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Selective staining as a tool for wood fibre characterization

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Abstract

A method of selective staining is proposed for wood fibre surface characterization. A number of dyes have been tested to determine their ability to stain fibres. Three groups of dyes are considered: the first group has good affinity for the lignin component of fibres (cationic dyes), the second group has good affinity for hemi-cellulose (cationic phthalocyanine dyes) and the third group are dyes with an affinity for the cellulose in the fibres (anionic direct dyes). The mechanism for dye-bonding to different parts of fibre components is also suggested.

Keywords: Wood fibre components; Selective staining; Cationic dyes; Cationic phthalocyanine dyes; Anionic direct dyes

1. Introduction

Changes in papermaking technology extend into the delignification process with oxygen and chlorine-free bleaching. These interventions into the traditional pulping technology result in chemical changes in both the lignin and the lignin-free portions of the fibre surface. Fibre surface quality has an important role in sheet-paper formation, in retention of papermaking additives, dyes inclusive, in brightness stability, in surface sizing and printing, in recyclability, etc. Many up-to-date techniques are used for studying fibre surfaces. There are methods of electron microscopy, spectroscopy, NMR, ESCA, methods based on poly-electrolyte and potentiometric titrations. None of these techniques tell us whether these functional groups belong to the lignin or to the carbohydrates.

In papermaking, coloring of fibres has been used for years [1]. Three categories of dyes have been usually used, namely, anionic, direct and cationic.

1.1. Anionic and direct dyes

These are Na- or K-salts of corresponding dyeing acids. They are negatively charged and show no natural affinity for the cellulose component of fibres (neither for unbleached nor for bleached). Because the fibres are negatively charged, the adherence of anionic dyes to the fibres is obtained with the help of added sizing agents. Direct dyes are anionic or zwitterionic and have affinity for the cellulose component of fibres, mostly because of their size and poor solubility in water.

1.2. Cationic dyes

These are chlorides, hydrochlorides, sulphates, oxalates or other salts of dyeing compounds. Aqueous solutions of these dyes are acidic, in many cases very sensitive to free alkalis and are soluble in aqueous acid media. Cationic dyes have an affinity for acid materials

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like lignin that are present in unbleached or in sawdust pulp. Cationic dyes have a very weak affinity for pure cellulose and if these dyes are used for bleached fibres, a retention-aid agent is needed. The affinity of cationic dyes for non-cellulose compounds is used for selective staining of fibres (fibre-furnish analyses are based on this principle in accordance with the ISO, SCAN and Tappi standards).

In understanding of textile dyeing, an essential progress has been made recently. Timofei et al. [2] for example, made a detailed study on fibre–dye binding using quantitative-structure activity relationship (QSAR). Porter [3] developed an equilibrium equation to correlate the data that account for positive and negative dye interactions on the fibres. The interactions between dyes and fibres were studied also during enzymatic treatment of cellulose fibres [4,5].

Other researchers have been studying fibre structure using selective staining of fibres. Srebotnik and Messner [6] examined the possibilities of observing the delignification process with differential staining of wood cross-sections using the principle of selective staining of lignin with Safranine dye and of the lignin-free part of fibres with Astra Blue dye. Yu et al. [7] used selective staining of cellulose components to determine the pore structure of fibres. Liu et al. [8] followed the inhomogeneity of delignification by measuring the change in fluorescence after staining fibres with selected dyes. Moss et al. [9] observed the degree of delignification by measuring the intensity of natural fluorescence of lignin in the fibres using Confocal Scanning Microscopy (CLSM).

The aim of our work is to determine the groups of dyes that would selectively stain the reference fibres. In our case, these fibres are unbleached softwood kraft pulp used here as lignin-containing fibres. The same fibres from which lignin is removed by oxidation are used as hemi-cellulose and cellulose containing fibres. Cellulose fibres prepared from them react as pure cellulose. Measuring the intensity of dyes adsorbed on these fibres we intend to create few groups of dyes having suitable affinity for lignin, hemi-cellulose and cellulose part of fibres. Then we try to explain the mechanism of dye bonding to functional groups (active sites) on the fibre surface.

2. Materials and methods

Three separate and independent methods were chosen to determine the affinity of different dyes for the different chemical components of fibres.

For a preliminary evaluation of dye affinity using capillary rise, a wide range of dyes was chosen. They can be classified into groups, as indicated below and as shown in Table 1. They are identified by their common name and Color Index Number [10], which encodes its chemical structure.

The oil-soluble dyes have great affinity for hydrophobic, especially aromatic structures and were expected to stain lignin. Amino-anthraquinone disperse dyes are less hydrophobic than oil-soluble dyes and less basic than other cationic dyes. pH-dependent anionic dyes contain phenolic and/or carboxylic groups as acid-functional groups, whereas pH-independent anionic dyes contain the sulphonic groups. Zwitterionic dyes contain both anionic as well as cationic groups. pH-dependent cationic dyes contain primary, secondary or tertiary amino groups. pH-independent cationic dyes contain quaternary ammonium structures. Direct dyes are anionic dyes, having an affinity for cellulose.

The affinity of the most promising of them was evaluated subsequently using optical reflectance. The results were checked by evaluation of dye affinity using model substances.

2.1. Determination of the affinity of dyes for the various chemical components of fibres

The methods of dye preparation and fibre and sheet preparation are described in the Appendix A.

2.2. Evaluation of dye affinity using capillary rise

The affinity of dyes for fibres in paper sheets can be assessed from the capillary rise of the dye when a sheet of paper is introduced vertically into a dye solution. Those dyes with the highest affinity showed the lowest rise of the dye, compared with the rise of the solvent itself. Dyes with no affinity would rise to the same height as the solvent. The ratio of the heights, $h_{\mathrm{dye}}/h_{\mathrm{solvent}}$ is taken as a measure, Q_h, of the affinity of the dye for the fibres in the sheet.

Dye solutions were prepared as indicated in Table 1. Capillary rise was measured on two sets of sheets. One set consisted of hand-sheets of kraft pulp with Kappa number of 30 and the results are presented as Q_{h30}. The other set consisted of chromatography paper that was lignin-free. These results are noticed as Q_{h0}. The ratio of relative capillary rise in the two sheets, $QQ_h = Q_{h0}/Q_{h30}$ was taken as a measure of the dye's affinity for lignin.

The results are displayed in Table 1.

2.3. Evaluation of the affinity of dyes using optical reflectance

In this experiment, sheets were made from three pulps that differed greatly in chemical composition.

Unbleached softwood kraft pulp is used here as an example of lignin-containing fibres (L). The same fibres from which the lignin was removed by oxidation are used as hemi-cellulose and cellulose containing fibres

Table 1
Results of preliminary dyeing tests (solvent: 0.01 M pH = 5 acetate buffer, added a: 10, b: 20, c: 30, d: 50, e: 70% EtOH; f: 96% EtOH without buffer; M: MeOH added instead of EtOH)

Group of dyes	Common name	C.I.	Solvent	Q_{h30}	Q_{h0}	QQ_h
Oil-soluble dyes	Solvent Yellow 58	11129	c	0.54	0.74	1.4
	Sudan Orange G	11920	d	0.89	0.84	0.9
	Sudan Red G	12150	e	0.86	1.00	1.2
	Citrus Red	12156	d	0.39	0.87	2.2
	Oil Red O	26125	d	0.71	0.49	1.4
Anionic dyes, pH-dependent	Brilliant Red R	15800	f	0.73	1.00	0.7
	Phenolphthalein			1.00	1.00	1.0
	Fluorescein	45350	c M	0.64	0.64	1
	Eosin	45380	a M	0.40	0.32	0.8
	Ethyl Eosin	45386	b	0.02	0.28	**14**
	Alizarin	58000		0	0	
Anionic dyes, pH-independent	Alcian Blue	74220			0.52	
	Sirius Light Turquoise Blue	74180			0.14	
Cationic dyes, pH-dependent	Bismarck Brown 1	21000	c	0	0	
	Bismarck Brown 4	21010	d	0.04	0.08	2
	Astra Fuchsin	42520	d	0.39	0.85	2.2
	Crystal Violet	42555	d	0.26	1	3.8
	Rhodamine B	45170		0.09	0.78	9
	Rhodamine B	45170	d	0.28	1	3.5
			b	0.02	0.98	49
	Ethyl Rhodamine B	45175		0.11	0.5	4.4
	Ethyl Rhodamine B	45175	d	0.01	1	**74**
			b	0.26	1	3.8
	Acriflavin	46000	d	0.075	0.47	6.2
	Acridine Orange	46005	d	0.14	1	7.1
	Acridine Yellow G	46025	d	0.13	1	7.8
	Safranine O	50240	b	0.01	0.27	20.3
	Nile Blue	51180	b	0.03	0.4	15.1
Cationic dyes, pH-independent	Madder Red	12245	b	0.01	0.1	**14**
	Straw Yellow	12719	b	0.04	0.61	17.2
			c	0.01	0.49	**37**
	Thionin	52000	d	0.04	0.40	9.9
	Azur A	52005	d	0.05	0.44	9.6
	Azur B	52010	d	0.03	0.42	**13**
	Methylene Blue	52015	d	0.05	0.36	6.7
	Azur C	52002	d	0.04	0.47	11.8
Disperse dyes	Celliton Blue	61505	d	1.00	0.73	0.7
	Solvent Blue 63	61520	e	1.00	1.00	1
	Solvent Blue 36	61551	e	1.00	1.00	1
	Disperse Blue 1	64500	d	0.82	0.34	0.4
Direct dyes, anionic, pH-independent	Chicago Blue RW	24280		0	0	
	Sirius Grey V	25040		0	0	
	Chlorantine Fast Red	29065		0	0	
	Sirius Supra Blue BRR	34140		0	0	
	Sirius Light Green BB	34270		0	0	
	Sirius Light Grey R	35870		0	0	
Zwitterion dyes	Methyl Red	13020		0	0	
	Metanil Yellow	13065	b	0.34	0.76	2.3
	Orange IV	13080	b	0.32	0.67	2

Bold: the best selectivities.

Table 2
Lignin and carbohydrate content (%) of test fibres

Content, %	*L* fibres	*H* fibres	*C* fibres
Klason lignin	2.41	0.11	0.55
Xylose	2.34	3.03	Traces

(*H*). Cellulose prepared from this pulp is used as an example of pure cellulose (*C*). The lignin, cellulose and hemi-cellulose contents of these fibres are given in Table 2.

The optical reflectance spectra of the sheets were measured using a McBeth Color Spectrophotometer over a wavelength range of 360 nm–740 nm for all stained and unstained preparations. Dye absorption was calculated from reflectance values for the whole measuring region according to the Kubelka–Munk equation.

$$R_\infty = R_{vz}/R_0$$

$$\text{Absorbance } K/S = (1 - R_\infty)^2/2R_\infty, \quad (1)$$

where R_{vz} is the reflectance of the stained fibres and R_0 is the reflectance of the unstained fibres.

The results are displayed in Tables 3–5.

2.4. Evaluation of dye affinity using model substances

We tested the affinity of selected dyes for isolated non-fibrous components of pulps.

To represent hemi-cellulose we used Xylan (Roth); Galactan (Aldrich-Chemie); Mannan and Polygalacturonic acid (Sigma); Apple pectin, degree of esterification: 70–75% (Fluka).

To represent lignin we used softwood kraft lignin powder, isolated from black liquors (KCL, Finland) and Novolac resin (laboratory synthesised).

The results are shown in Table 6.

3. Results

3.1. Evaluation of dye affinity using capillary rise

Evaluation of dye affinity using capillary rise (Table 1), which is in fact a comparison of the affinity of dyes for the lignin-containing and lignin-free fibres, shows that Ethyl Eosin and tested cationic dyes have a higher affinity for lignin-containing fibres than for lignin-free fibres. Some of the tested oil-soluble dyes and some zwitterionic dyes have not much more affinity for lignin than for lignin-free fibres. Direct dyes, which are known to have an affinity for cellulose [2] proved to have high affinity for both types of tested fibres.

If we compare the dyes of similar structure in Table 1, we see the following affinity series for them:

1. Sudan Orange G (C.I. 11920) < Sudan Red G (C.I. 12150) < Citrus Red (C.I. 12156);
2. Eosin (C.I. 45380) < Ethyl Eosin (C.I. 45386);
3. Orange IV (13080) < Metanil Yellow (13065);
4. Rosolic acid (data not shown) <<< Para-rosaniline (C.I. 42500) < Crystal Violet (C.I. 42555);
5. Rhodamine B (C.I. 45170) < Ethyl Rhodamine B (C.I. 45175);
6. Acriflavin (C.I. 46000) < Acridine Orange (C.I. 46005) < Acridine Yellow G (C.I. 46025);

Table 3
Staining of reference fibres with dyes having affinity for lignin (solvent: 0.01 M pH = 5 acetate buffer)

Dyes, concentration	C.I.	λ_{max} (nm)	Absorbance Ku–Mu at λ_{max}			Selectivity			
			C	*H*	*L*	*H*–*C*	*L*–*H*	*H*/*C*	*L*/*H*
Anionic									
Ethyl Eosin, 5×10^{-4} mol/L[a]	45386	540	0.008	0.001*	0.025	−0.007	0.024	0.1	25
Cationic									
Madder Red, 5×10^{-5} mol/L	12254	510	0.056	0.023	0.593	−0.033	0.570	0.4	26
Straw Yellow, 5×10^{-4} mol/L[a]	12719	400	0.010	0.004	0.091	−0.006	0.087	0.4	23
Auramine O, 5×10^{-4} mol/L[a]	41000	450	0.007	0.002	0.292	−0.005	0.290	0.3	146
Para-rosaniline, 5×10^{-4} mol/L[a]	42500	550	0.059	0.056	1.137	−0.003	1.081	0.9	20
Crystal Violet, 5×10^{-5} mol/L	42555	590	0.001*	0.001*	2.819	0.000	2.818	1.0	**2820**
Ethyl Rhodamine B, 5×10^{-5} mol/L	45175	570	0.024	0.001*	1.686	−0.023	1.685	0.0	**1690**
Safranine O, 5×10^{-5} mol/L	50240	530	0.001*	0.001*	0.499	0.000	0.468	1.0	499
Azure B, 5×10^{-5} mol/L	52010	650	0.001*	0.001*	1.271	0.000	1.271	1.0	**1270**
Methylene Blue, 5×10^{-5} mol/L	52015	670	0.001*	0.001*	1.530	0.000	1.530	1.0	**1530**
Acridine Orange, 1×10^{-4} mol/L	46005	490	0.446	1.022	2.331	0.576	1.309	2.3	2.3
Nile Blue, 5×10^{-5} mol/L	51180	630	0.272	0.084	2.022	−0.188	1.938	0.3	24

C = cellulose, *H* = kraft softwood pulp treated with $KMnO_4$, *L* = kraft pulp. *If the absorbance was zero than the figure 0.001 was selected to express the selectivity ratio. Bold: the best selectivities.

[a] Addition of 20% of EtOH.

Table 4
Staining of reference fibres with the dyes having affinity for hemi-cellulose (solvent: 0.01 M pH = 5 acetate buffer)

Dyes, concentration	C.I.	λ_{max} (nm)	Absorbance Ku–Mu at λ_{max}			Selectivity			
			C	*H*	*L*	*H*–*C*	*L*–*H*	*H*/*C*	*L*/*H*
Alcian Blue–pyridine variant, 5×10^{-5} mol/L		620	0.123	0.808	0.933(630 nm)	**0.686**	0.125	**6.6**	1.2
		670	0.110	0.700	0.898(680 nm)	**0.590**	0.198	**6.4**	1.3
		670/620	0.894	0.866	0.962	−0.028	0.096	1.0	1.1
Alcian Blue, 1×10^{-4} mol/L	74240	620	0.323	0.442	0.728	0.119	0.286	1.4	1.6
		680	0.203	0.348	0.615	0.145	0.267	1.7	1.8
		680/620	0.628	0.787	0.845	0.159	0.058	1.3	1.1
Cu(II) tetraaza phthalocyanine, 1×10^{-4} mol/L[a]		610	0.228	0.366(600 nm)	0.782	0.138	0.416	1.6	2.1
		660	0.150	0.259	0.708	0.109	0.449	1.7	2.7
		660/610	0.658	0.708	0.905	0.050	0.195	1.1	1.3
Astra Blue, 5×10^{-5} mol/L		620	0.376	1.247	1.404	**0.871**	0.157	**3.3**	1.1
		670	0.265	1.100	1.497	**0.835**	0.397	**4.2**	1.4
		670/620	0.705	0.882	1.066	0.177	0.184	1.3	1.2

C = cellulose, *H* = kraft softwood pulp treated with $KMnO_4$, *L* = kraft pulp. Bold: the best selectivities.
[a] Addition of 50% acetic acid.

7. Methylene Blue (C.I. 52015) < Azure A (C.I. 52005) < Thionin (C.I. 52000) < Azur C (C.I. 52002) < Azure B (C.I. 52010).

Let us present only those functional groups that are different in dyes of the affinity series mentioned above and that seem to be the cause of differences in the affinity of mentioned dyes for tested fibres:

1. $-OH < -OCH_3 < (-OCH_3)_2$;
2. $-COOH <$ COOEt;
3. *para*-$SO_3^- <$ *meta*-SO_3^-;
4. $(-OH)_3 <<< (-NH_2)_3 < (-N(CH_3)_2)_3$;
5. $-COOH <$ COOEt;
6. $(-NH_2)_2 < (-N(CH_3)_2)_2 < (-NH_2)_2 + (-CH_3)_2$;
7. $(-N(CH_3)_2)_2 < -NH_2 + -N(CH_3)_2 < (-NH_2)_2 < -NH_2 + -NHCH_3 < -NHCH_3 + -N(CH_3)_2$.

The first two series of dyes do not contain positively charged structures. In the first series, the dyes contain none, one, and two methoxyl groups, respectively. In this series of oil-soluble dyes, the number of methoxyl groups is the most influential factor regarding the affinity for lignin.

In the second and the fifth series of dyes, the ester group gives rise to higher affinity for lignin than the free carboxyl group.

The third series contains two isomeric zwitterionic dyes having a low and not very different relative affinity for lignin. They contain a sulphonate group and an arylamino group.

Table 5
Staining of reference fibres with dyes having affinity for cellulose (solvent: 0.01 M pH = 5 acetate buffer)

Dyes, concentration	C.I.	λ_{max} (nm)	Absorbance Ku–Mu at λ_{max}			Selectivity			
			C	*H*	*L*	*H*–*C*	*L*–*H*	*H*/*C*	*L*/*H*
Zwitterionic direct									
Direct Blue 1, 5×10^{-5} mol/L	24410	640	0.175	0.064	0.100	−0.111	0.036	0.4	1.6
Anionic									
Direct Red 81, 5×10^{-5} mol/L	28160	400	0.239	0.012	0.036	−0.227	0.024	**0.1**	3.0
		530	0.939	0.160	0.274	**−0.779**	0.114	**0.2**	1.7
Direct Yellow 50, 5×10^{-5} mol/L	29025	420	1.566	0.635	0.659	**−0.931**	0.024	0.4	1.0
Direct Black 71, 5×10^{-5} mol/L	25040	640	0.198	0.152	0.236	−0.046	0.084	0.8	1.6
Direct Black 75, 5×10^{-5} mol/L	35870	640	0.046	0.010	0.063	−0.036	0.053	**0.2**	6.3
Direct Black 71, 5×10^{-5} mol/L[a]	25040	640	0.003	0.001	0.015	−0.002	0.014	0.3	15.0
Direct Black 75, 5×10^{-5} mol/L[a]	35870	640	0.099	0.054	0.134	−0.045	0.080	0.5	0.4
Cu(II) Phthalocyanine tetrasulfonate, 5×10^{-4} mol/L		600	0.068	0.011	0.008	—0.057	−0.003	**0.2**	0.7

C = cellulose, *H* = kraft softwood pulp treated with $KMnO_4$, *L* = kraft pulp. Bold: the best selectivities.
[a] Addition of 20% of EtOH.

Table 6
Staining of model substances with selected dyes, Ku–Mu units[a]

Dyes	Color index no., C.I.	λ (nm)	Xylan	Polygalacturonic acid	Pectin	Kraft lignin – softwood	Novolac resin	L/X[b]	N/X[b]
Crystal Violet	42555	590	1.389	0.900	0.933	3.329	5.554	2.4	4.0
Ethyl Rhodamine B	45175	570	0.797	0.421	0.358	1.770	1.885	2.2	2.4
Methylene Blue	52015	670	0.279	0.005	0.009	0.848	0.709	3.0	2.5
Safranine	50240	530	0.201	0.042	0.008	0.602	0.517	3.0	2.6
Acridine Orange	46005	490	0.711	0.321	0.160	3.820	5.936	2.4	8.4
Astra Blue		600	3.273	2.480	2.990	0.935	0.762	0.29	0.23
Astra Blue		670	0.786	0.453	0.719	0.622	0.628	0.79	0.80
Astra Blue[c]			4.16	5.47	4.16	1.50	1.21	0.35	0.29
Alcian Blue pyridine variant		600	0.604	0.394	0.000	0.242	1.380	0.40	2.3
Alcian Blue pyridine variant		670	0.220	0.117	0.000	0.108	1.540	0.49	7.0
Alcian Blue pyridine variant[c]			2.75	3.37		2.24	0.90	0.89	0.33
Oil Red O	26125	520				2.285	0.063[d]		
Citrus Red	12156	520				0.083	0.058[d]		

At all tested substances, Direct Blue 1, Direct Red 81 and Direct Black 75 gave rise to absorbance of 0.002 or less.
[a] Galactan did not precipitate using our procedure. Mannan was not stained by tested dyes (absorbance of 0.002 or less).
[b] L/X – kraft lignin-to-xylan selectivity, N/X – Novolac to xylan selectivity.
[c] A_{600}/A_{670}; ratio of absorbance at 600 and 670 nm.
[d] The precipitate blocked the filtration.

The fourth series contains one (at pH = 5) uncharged (Rosolic acid) and two positively charged members. Here, the presence of positive charge has a profound influence. From the point of view of functional groups we can see that phenolic groups (cf. also Eosin) do not give rise to an affinity for lignin, whereas the amino groups do, and the affinity is greater if they are alkyl substituted. The contribution of alkyl substitution of amino groups is also evident in the sixth series. The last three series do contain a single positive charge in the structure and they are quite selective for lignin. All of them contain amino groups.

Among the tested groups of dyes, those belonging to cationic and direct dyes proved to be useful for our purpose. The best representatives of them as well as Ethyl Eosin were included in additional tests together with some phthalocyanine dyes.

3.2. Evaluation of dye affinities using optical reflectance

For better formation of dye groups, special test fibres were selected (Table 2): *L* fibres contain lignin, hemi-celluloses and cellulose, *H* fibres contain hemi-celluloses and cellulose, but little lignin, whereas *C* fibres are mostly cellulose with traces of hemi-celluloses and little lignin.

The results of staining are presented in Tables 3–5. Looking at the results, we can confirm the general perception: cationic dyes mostly have affinity for lignin-containing fibres, and only one of the anionic dyes shows that affinity to a substantial extent (Table 3). Furthermore, most dyes for which we noticed an affinity for hemi-cellulose containing fibres are cationic phthalocyanine dyes (Table 4). In the group of dyes with an affinity for *C* fibres (cellulose) we used only anionic direct dyes (Table 5).

Among the cationic dyes that show an affinity for lignin (Table 3) we noticed the highest relative selectivity using Crystal Violet (with selectivity ratio $L/H = 2820$), followed by Ethyl Rhodamine B (1690), then Methylene Blue (1530), Azure B (1270), Safranine O (500) and, finally, Auramine O (150). Other tested dyes, the anionic dye Ethyl Eosin, C.I. 45386 and the cationic dyes: Pararosaniline, C.I. 42500, Straw Yellow, C.I. 12719, Madder Red, C.I. 12245, and Nile Blue, C.I. 51180, show a weaker selectivity for lignin, with a ratio of no more than 20–26. Nile Blue also shows some selectivity for cellulose and less for the hemi-celluloses. Acridine Orange, C.I. 46005, on the other hand, shows a low selectivity ratio, $L/H = 2.3$ and $H/C = 2.3$, as well. The selectivity ratios presented in Tables 3–5 are not to be compared with those in Table 1 since they were obtained by different methods.

The dyes that show selectivity for the hemi-cellulose part of fibres (Table 4) are cationic phthalocyanine dyes. A special characteristic of these dyes is that they have two absorption peaks when they are adsorbed on fibres, but not in the water solution. One peak is positioned near 620 nm and the other near 670 nm. The highest relative selectivity for hemi-celluloses has Alcian Blue pyridine variant with $H/C = 6.6$ and 6.4, followed by Astra Blue (3.3 and 4.2), Acridine Orange (2.3, Table 3), and Cu(II) tetraaza phthalocyanine (1.6 and 1.7). These dyes have slightly more affinity for lignin than for hemi-celluloses, Cu(II) tetraaza phthalocyanine (2.1 and

2.7) ~ Acridine Orange (2.3) > Alcian Blue (1.6 and 1.8) > Alcian Blue pyridine variant (1.2 and 1.3) > Astra Blue (1.1 and 1.4). No dye was found more selective for hemi-celluloses than Alcian Blue pyridine variant and Astra Blue.

For all phthalocyanine dyes the ratio of both absorption peaks in the spectrum of each dye is also calculated (Table 4). This ratio increases in the series $C < H < L$, with Alcian Blue pyridine variant as an exception, $H < C < L$.

Since cationic dyes have an appreciable affinity for lignin, we first selected from among those having an affinity for cellulose the direct dyes having as few cationic groups as possible. The C fibres were the most intensively stained with Direct Red 81 and Direct Yellow 50, followed by Direct Black 71 and Direct Blue 1 (Table 5). The most selective for cellulose are Direct Red 81, Direct Black 75, and Cu(II) Phthalocyanine tetrasulphonate. Unfortunately, the latter two dyes stain cellulose only faintly.

3.3. Evaluation of dye affinity using model substances

To ensure the correctness of the conclusions drawn above, we tested the affinity of some dyes for some polysaccharides making up hemi-cellulose as well as for kraft softwood lignin extracted from black liquors and a phenol–formaldehyde condensation product (Novolac resin) as a lignin model (Table 6). Galactan could not be precipitated using our method. Mannan was not stained by any of the tested dyes.

During lignin precipitation, tested oil-soluble dyes stained lignin relatively effectively; Novolac resin was stained too, but its precipitate blocked the filter surface and only a small part of it was available for measurement (Table 6).

The dyes considered selective for lignin stained lignin and Novolac resin, whereas they also stained the polysaccharides, but to a lower extent:

Crystal Violet: Novolac > lignin > xylan > pectin > polygalacturonic acid

Ethyl Rhodamine B: Novolac > lignin > xylan > polygalacturonic acid > pectin

Methylene Blue: lignin > Novolac > xylan > pectin > polygalacturonic acid

Safranine: lignin > Novolac > xylan > polygalacturonic acid > pectin

Acridine Orange: Novolac > lignin > xylan > polygalacturonic acid > pectin

This is also reflected in lignin-to-xylan (L/X) and Novolac resin-to-xylan (N/X) selectivity, which amounts to between 2 and 4. Acridine Orange and Crystal Violet stained lignin and Novolac resin the most intensively. They also have the highest N/X selectivities, especially Acridine Orange. The dyes selective for lignin stained pectin and the polygalacturonic acid to a lower degree than xylan.

The dyes found to be selective for hemi-cellulose, Astra Blue and Alcian Blue pyridine variant stained everything except mannan. Their absorption peaks (at 600 and 670 nm) relate to L/X or N/X selectivity, being the most selective to xylan. Their intensities are at:

600 nm peak: xylan > pectin > polygalacturonic acid > lignin > Novolac resin,

670 nm peak: xylan > pectin > Novolac resin > lignin > polygalacturonic acid.

The peak height ratio A_{600}/A_{670} (ratio of peak heights at 600 nm and 670 nm) in both cases reflects the highest selectivity for polygalacturonic acid:

Astra Blue: Polygalacturonic acid > pectin = xylan > lignin > Novolac

Alcian Blue pyridine variant: Polygalacturonic acid > xylan > lignin > Novolac >>> pectin.

The peak height ratio A_{600}/A_{670} of the dyes Astra Blue and Alcian Blue pyridine variant bound to fibres is thus an indicator for the presence of anionic, i.e. mainly carboxylic groups on the surface of the fibres.

Direct dyes did not stain any test substance.

There is an important dependence on the preparation method for testing the dye affinity for lignin. Films formed from dioxane or acetone solution of lignin or Novolac resin were much less intensively stained (results not shown) than if stained during precipitation.

4. Discussion

4.1. Evaluation of dye affinity using capillary rise (Table 1)

This evaluation raises several questions. On the one hand, the oil-soluble dyes were expected to stain the aromatic structures of lignin effectively. They intensively stain lignin and its model compound in solution (Table 6), but much less in film, and they do not effectively stain lignin contained in kraft fibres of kappa No. 30. This indicates, on the one hand, that the residual or the re-deposited lignin in fibres is so compact that the dyes cannot penetrate it. This compactness is quite probable since the tests were performed well below the glass transition temperature (T_g) of lignin, which is 130–150 °C [11]. On the other hand, it indicates that the hydrophobic parts of lignin in fibres are not exposed to the surrounding solution being protected by the hydrophilic structures not stained by oil-soluble dyes. Thus, the hydrophobicity of the dyes does not seem to be of crucial importance in dying lignin in the fibres.

Another question is whether the negatively charged groups in lignin are responsible for the difference in affinity of anionic vs. cationic dyes. At first glance the answer is *yes*, since cationic dyes beyond all doubt have a higher affinity for the lignin-containing fibres than the anionic dyes.

But there is also another phenomenon. Let us consider the affinity series of dyes of similar structure presented in the Results section. The presence of positive charge is no doubt of highest importance for the dye affinity for lignin but it is obviously not the only prerequisite. It seems to be followed by two additional features. One of them is a high electron density in some peripheral functional groups, especially when enhanced by methyl or other alkyl groups. The other one is the steric shielding contributed by the aforementioned methyl or other alkyl groups. Oil-soluble dyes indicate that the hydrophobicity of methyl groups is of minor importance.

The consequence of these additional effects was demonstrated earlier. For the pair of dyes Eosin < Ethyl Eosin it was observed that non-ionic acidic sorbents (e.g. silica) as well as non-ionic acidic solvents (e.g. phenols) have more affinity for Ethyl Eosin than for Eosin [12]. From these facts it follows that non-ionised phenolic groups in lignin also contribute importantly to the affinity of dyes for lignin, forming hydrogen bonds with them.

To be effective, the phenolic groups of lignin must lie on the lignin surface accessible to a water solution of dye. This is quite probable since during the kraft delignification process the phenolic groups of lignin are largely dissociated at the high pH of the black liquor and thus tend to be oriented towards the fibre–water interface where they remain fixed.

Now we can explain the reasons for the affinity of dyes for lignin. Some of the lignin phenolic groups placed at the fibre–water interface ionise in contact with cationic dyes to enable a strong ionic interaction. But several, if not most, of the lignin phenolic groups interact as non-ionised acidic groups, i.e., by donating hydrogen to form hydrogen bonds with electron-rich groups in the dyes.

4.2. Evaluation of dye affinity using model substances (Table 6)

The above explanation is also supported by the evaluation of dye affinity using model substances. The affinity of dyes selective for lignin in the series Lignin and Novolac > xylan > pectin and polygalacturonic acid shows beyond any doubt that the dyes' affinity for phenolic groups is much higher than their affinity for carboxylic groups, which are the characteristic functional groups of pectin and polygalacturonic acid and largely dissociated, up to >90% at pH 5, where the tests were performed.

There is also some influence of steric hindrance in lignin as well as in dyes. In lignin, the overall steric hindrance at the phenolic OH group is softwood kraft lignin < Novolac resin. Comparing the absorbencies of Safranine and Methylene Blue on test substances, the series is softwood kraft lignin > Novolac resin. The series is different for the other cationic dyes: it is Novolac resin > softwood kraft lignin (Table 6). So, the steric hindrance at the phenolic OH group of lignin may or may not be important.

The situation is more coherent among the dyes. Here, alkyl substituents on the nitrogen or oxygen atoms give rise on the one hand to the higher electron density on these atoms, enhancing the strength of hydrogen bonds. On the other hand, they give rise to a lower number of possible hydrogen bonds with water as well, as they sterically shield the hydrogen bonds formed between lignin and dyes from the water solution. This effect adds additional stability to the hydrogen bonds formed.

4.3. Staining the L fibres (Table 3)

Subsequent evaluation of selected dye affinities by optical reflectance using as test fibres the lignin, hemi-celluloses and cellulose containing fibres (*L* fibres), hemi-celluloses and cellulose containing fibres (*H* fibres), and cellulose containing fibres (*C* fibres); Table 3, supports the above conclusions. Additional testing (Table 3) shows that *N*-alkyl-triphenyl-methane (Crystal Violet), xanthene (Ethyl Rhodamine B), and thiazine dyes (Azure B, Methylene Blue) having a delocalised positive charge in their aromatic system and basic groups on their periphery stain lignin better than the azo dyes (Madder Red, Straw Yellow) having the localised positive charge but no basic groups on their periphery.

There is the question of whether these dyes are ionised on fibres or not. The cationic dyes Madder Red, C.I. 12,245, Straw Yellow, C.I. 12,719, Azure B, C.I. 52,010, and Methylene Blue, C.I. 52,015, contain a quaternary ammonium structure that is ionised at any pH level. The dyes Acridine Orange, C.I. 46,005, Para-rosaniline, C.I. 42,500, Crystal Violet, C.I. 42,555, Ethyl Rhodamine B, C.I. 45,175, Safranine O, C.I. 50,240, and Nile Blue, C.I. 51,180, contain primary and/or tertiary ammonium structures that can lose their ionisation in strongly alkaline solutions. At pH = 5, one of their functional groups is ionised. Ethyl Eosin, on the other hand, exists at pH 5 partly in non-ionised form and largely as a mono-anion [12]. Only the ionised form absorbs strongly at λ_{max} presented in Table 3.

4.4. Staining the H fibres (Table 4)

We have to bear in mind that hemi-cellulose molecules consist of main- and side-chain polysaccharides also containing uronic acids. They carry many

free −OH and −COOH groups to bind cationic dyes. At pH 5, the carboxyl groups of uronic acids are largely dissociated, up to >90%. Cationic phthalocyanine dyes bind most effectively to hemi-cellulose and show a special but not very well-expressed selectivity for hemi-celluloses. These dyes' absorbancies of stained *L*- and *H*-fibres are almost alike, so this is another confirmation that in *L* fibres the lignin structures are covered with hemi-celluloses that are covalently bound to lignin structures. This is supported by the fact that using Alcian Blue pyridine variant, the selectivity expressed with the L/H ratio for the peak at 620 or 670 nm relates to tested fibres 1.2 or 1.3 (Table 4), or using Astra Blue it is 1.1 or 1.4, respectively, whereas testing kraft lignin and xylan (Table 6) it is 0.40 or 0.49 for Alcian Blue pyridine variant as well as 0.29 or 0.79 for Astra Blue. Comparing these values we can state that the affinity of these dyes is better for polysaccharides than for lignin. Among the cationic dyes of smaller size than the phthalocyanine dyes, Acridine Orange has a special position with its selectivity $L/H = H/C = 2.3$. To a certain point, a similar situation holds for Nile Blue staining. The phenyl rings in both dyes are condensed; they are prone to form aggregates and this seems to favour the hemi-cellulose structure. The phenyl rings in the dyes with the best selectivity for lignin are either not condensed and may perform free rotation, or the molecules are small. The phthalocyanine dyes, with their circular shape of condensed heterocyclic and phenyl rings, seem to suit the hemi-cellulose structures best. Do they (or their aggregates) fit into cavities formed on lignin removal? This question deserves a separate study.

4.5. Staining the C fibres (Table 5)

The molecular structure of tested di- or poly-azo direct dyes (Table 5) is ribbon-like. As such they are likely adsorbed on cellulose microfibril surfaces [2,4]. Actually their bond to fibres is simple adsorption but depends on the amino or OH groups present in the dye molecules that are able to form hydrogen or van der Waals bonds with primary OH groups on fibre surfaces [3,5,13,14].

Among the direct dyes it could be expected that the dyes having the most extended structure containing the most aromatic systems and the lowest SO_3/Ar ratio would be the most effective. This is not a fact, since the tetra-azo dye Direct Black 75 and the Cu(II) Phthalocyanine tetrasulphonate stain the fibres least intensively. On the other hand, the fibres are most intensively stained by those dyes having an amido group in their structure, i.e., by the di-azo dyes Direct Yellow 50 and Direct Red 81. This fact stresses again the importance of hydrogen bond-forming groups in the dye structure for their affinity for cellulose.

4.6. Differential staining

An additional purpose of the present study was also to select those dyes with absorption maxima in the visible spectrum as far as possible from each other, so that they would also be useful for differential staining. Among the dyes selective for lignin useful for the blue–violet region would be Crystal Violet ($\lambda_{max} = 590$ nm), Methylene Blue ($\lambda_{max} = 670$ nm), and Azure B ($\lambda_{max} = 650$ nm), for the red region Ethyl Rhodamine B ($\lambda_{max} = 560$ nm) and Safranine O ($\lambda_{max} = 530$ nm), and for the yellow region Auramine O ($\lambda_{max} = 450$ nm). All the hemi-cellulose selective dyes except Acridine Orange are blue. So, to stain selectively all three main components of fibres, a combination of Ethyl Rhodamine B or Safranine O, Astra Blue or Alcian Blue pyridine variant, and Direct Yellow 50, or a combination of Auramine O, Astra Blue or Alcian Blue pyridine variant, and Direct Red 81 would come into consideration. The most selective lignin stain, Crystal Violet, does not fit well into these combinations.

5. Conclusions

With the method of selectively staining the fibres we form three groups of dyes having an affinity either for lignin or for hemi-cellulose or for cellulose. The absorption peak ratio of the dyes having an affinity for hemi-cellulose indicates the presence of carboxylic groups. The explanation of bonding mechanisms between the functional groups of the dye structures and different active sites of the components on the tested fibre surfaces will help us to understand the bonding abilities between different papermaking fibres themselves and with paper additives. Selective staining can be a tool for the detailed study of fibre surfaces depending on its origin and its technology.

Appendix A

A1. Preparation of dye solutions

Dyes were dissolved in 0.01 M pH = 5 acetate buffer at concentrations of 5×10^{-5} mol/L or 5×10^{-4} mol/L (with the addition of 20% EtOH, if the dye was not sufficiently soluble in water).

A2. Preparation of fibres for staining

A2.1. Unstained fibres

A sample of 0.01 g of pulp was weighed and dispersed in deionised water. Microscope preparations were made according to the TAPPI standard method [15]. The fibres were divided into three equal parts and spread

over exactly defined round areas of 2.83 cm^2 on a glass slide. The preparations were placed on an infrared laboratory drier at 50 °C for 20 min. Each sheet consisted of 1.50 ± 0.05 mg of air-dry fibres.

A2.2. Staining procedure

The same amount of fibres was weighed for staining. The fibres were placed on a watch glass and 2 ml of dye solution was added. The ratio of dye to fibre was 10^{-5} mol/g or 10^{-4} mol/g, depending on the dye concentration. Reaction time was 5 min. During this time the fibres were well dispersed and mixed with the dye solution using a dissecting needle to enable all the fibres to contact the dye solution. At the end of the reaction time the fibre suspension was placed on a small stainless steel screen and quickly washed with cold water. The procedure was continued as described for unstained preparations.

A3. Staining procedure for polysaccharides

Solutions (2%) of each polysaccharide were prepared in deionised water. A polysaccharide solution (0.25 ml) was placed in a glass tube to which 2.25 ml of dye solution was added. The time for staining was 5 min. Then 2.1 ml of Na_2SO_4 (10% solution in 10% MeOH) was added to the solution and the polysaccharide was allowed to precipitate completely. The polysaccharide precipitate was then filtered through a Nucleopore Polycarbonate membrane and washed well with the Na_2SO_4 solution. Then the precipitate on the polycarbonate filter was carefully placed between 1 in. × 3 in. glass slides for measurement of the color intensity.

The same procedure was used for the unstained polysaccharide precipitates.

A4. Staining procedure for lignin

Softwood kraft lignin was dissolved to form a 1% dioxane solution. The Novolac resin was dissolved in acetone. Of the lignin solution, 0.2 ml was placed in a glass tube, mixed with two successive 0.5 ml portions of 5×10^{-5} M dye solution in acetate buffer (0.01 M, pH 5). After 5 min, 7 ml of deionised water was added to precipitate the lignin sample. The precipitate was then filtered through the polycarbonate filter and treated as above.

A5. Staining the lignin models with oil-soluble dyes

Two oil-soluble dyes were selected to test the staining ability of lignin models: Oil Red O, C.I. 26,125 and Citrus Red, C.I. 12,156. The dyes were dissolved in 70% EtOH. The procedure of precipitating the stained solution of lignin was the same as described above, using 50% EtOH as the precipitating agent. The results are displayed in Table 6.

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