaker interactions with hydrophilic compounds and a strong negative correlation between $\log P$ and R_f values. This behavior is probably due to the concerted effects of the Lewis acidic NH group in addition to the Lewis basic carbonyl group and the hydrophobic benzene ring, resulting in a wide availability to various functional groups as in the cases of HPLC stationary phases [5–8,10] and extraction phases for hydrophobic hormones [13]. In addition, the PI-modification reduced the water absorption rate of FP, further supporting the hydrophobicity of the phenylcarbamate groups (2–3 and 5–7 min for 3-cm-development of water on FP and PI-FP, respectively). For these results, we determined PI-FP, which showed the most significant change in separation behavior and the strongest correlation between $\log P$ and R_f values, as the most effective modified FP for further detailed analysis with the comparison with FP.

3.3. Development of Dyes on FP and PI-FP

FP and PI-FP were used as the stationary phases, and the development behavior was investigated in detail using the eight dyes shown in Figure 3. Water, water/methanol = 3/2, isopropanol/water = 3/1, and acetone/water = 9/1 were used as eluents.

Separation using FP showed a very contrastive separation behavior. Elution with highly polar solvents such as water or water/methanol = 3/2 resulted in high R_f values close to 1 for the dyes with $\log P$ below 1.7 and R_f values below 0.1 for the dyes with $\log P$ above 1.9. As a result, there was no strong correlation between $\log P$ and R_f values. By contrast, the development with polar eluents, water, water/methanol = 3/2, and isopropanol/water = 3/2, worked for PI-FP, showing a strong negative correlation between $\log P$ and R_f values, and the $\log P$ of separable compounds was expanded to -2.8 – 3.6 . However, a clear correlation was not

found for development with the less polar isopropanol/water = 3/1, isopropanol/water = 9/1, and acetone/water = 9/1. This result may be due to the decrease in hydrophilicity of the stationary phase in PI-FP. Table 3 shows the solubility parameters (SP values) [26] of the eluents used in this study, and Figure 7 shows the relationship between the SP values and the correlation coefficient R^2 . The R^2 of FP became lowest at the SP value of 33, and correlations for other SP values were also low. The point with the weakest correlation suggests the close polarities of the stationary phase and the eluent, with which the $\log P$ is not a crucial factor controlling the separation behavior. For this presumption, the probable SP value of FP is around 33. On the other hand, the minimum SP value for PI-FP was 26, agreeing with the hydrophobicity of PI-FP being higher than that of FP. The larger difference in the polarity and eluents is a factor of more efficient separation with PI-FP. Other parameters, $\log S$, another parameter indicative of polarity, and the PSA, the surface area of all polar molecules in a compound, showed lower correlation than $\log P$ (Supplementary Data).

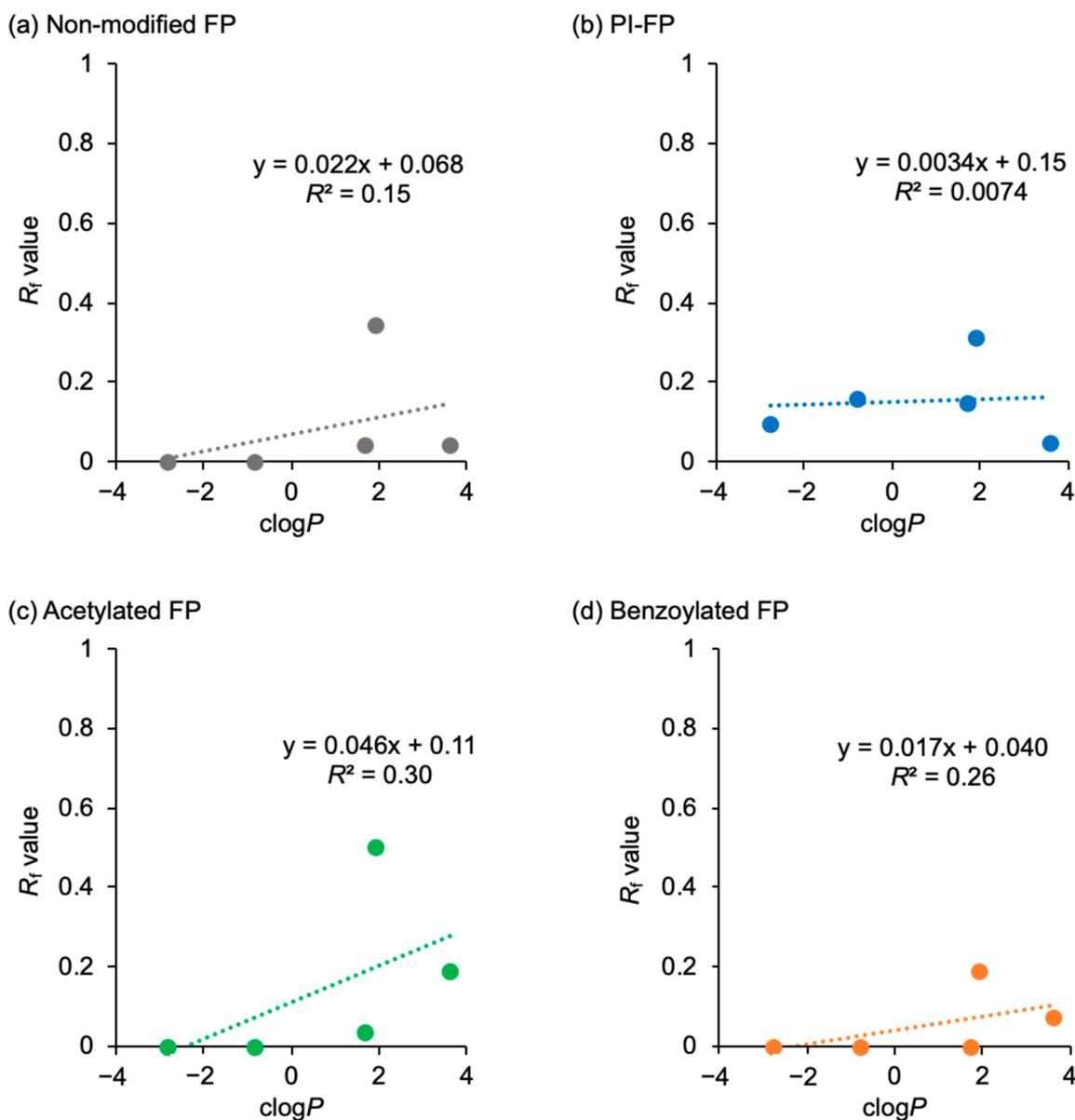


Figure 5. Correlation between $\log P$ and R_f values in elution with isopropanol/water = 9/1 ((a): nonmodified, (b): PI-modified, (c): acetylated, and (d): benzoylated FP as stationary phase).

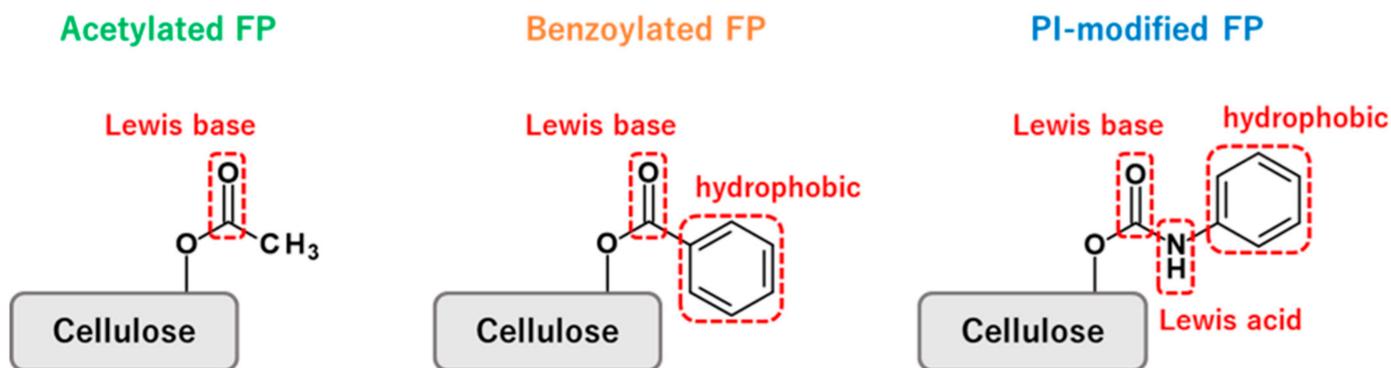


Figure 6. Expected effect of chemical structures on separation.

Table 3. Eluents and their solubility parameters.

Eluents	Solubility Parameter
water	48
water/methanol = 3/2	40
isopropanol/water = 3/2	33
isopropanol/water = 3/1	29
isopropanol/water = 9/1	26
acetone/water = 9/1	22

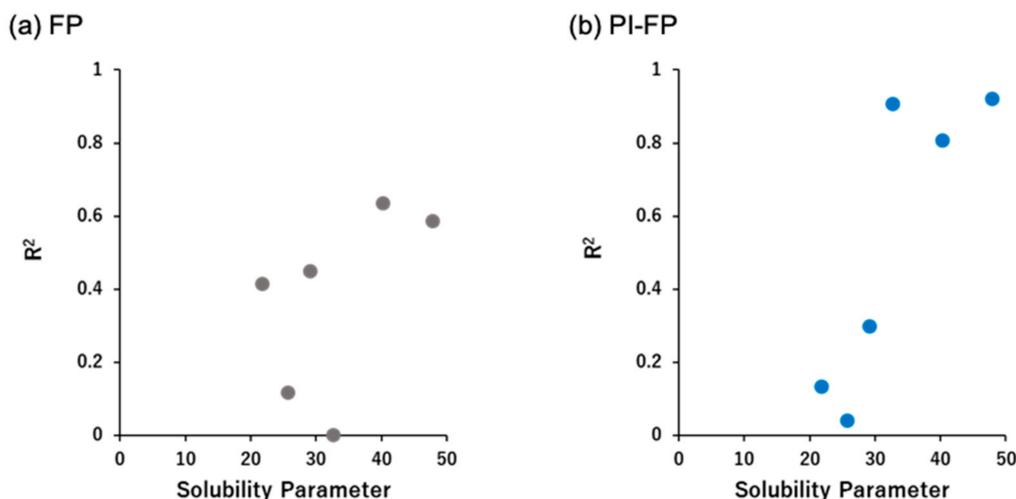


Figure 7. Relationship between solubility parameter of eluent and R^2 of correlation between $\log P$ and R_f value ((a): FP and (b): PI-FP).

3.4. Separation of Dyes with PI-Modified Paper

We applied this concept for paper-based preparative separation by using a thread-shaped stationary phase. The stationary phase was made of PI-modified wiping paper (PI-WP), obtained by applying the same method as that for PI-FP to a wiping paper (WP), twisted into a thread shape (Figure 8a), and one end of this thread-like stationary phase was immersed in a mixed solution of Brilliant Blue fcf with the $\log P$ value of 1.71 and Tartrazine with the $\log P$ value of -2.79 in a mixed solution of i PrOH/water = 3/2 (Figure 8b).

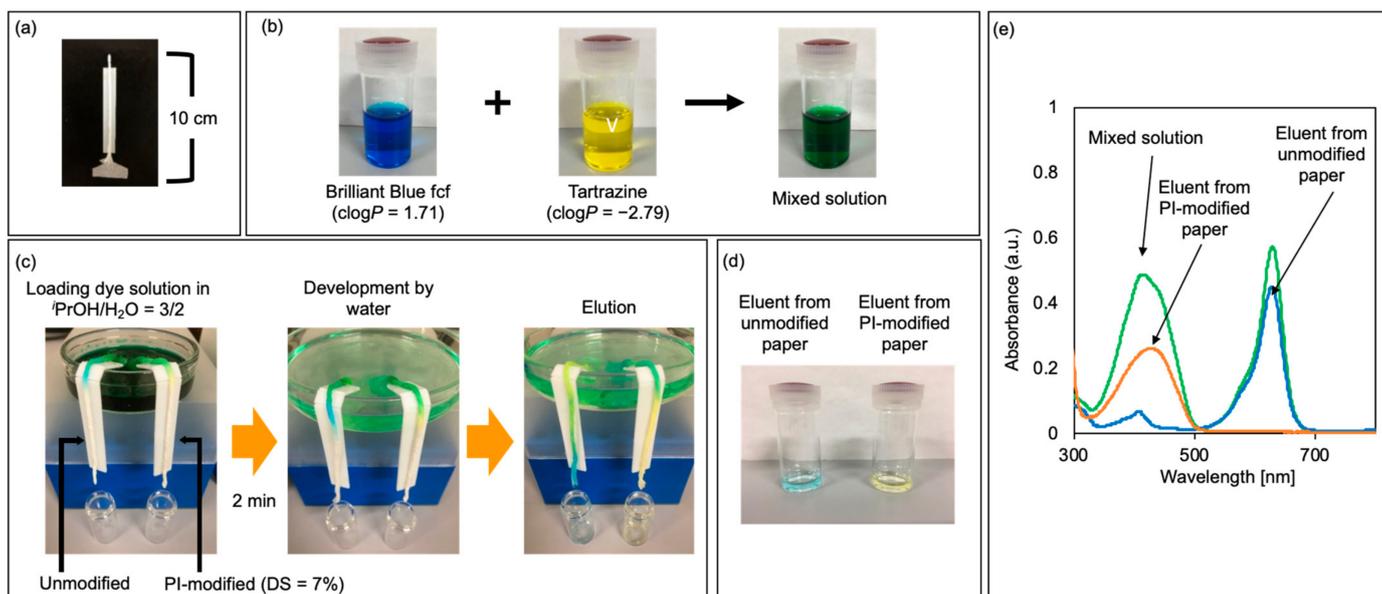


Figure 8. Photo images of (a) thread-like stationary phase of twisted wiping paper, (b) aqueous solutions of dyes, (c) chromatographic separation of dyes with unmodified and PI-modified stationary phases, and (d) eluents from the stationary phases. (e) UV-vis spectra of the mixed solutions of Brilliant Blue fcf and the eluents from the stationary phases.

The two components were separated by the elution with water through the thread-like stationary phase (Figure 8c). The tip of the stationary phase was immersed in the mixed solution, and another tip was hung down to a glass vial. After 2 min of this charging process, the solution was changed to water for elution. As a result, a blue fraction originating from Brilliant Blue fcf was eluted from WP, while a yellow fraction containing Tartrazine was eluted from PI-WP (Figure 8d). This behavior, consistent with the separation behavior described for FP and PI-FP, indicates that separation is possible even with a thread-like stationary phase, as in the case of FP.

UV-vis spectra of the eluates showed that the blue eluates obtained from WP matched Brilliant Blue fcf and that the yellow eluates obtained from PI-WP matched Tartrazine (Figure 8e). This result demonstrates that thread-like paper stationary phases enable preparative separation.

4. Conclusions

This work expanded paper chromatography toward substances unavailable for conventional paper chromatography by modification with PI. The modification under controlled conditions attained the modification with DS around 5% maintaining the morphology of the paper. PI-FP, filter paper modified with phenyl isocyanate, developed dyes with positive and negative $\text{clog}P$ values separately following the order of $\text{clog}P$. The modification with PI could be applied to the preparative separation of dyes using thread-like paper modified with PI. This approach is potentially scalable and may allow the recycling of stationary phases. The use of modified paper will provide inexpensive analysis and preparative separation of a wide range of organic compounds.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/technologies11010001/s1>, Figure S1: correlation between $\text{clog}P$ and R_f values in development on FP eluted by various eluents; Figure S2: correlation between $\text{clog}P$ and R_f values in development on PI-FP eluted by various eluents; Figure S3: correlation between $\text{clog}S$ and R_f values in development on FP eluted by various eluents; Figure S4: correlation between $\text{clog}S$ and R_f values in development on PI-FP eluted by various eluents; Figure S5: correlation between PSA and R_f values in development on FP eluted by various eluents; Figure S6: correlation between PSA

and R_f values in development on PI-FP eluted by various eluents; Figure S7: Relationship between solubility parameter of eluent and R^2 of correlation between $\log S$ and R_f value in developments using FP and PI-FP; and Table S1: $\log S$ and PSA of analytes.

Author Contributions: Conceptualization, B.O.; methodology, S.K. and B.O.; validation, B.O. and Y.M.; formal analysis, S.K.; investigation, S.K.; resources, S.K.; data curation, S.K.; writing—original draft preparation, B.O. and S.K.; writing—review and editing, B.O. and Y.M.; visualization, B.O. and S.K.; supervision, B.O.; project administration, B.O.; funding acquisition, B.O. and Y.M. All authors have read and agreed to the published version of the manuscript.

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