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DETERMINATION OF ANTHRAQUINONES IN PULPING MATERIALS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING ON-LINE POST-COLUMN DERIVATIZATION

NOBUTOSHI KIBA*, MOTOHARU TAKAMATSU and MOTOHISA FURUSAWA
Department of Chemistry, Faculty of Engineering, Yamanashi University, Kofu-shi 400 (Japan)
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SUMMARY

A selective assay for anthraquinones in black liquors and papers was developed based on the reduction of anthraquinones to anthrahydroquinones. Anthraquinones were extracted from the samples and portions of the extracts were analysed by high-performance liquid chromatography on a column of Jasco Finepak SIL C_{18} (10 μm). Elution was effected with acetonitrile–0.3 m*M* hydrochloric acid (70:30) at 0.75 ml/min and the eluate was mixed with 0.2 *M* sodium dithionite in 0.5 *M* sodium hydroxide (0.50 ml/min) and water (0.25 ml/min); the absorbance at 500 nm was measured. The method was suitable for the range 0.3–80 nmol in 50-μl volumes of sample injected.

INTRODUCTION

Anthraquinone (AQ) and anthraquinone derivatives catalyse delignification and stabilize carbohydrates during the alkaline pulping of wood[1–3], which has led to industrial applications in pulping mills. As a result, there is great interest in monitoring the levels of these compounds in the spent liquors and the final pulp products and a simple and selective method for their determination was required. Several methods have been applied to this problem, including gas chromatography[4,5], liquid chromatography[6–11], polarography[12] and spectrophotometry[13].

Selective spectrophotometric determinations of AQ have been based on measurement of the red colour generated by the reduction of AQ in alkaline solution[14–16]. Anthraquinone derivatives such as 2-methylanthraquinone (MAQ), 2-ethylanthraquinone (EAQ), anthraquinone-2-sulphonic acid (AQS) and anthraquinone-2-carboxylic acid (AQC) are also coloured. Therefore, mixtures of anthraquinones have to be separated by some technique prior to spectrophotometric determination of the individual components.

The reduction products of anthraquinones are extremely susceptible to air oxidation. Therefore, batchwise operations have to be performed under an inert atmosphere[14]. A continuous-flow system is well suited to the detection of such unstable species.

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In this work, reversed-phase high-performance liquid chromatography (HPLC) was used to analyse the anthraquinones utilizing post-column derivatization with sodium dithionite.

EXPERIMENTAL

Apparatus and reagents

A schematic diagram of the HPLC system is shown in Fig. 1. Sample solutions were analysed at room temperature on a column (250 × 4.6 mm I.D.) (H) of Jasco Finepak SIL C_{18} (10 μm). The mobile phase, acetonitrile–0.3 m*M* hydrochloric acid (70:30) (B), was pumped by a piston pump (BIP-1; Jasco, Tokyo, Japan) at 0.75 ml/min. The eluate was mixed with 0.2 *M* sodium dithionite in 0.5 *M* sodium hydroxide (0.50 ml/min) (C) and water (0.25 ml/min) (A). The colour was allowed to develop in PTFE tubing (100 cm × 0.5 mm I.D.) (I) at 30°C (M). The absorbance at 500 nm was measured with a spectrophotometer (Uvidec 100-IV; Jasco) fitted with a flow cell (length 10 mm, volume 8 μl) (J). A syringe-loading injector with a 50-μl loop (KHP U1 130; Kyowa Seimitsu, Tokyo, Japan) (G) and a Model FBR-251A recorder (TOA Electronics, Tokyo, Japan) (K) were used.

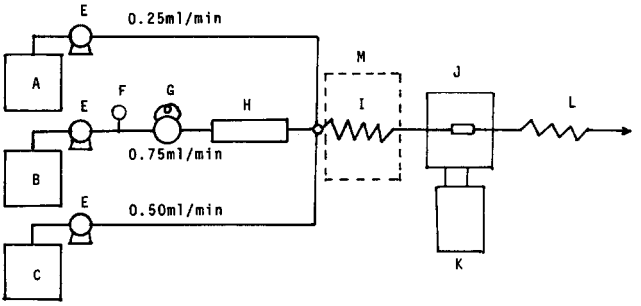

Fig. 1. Schematic diagram of the HPLC system for the determination of anthraquinones by post-column derivatization. A = Water; B = eluent (acetonitrile–0.3 m*M* hydrochloric acid, 70:30); C = reagent solution (0.2 *M* sodium dithionite in 0.5 *M* sodium hydroxide); E = pump; F = pressure gauge; G = injector; H = separation column (Finepak SIL C_{18} 10 μm, 250 × 4.6 mm I.D.); I = mixing coil (100 cm × 0.5 mm I.D.); J = spectrophotometric detector (Jasco Uvidec 100-IV, flow cell length 10 mm, cell volume 8 μl, wavelength 500 nm); K = recorder; L = back-pressure coil (500 cm × 0.25 mm I.D.); M = thermostat (30°C).

All reagents were of the highest purity available. Anthraquinones were purchased from Tokyo Kasei (Tokyo, Japan). Sodium dithionite and acetonitrile were obtained from Nakarai Chemicals (Kyoto, Japan).

Procedure

Analysis of black liquor. Black liquor (30 ml) was extracted twice with cyclohexane (50 ml) in a 200-ml separating funnel by shaking for 5 min on a shaker and leaving for 10 min. The total extracts were evaporated using a rotary evaporator under reduced pressure and the resulting residue was transferred into a 25-ml volumetric flask with acetonitrile (10 ml). For the complete extraction of AQS and AQC

another aliquot of the liquor (30 ml) was applied directly to an XAD-2 column (100 mesh; 300 × 8 mm I.D.), which was then washed with water until the washings were colourless. Elution was carried out with acetonitrile (10 ml). The eluate was collected in the 25-ml volumetric flask and the volume was adjusted to 25 ml with acetonitrile. A portion of the solution was filtered with a membrane filter (0.45 μm) and analysed by HPLC.

Analysis of papers. The sample (*ca.* 10 g) was cut into strips, placed a Soxhlet extractor and extracted with methylene chloride for 1 h. The extract was evaporated using a rotary evaporator under reduced pressure. The residue was transferred into a 10-ml volumetric flask with acetonitrile. The solution was analysed by HPLC in a similar manner to above.

RESULTS AND DISCUSSION

Reduction of anthraquinones

A number of aqueous organic solvent systems were screened for their ability to dissolve anthraquinones, sodium dithionite, sodium hydroxide and colored products. Aqueous solutions of methanol, ethanol, isopropanol, 2-ethoxyethanol, tetrahydrofuran, 1,4-dioxane, acetone, acetonitrile or dimethyl sulphoxide were satisfactory solvent systems. Of these, a 50% aqueous solution of acetonitrile, isopropanol or acetone was suitable for the colour development (Table I). In these solutions the colour was fully developed immediately on mixing, but the colour intensity in the aqueous isopropanol and aqueous acetone was unstable.

The reduction products absorbed light at about 420 nm and about 500 nm. In this method a detection wavelength of 500 nm was chosen because of the selectivity. All compounds showing higher absorption at about 400 nm, such as fluorescent whitening agents in papers and unknown components in black liquors, could not always be separated completely from the anthraquinones by the HPLC.

The dithionite concentration required was more than 30 times that of anthraquinones in order to attain full colour development in aqueous acetonitrile. A sodium dithionite concentration of 0.2 *M* was chosen. The stability of dithionite increases

TABLE I

EFFECT OF ORGANIC SOLVENT ON THE COLOUR DEVELOPMENT OF THE SODIUM SALT OF ANTHRAHYDROQUINONE

Anthraquinone, $5.0 \cdot 10^{-5}$ *M*; reaction temperature, 30°C.

50% aqueous organic solvent	*Absorbance at 500 nm*
Acetonitrile	0.250
Isopropanol	0.250
Acetone	0.249
Tetrahydrofuran	0.232
1,4-Dioxane	0.219
2-Ethoxyethanol	0.208
Ethanol	0.205
Methanol	0.183
Dimethyl sulphoxide	0.182

with increase in basicity; a freshly prepared 0.2 *M* solution of sodium dithionite in 0.5 *M* sodium hydroxide remained stable for 24 h. When 0.2 *M* sodium dithionite in 0.5 *M* sodium hydroxide solution was mixed with aqueous acetonitrile at below 20°C, ghost peaks was observed because of incomplete mixing in the mixing coil. This problem was almost overcome at temperatures above 25°C.

The peak height was constant between 25 and 45°C. The effect of the length of the mixing coil (0.5 mm I.D.) on the peak height was studied over the range 0.5–2.0 m at a flow-rate of 1.5 ml/min (dithionite solution 0.5 ml/min, aqueous acetonitrile 0.75 ml/min and water 0.25 ml/min). The results are shown in Fig. 2. The decrease in peak height with increasing coil length is due to the peak broadening. With a 0.5-m coil, the baseline was unstable owing to incomplete mixing. The peak area was not dependent on the length of the mixing coil up to 3.0 m.

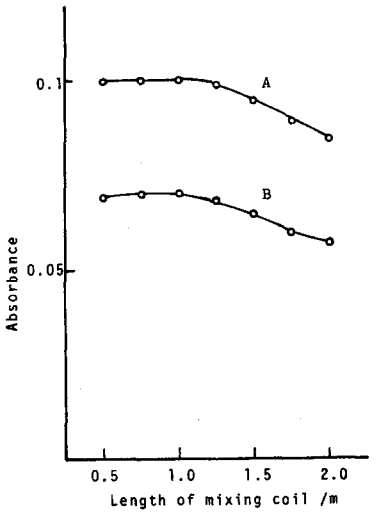

Fig. 2. Effect of length of mixing coil on peak height. Mixing coil, 0.5 mm I.D.; flow-rate, 1.5 ml/min; sample, 10 nmol per 50 µl. A, Anthraquinone; B, anthraquinone-2-carboyxlic acid.

Separation

The separation of the anthraquinones was investigated by using HPLC and the continuous-flow reaction system. A clear separation was obtained by using as the mobile phase acetonitrile–0.3 m*M* hydrochloric acid (70:30) at a flow-rate of 0.75 ml/min. As the eluent was not well mixed with the reagent solution, water was added at a flow-rate of 0.25 ml/min. The separation parameters of the anthraquinones are given in Table II. Although the number of theoretical plates and the asymmetry factor for AQS were poor, the effect of dispersion in the mixing coil on the chromatographic resolution was negligibly small for all the anthraquinones. 9,10-Phenanthraquinone (PQ) behaves similarly to the anthraquinones and its reduction product absorbs light at 500 nm[14]. PQ did not interfere in the determination of the anthraquinones, because its retention time was 8.50 min under the chromatographic conditions used.

A calibration graph of peak area against amount of anthraquinones in the

TABLE II

SEPARATION PARAMETERS OF ANTHRAQUINONES

k' = Capacity factor; N = number of theoretical plates; R_s = resolution; α = separation factor; A_s = asymmetry factor.

Anthraquinone	*Retention time (min)*	k'	$N(\times 1000)$	R_s	α	A_s
AQS	3.0	−0.17	0.46			1.70
				7.41	−6.88	
AQC	7.8	1.17	1.93			1.06
				5.44	1.95	
AQ	11.6	2.28	3.96			1.00
				3.23	1.32	
MAQ	14.4	3.00	4.02			1.05
				2.37	1.22	
EAQ	16.9	3.67	3.90			1.05

aliquot injected (50 μl) covering the range 0.3–80 nmol was linear and passed through the origin. The peak areas of MAQ, WAQ, AQS and AQC relative to AQ = 100% were 93.3, 87.7, 83.4 and 76.7%, respectively. The detection limits of AQ, MAQ, EAQ, AQS and CAQ were 0.2, 0.2, 0.2, 0.1 and 0.2 nmol, respectively.

Recovery

Anthraquinones was recovered from artificial black liquor containing lignin (1 g) and glucose (1 g) in 1 *M* sodium hydroxide solution by the extraction procedure described above. The results are shown in Table III. The small losses observed in the experiments relating to small amounts of anthraquinones are without practical significance.

This extraction procedure is too time consuming to be suitable for routine analysis. The acetonitrile extraction method[6] was rapid, but the extract was strongly basic and damaged the ODS column. It was preferable to use a water-immiscible solvent such as cyclohexane, chloroform or ethylene chloride. Chloroform and ethylene chloride produced emulsions that were not sharply separated.

TABLE III

RECOVERY OF ANTHRAQUINONES FROM ARTIFICAL BLACK LIQUOR

Anthraquinone	*Added (μmol)*	*Recovered (μmol)*	*Anthraquinone*	*Added (μmol)*	*Recovered (μmol)*
AQ	25.1	25.1	AQS	24.7	24.2
	2.51	2.48		2.47	2.40
	0.63	0.62		0.62	0.60
MAQ	24.8	24.8	AQC	24.8	24.3
	2.48	2.46		2.48	2.41
	0.62	0.61		0.62	0.60
EAQ	25.0	24.9			
	2.50	2.48			
	0.63	0.62			

Application

Table IV gives the results of the HPLC determination of anthraquinones in black liquor and papers. The extracts from papers fluoresced blue, which is attributed to the fluorescent whitening agents. Fig. 3 is a chromatogram of the extract from paper I. The anthraquinone peaks were sharp and there were no peaks based on other components.

TABLE IV

ANALYTICAL RESULTS FOR ANTHRAQUINONES IN BLACK LIQUOR AND PAPERS

Sample	*Concentration of anthraquinone (ppm)*				
	AQ	*MAQ*	*EAQ*	*AQS*	*AQC*
Liquor (60.0 ml)	103*	35*	24*	17*	17*
Paper I (8.524 g)	8.8	3.0	2.7	1.9	1.9
Paper II (11.722 g)	2.9	–	–	–	–

* μg/ml sample.

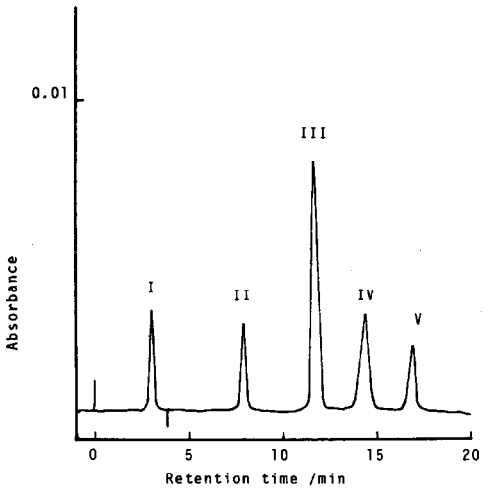

Fig. 3. Chromatogram of extract from paper I (Table IV). Paper I (8.524 g) was extracted with methylene chloride, the extract was evaporated and the residue dissolved in 10 ml of acetonitrile. A 50-μl portion of the solution was injected. Conditions as Fig. 1. I = AQS; II = AQC; III = AQ; IV = MAQ; V = EAQ.

CONCLUSIONS

A post-column derivatization reaction is proposed for the HPLC determination of anthraquinones. Optimum reagents concentrations and chromatographic conditions were evaluated. The results demonstrate the applicability of this approach to the problem of the determination of residual anthraquinones in pulping materials. The proposed method is inferior in sensitivity to the conventional liquid chromatographic methods[6–11]. However, the use of post-column derivatization results in a significant increase in selectivity.

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