農薬生産用農産食料製造-澱粉粒の染料吸着(農芸化学科)

Pesticide-producing Agricultural Food Processing. Dye adsorption onto starch granules*

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I INTRODUCTION

Anthocyans and the dyes having absorbance peaks at 500–600 nm could affect the aphid arrestivity of green leaves and, hence, would be utilized as insect deterrents[2]. Since, in order to develop anthocyan pesticides[2,3], a proper adsorbent was required, starch granules were selected as the first adsorbent to be investigated.

There have been some reports on dye adsorption onto various starches and their derivatives[1,4,5]. Nevertheless, the number of precise studies on the basic relationships between the dye adsorption and the properties of starches is about nul. The present work was carried out in order to clarify the effect of granular size of starches on the dye adsorption using 13 starches and 3 acidic dyes, 3 basic dyes, and 3 amphichroic dyes.

II MATERIALS AND METHODS

1 Starch preparation

The starches prepared from corn, potato, sweet potato, and wheat were obtained from Wako Pure Chemical Co., Tokyo. The rest of the starches used were prepared in author's laboratory as shown in Table 1. Starches from alocasia (stem), cycad, ginger, yam, and canna were prepared by repeated water washing. Each plant source was homogenized with a blender, filterated through double gauze layer three times to remove tissue fragments, washed with water repeatedly by precipitation, and air-dried. The starch from the stem of alocasia was prepared by removing the layer of raphides by a use of a siphon in every water washing. The starches of taro, wet taro, and alocasia (seed) were prepared by repeated alkali steeping, followed by repeated water washing to obtain neutral solutions. The starch of garcinia (seed) was prepared by repeated acid steeping, followed by repeated water washing to obtain a neutral solution.

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Table 1. The source plant, organ, method of preparation, and granule size of the starches used

Starch No.	Source plant	Organ	Method of preparation*	Granule size (μ)**
1	*Colocasia a.* S.	Tuber	b	1
2	*Colocasia e.* S.	Tuber	b	3
3	*Alocasia o.* C. K.	Stem	a	4
4	*Cycas r.* T.	Seed	a	7
5	*Garcinia s.* M.	Seed	c	9
6	*Zingiber o.* R.	Rhizome	a	12
7	*Alocasia o.* C. K.	Seed	b	15
8	*Zea m.* L.	Seed	d	18
9	*Ipomoea b.* L.	Tuber	d	20
10	*Dioscorea b.* D.	Tuber	a	23
11	*Triticum a.* L.	Seed	d	25
12	*Solanum t.* L.	Tuber	d	30
13	*Canna i.* L.	Rhizome	a	45

* a : Repeated washing with water, b : Alkali steeping method.
c : Acid steeping method, d : Commercial sample
** Longitudinal size

2 Measurement of granule size

An optical microscope with a micrometer was used for the measurement of the size of starch granules. The granule size of the starches used in this work was expressed as an average value of the longitudinal diameters measured on 20 granules. The values obtained are shown in Table 1.

3 Dyes

Erythrosine, phloxine, and tartrazine as acidic dyes, methylen blue, methyl violet, and fuchsine basic as basic dyes, and methyl orange, bromocresol green, and methyl red as amphichroic dyes were all obtained from Wako Pure Chemical Co., Tokyo.

4 Dye adsorption from solution

Dye adsorption onto starch granules was carried out in water and methanol by the same procedures. Into the test tube containing 10 ml of the respective dye solution whose absorbance was adjusted to 0.5 at the wavelength of the absorbance peak, 0.1 g of starch was added, stoppered, and left standing at 24°C in a dark room for 24 hr, at which the solution was centrifuged and then used for measurement of the absorbance of the supernatant with a Hitachi (Model 139) spectrophotometer. The amount of adsorption was expressed as the percentage of a decrease in the absorbance with respect to the initial absorbance of the dye solution used. The effect of ions on the adsorption was tested by the procedures stated above after preparing 0.1 M NaCl, 0.1 M KCl, 0.05 M $CaCl_2$, and 0.05 M $BaCl_2$ aqueous solutions.

5 Effect of solvent evaporation on dye adsorption

To 0.5 g of starch granules in a sharlei (4.5 cm in diameter) 2 ml of a dye solution in methanol, ethanol, propanol, and butanol, whose absorbance was adjusted to 1.0 at the wavelength of the absorbance peak, was added, mixed, and then left standing for air-drying at 24°C in a dark room for 3–4 hr. The dry, dyed starch was washed with the respective solvent thoroughly. The absorbance of the dye eluted by the respective solvent was determined after volume adjustment to 10 ml, and the percentage loss of the dye was evaluated with respect to that of the initial dye solution to obtain the amount of adsorption.

III RESULTS AND DISCUSSION

1 Dye adsorption in aqueous solutions

The amounts of dye adsorption onto the starch granules used in the present work in aqueous solutions are shown in Table 2. Acidic dyes showed larger dye adsorption onto small size granules

Table 2. Percentage adsorption of acidic dyes, basic dyes, and amphichroic dyes onto various starches in distilled water

Starch	Acidic dyes			Basic dyes			Amphichroic dyes		
No.	Er	Ph	Ta	MB	MV	Fu	MO	MR	BG
1	65.2	41.4	29.6	47.3	59.3	47.0	11.6	3.2	13.9
2	62.4	38.0	29.8	46.0	62.1	53.4	13.8	2.2	11.9
3	60.2	34.0	29.0	47.1	60.0	54.0	16.0	4.0	9.5
4	46.7	26.9	13.7	37.6	57.5	40.5	19.8	5.2	3.2
5	56.2	35.3	25.1	39.3	40.4	46.6	20.4	4.6	9.3
6	50.4	28.4	23.2	38.0	55.7	41.3	18.2	4.8	8.0
7	47.3	25.3	19.8	34.3	56.0	37.4	20.0	6.0	11.8
8	53.1	58.1	13.3	29.5	54.7	29.5	22.0	5.0	11.3
9	38.3	19.0	9.8	39.4	59.8	39.4	12.4	5.0	5.0
10	10.1	1.7	0.0	42.5	56.7	42.5	4.6	2.2	0.0
11	48.3	44.0	15.7	31.9	49.6	31.9	13.6	4.2	11.1
12	3.7	0.0	0.0	70.0	69.5	72.2	0.4	0.0	0.0
13	4.0	0.0	15.7	75.3	78.9	62.8	0.0	0.0	0.0

Er : Erythrosine, Ph : Phloxine, Ta : Tartrazine, MB : Methylene blue, MV : Methy violet, Fu : Fuchsine basic, MO : Methyl orange, MR : Methyl red, BG : Bromocresol green

inspite of minor variations among the pigments used. This tendency was observed clearly in erythrosine, having 60% adsorption onto small granules and only 5% adsorption onto large granules. Basic dyes showed a greater adsorption onto large size granules with non-continuous way with respect to granule size at 30 μ. In all region of the sizes studied, the amount of adsorption of the basic dyes was higher than that of the acidic or amphichroic dyes. Amphichroic dyes showed the tendency being similar to the acidic dyes with the smallest adsorption among three kinds of the dyes used in this work.

In general, the dye adsorption onto starch granules in aqueous solutions was greater with basic dyes, the next with acidic dyes, and the least with amphichroic dyes. The above results indicated that the dye adsorption in aqueous solutions was mainly due to ionic bond formation at granular surface and inner phase of the starch granules.

2 Effect of ions on dye adsorption

The results of dye adsorption experiments at the presence of various ions are shown in Table 3

Table 3. Percentage adsorption of erythrosine (Er), methylene blue (MB), and methyl orange (MO) onto various starches in 0.1 M NaCl, 0.1 M KCl, and 0.05 M $CaCl_2$ solutions

Starch No.	Er			MB			MO		
	NaCl	KCl	$CaCl_2$	NaCl	KCl	$CaCl_2$	NaCl	KCl	$CaCl_2$
1	26.4	28.8	31.2	5.7	1.9	2.9	19.5	12.8	15.6
2	22.6	31.9	25.9	7.6	0.0	0.9	11.0	8.0	14.4
3	23.0	28.4	23.2	8.0	2.2	2.0	12.4	8.9	12.4
4	8.7	15.4	16.3	5.7	4.3	1.4	13.9	13.4	11.3
5	16.5	25.9	21.2	15.6	8.1	6.1	17.3	19.9	15.8
6	19.2	35.3	22.8	10.1	7.4	7.0	16.4	18.0	18.3
7	17.4	23.2	20.8	9.8	7.2	6.8	17.3	19.7	19.5
8	36.5	42.1	41.1	6.8	3.8	2.6	17.2	19.6	14.2
9	17.2	27.3	23.3	8.4	6.0	4.5	9.6	19.7	16.8
10	7.8	12.0	14.5	9.6	3.4	2.8	15.2	15.3	12.1
11	23.7	32.9	31.0	10.9	3.4	1.9	15.7	19.6	15.6
12	6.3	10.3	15.5	6.8	4.5	4.0	13.7	15.5	14.2
13	7.1	10.9	15.3	19.3	18.4	13.7	13.7	13.3	13.3

for erythrosine, methylene blue, and methyl orange.

Erythrosine, an acidic dye, showed a decrease to 30% from 65% at a smaller granule size than 10 μ and slightly increased at a larger granule size, indicating a less evident tendency comparing with those in distilled water. Methylene blue, a basic dye, showed a notable decrease in dye adsorption all over the range of the granule size showing almost constant amounts over the whole range studied. Methyl orange, an amphichroic dye, did not show any ionic effect on the dye adsorption at the region smaller than 23 μ. But, at sizes greater than the value, 10–20% adsorption was observed while no adsorption was observed in the case of distilled water. As a result, there was no variation in dye adsorption with respect to granule size.

The ionic concentration dependence of dye adsorption onto potato starch is shown in Table 4.

Dye	Cation	Ionic strength 0.01	0.025	0.05	0.10
Er	Na	33.2	30.7	27.3	17.2
Er	K	39.3	36.2	32.6	27.3
Er	Ca	45.9	42.9	48.7	23.3
Er	Ba	55.1	41.9	39.2	33.9
MB	Na	22.0	20.4	10.4	8.4
MB	K	22.9	13.9	8.4	6.0
MB	Ca	10.4	7.8	5.3	4.5
MB	Ba	8.0	7.6	6.2	6.6
MO	Na	19.2	17.7	17.5	9.6
MO	K	22.5	20.8	20.3	19.7
MO	Ca	24.1	22.7	21.6	16.8
MO	Ba	27.1	23.3	20.1	20.1

Table 4. The effect of cations on the adsorption of erythrosine (Er), methylene blue (MB), and methyl orange (MO) onto potato starch

Erythrosine and methyl orange gave an initial increase and a decrase at higher concentrations whereas methylene blue decrased monotonically as increasing ionic concentration. Divalent cations generally gave greater effects on the dye adsorption especially on methylene blue, indicating the major involvement of cations in the observed effects. It is noteworthy that an acidic dye and an amphichroic dye had the same tendency as to the action of cations.

The ionic interaction with dye adsorption onto starch granules and the variations in dye adsorption due to the ionic characteristics of the dyes as described in the previous section clearly indicated that the ionic bond formation was the major mechanism of dye adsorption in aqueous solutions.

3 Dye adsorption in methanol solution

The amounts of dye adsorption in methanol sulutions onto starches, Nos. 3, 4, 9, 12, and 13, are shown in Table 5. Erythrosine showed small adsorption onto two starches. Methylene blue

Starch No.	Er	MB	MO
3	0.0	4.1	2.4
4	0.0	2.1	0.3
9	4.5	3.1	0.0
12	0.0	13.8	0.0
13	1.1	11.7	0.0

Table 5. Percentage adsorption of erythrosine (Er), methylene blue (MB), and methyl orange (MO) onto various starches in methanol

showed slightly higher adsorption than other pigments, especially at a larger granule size. Methyl orange showed almost no adsorption onto all the starches used. Unlike aqueous solution, methanol solution was not a proper system for dye adsorption onto starch granules.

The results of small dye adsorption onto starch granules in methanol solutions would be explained as a consequence of inability in ionic bond formation due to the specific molecular orientation of methanol on dye solvilization, causing inhibition of ionic interaction between dye molecules and the ionic cites on and in the starch granules. This point also indicated the significance of ionic bond formation in the dye adsorption observed in this work.

4 Effect of solvent evaporation on dye adsorption

The effect of solvent evaporation on dye adsorption in methanol, ethanol, propanol, and butanol is shown in Table 6. On potato starch, ethanol gave the adsorption greater than 95% for

Table 6. Percentage adsorption of erythrosine (Er), methylene blue (MB), and methyl orange (MO) onto potato starch and sweet potato starch by solvent evaporation in methanol, ethanol, propanol, and butanol

Dye	Starch	Methanol	Ethanol	Propanol	Butanol
Er	Potato	78.2	96.4	91.7	20.0
Er	Sweet potato	92.0	95.3	81.9	30.0
MB	Potato	89.0	96.0	88.6	43.5
MB	Sweet potato	80.5	79.8	68.9	45.3
MO	Potato	84.0	98.3	95.2	35.3
MO	Sweet potato	89.5	97.7	85.7	44.1

all dyes while butanol gave farely small values. On sweet potato starch, methanol and ethanol gave the heighest adsorption while butanol gave extremely low values. From the above observations, methanol with a lower boiling point was used in the following experiment.

Dye adsorption by solvent evaporation, using methanol, onto various starch granules is shown in Table 7. Erythrosine gave adsorptions greater than 90% at smaller granule size and a decrease as the granule size increased. This tendency was similar to the dye adsorption in aqueous solutions. Methylene blue showed 45.3% adsorption onto taro starch and increased gradually as the granule size increased and reached to the highest value of 94%. Methyl orange, similarly to methylene blue, increased adsorption from the value of 28.4% onto taro starch as increasing granule size and reached to the heighest value of 93.5%. It was clearly shown that the solvent evaporation could enhance the amount of dye adsorption onto the starch granules in methanol solutions. A possible mechanism for the enhancement effect observed may be ionic bond formation by the dyes penetrated into the starch granules in a drying process from the outer layer and direct contact achieved by the removal of solvent from the solvation layer at a molecular level. This ionic bond may be considered to be stronger than the solvation force of the solvents used.

Table 7. Percentage adsorption of erythrosine (Er), methylene blue (MB), and methyl orange (MO) onto various starches by solvent evaporation in methanol

Starch No.	Er	MB	MO
1	91.4	45.3	28.4
2	90.5	54.0	80.0
3	91.6	61.5	41.7
4	94.1	77.5	85.0
5	89.6	78.8	80.4
6	86.8	80.4	82.7
7	80.3	82.5	83.8
8	61.4	59.5	84.5
9	92.0	80.5	89.5
10	77.3	80.0	85.5
11	82.7	81.5	93.5
12	78.2	89.0	84.0
13	69.5	94.0	88.0

From the above results, it became now evident that a solvent-evaporation method was proper for formulation of safe vision-inhibitors, the dyes adsorbed onto starch granules, as proposed elswhere[2,3].

IV SUMMARY

In order to develop dye vision-inhibitor, dye adsorption onto starch granules was studied using 13 starches, 3 acidic dyes, 3 basic dyes, and 3 amphichroic dyes. The amounts of dye adsorption were in the order of basic dyes〉 acidic dyes〉 amphichroic dyes. Among acidic dyes, small size starches gave a greater adsorption than large size starches. The effect of cations was greater with divalent ions than monovalent ions. At a higher ionic concentration (0.1 M), the adsorption of acidic dyes and basic dyes decreased while that of amphichroic dyes increased for some starches. Onto all starches, the amount of dye adsorption in methanol sulutions was about nul. When a solvent-evaporation method was applied to methanol, ethanol, propanol, and butanol solutions, dye adsorption was greater in methanol and ethanol and decreased as the number of carbons increased. Dye adsorption onto starches in methanol solutions with the solvent-evaporation method was generally greater than that in aqueous solutions. It was also shown that the adsorption of basic dyes and amphichroic dyes increased as the size of starch granules increased. The involvement of ionic bond formation at not only granular surface but also the inner phase of starch granules was proposed for the explanation of the results observed.

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農薬生産用農産食料製造 — 澱粉粒の染料吸着

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要　約

染料視覚阻害剤を開発する目的で，13種の澱粉，3酸性染料，3塩基性染料，及び3両性染料を用いて，澱粉粒への染料吸着を調べた。染料吸着量は，塩基性染料>酸性染料>両性染料であった。酸性染料中，小径澱粉粒が大径澱粉粒より高い吸着を示した。陽イオンの染料吸着に与える影響は，二価が一価より高かった。イオンの高濃度（イオン強度0.1）では，酸性染料及び塩基性染料の吸着は低下したが，両性染料は，特定の澱粉については増加を示した。全澱粉について，メタノール中での吸着は，ほぼ零に近かった。メタノール，エタノール，プロパノール，及びブタノール中での溶媒蒸発法による吸着では，メタノールとエタノールが高く，プロパノール，次にブタノールの順に低下した。メタノール蒸発法による染料吸着は，水溶液中の吸着より高かった。塩基性染料及び両性染料は，澱粉粒径が大となるに従い吸着量が高かった。これらの現象を説明するために，澱粉粒の表面及び内部に於けるイオン結合形成の可能性が提案された。

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