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Short communication

Determination of 9,10-dihydroxyanthracene and anthraquinone in Kraft pulping liquors by high-performance liquid chromatography

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Abstract

A simple chromatographic method was developed to determine the amount of anthraquinone (AQ) and its reduced form, the disodium salt of 9,10-dihydroxyanthracene (Na_2AQ), in white pulping liquors. The method is based on the different solubilities of AQ (insoluble) and Na_2AQ (soluble) in aqueous medium. Prior to analysis, samples (containing AQ + Na_2AQ) are filtered to remove the insoluble quinone (AQ) and oxidized with hydrogen peroxide. The resulting oxidized material, which corresponds to the initial amount of Na_2AQ present in the sample, is dissolved in N,N-dimethylformamide (DMF) and this final solution is analysed for AQ by high-performance liquid chromatography. The basic operating conditions are as follows: detection wavelength, 254 nm; flow-rate, 1.5 ml min^{-1}; temperature, 40°C; eluent, methanol–water (82:18, v/v); and volume of sample injected, 10 μl. The method is suitable for the determination of quinonoid compounds in the 5–2500 $\mu g\ ml^{-1}$ range in Kraft pulping liquor and the limit of detection is 0.5 $\mu g\ ml^{-1}$.

1. Introduction

Anthraquinone (AQ) is used in the pulping industry because it increases the cellulose yield and lowers the energy consumption during the alkaline pulping of wood [1]. AQ is reduced to the disodium salt of 9,10-dihydroxyanthracene (Na_2AQ) by the sodium sulfide present in the pulping liquor according to the following equation:

$$NaHS + 9\ NaOH + 4\ AQ \rightleftharpoons Na_2SO_4 + 4\ Na_2AQ + 5\ H_2O \quad (1)$$

However, the determination of Na_2AQ is difficult as it is present at very low levels and it is extremely susceptible to air oxidation [2]. Therefore, the concentration of reduced quinone must be determined in an indirect way. Several methods have been applied to solve this problem, including high-performance liquid chromatography (HPLC) [2–8], gas chromatography (GC) [9], GC–mass spectrometry [10], differential pulse polarography [11] and spectrophotometry [12]. These methods require extensive sample extraction and solvent concentration and their operating costs are high. In this paper, a simple, rapid and isocratic HPLC method is proposed for the measurement of the concentration of AQ and its reduced form, which is soluble in the

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pulping liquor. A minimum of sample preparation is necessary without solvent extractions where incomplete recoveries could occur. The method is based on the different solubilities of AQ and Na_2AQ in aqueous medium. The detection limits are in the low ppm range and the method is therefore a satisfactory alternative to previously reported techniques.

2. Experimental

2.1. Apparatus and conditions

A Perkin-Elmer (Norwalk, CT, USA) Series 2 liquid chromatograph, equipped with a Rheodyne Model 7105 injection valve (175-μl loop), an LC-75 UV detector and an LC-100 column oven, was used. The stainless-steel column (25 cm × 4.6 mm I.D.) was packed with 10-μm reversed-phase C_{18} Spherisorb ODS 2 (Supelco, Bellefonte, PA, USA). Chromatograms were obtained with a Varian (Sunnyvale, CA, USA) Model 4290 integrator. The operating conditions were as follows: detection wavelength, 254 nm; flow-rate, 1.5 ml min^{-1}; temperature, 40°C; and chart speed, 1 cm min^{-1}. The eluent was methanol–water (82:18, v/v).

2.2. Reagents

Anthraquinone was obtained from Aldrich (Steinheim, Germany). Prior to use, the mobile phase was filtered through a 0.45-μm pore-size HA membrane filter (Millipore, Bedford, MA, USA) and degassed for 50 min using an ultrasonic bath. The water used was prepared by glass distillation and filtration with a Milli-Q water-purification system (Millipore). Methanol and DMF were purchased form Merck (Darmstadt, Germany). All reagents were of analytical-reagent grade.

2.3. Procedure

To obtain artificial samples of white pulping liquor, containing 0.7–1.7 mol l^{-1} of sodium hydroxide, 0.1–0.3 mol l^{-1} of sodium sulfide and 0.005–2500 μg ml^{-1} of AQ, a set of experiments were carried out in a 1000-ml pressure vessel (Autoclave Engineers Group, Erie, PA, USA) at different temperatures. The reduction reaction was allowed to proceed under the different experimental conditions for 1 h to ensure that the maximum conversion of AQ to Na_2AQ was attained. Then, quinone compounds (AQ and Na_2AQ) were recovered from the pulping liquors by the procedure described below.

For the determination of Na_2AQ (see Fig. 1), since some of the initial quinone is reduced by the sodium sulfide present in pulping liquor to Na_2AQ according to Eq. 1, samples (10 ml) must be filtered to remove the unreacted AQ, which is insoluble in this medium. The filtered samples are oxidized with hydrogen peroxide (10 ml) and the resulting material (AQ suspension,

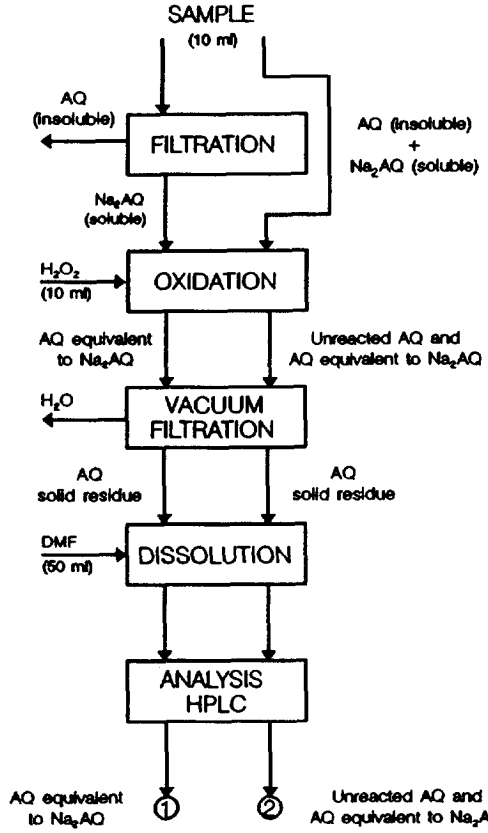

Fig. 1. Analytical procedure for AQ and Na_2AQ analysis in white pulping liquors.

which corresponds to the amount of Na_2AQ present in the sample) is separated by vacuum filtration as shown in Fig. 1, path 1. The solid residue is dissolved in 50 ml of DMF by shaking for 10 min, then an aliquot of this organic solution is analysed by HPLC.

For the determination of the total quinone (Na_2AQ soluble + AQ insoluble), the procedure is similar to that for Na_2AQ, but the samples are not filtered to remove the insoluble AQ (as shown in Fig. 1, path 2).

Standards of AQ in DMF in the concentration range 1–500 μg ml^{-1} were prepared in DMF by serial dilution.

3. Results and discussion

DMF does not interfere in the determination of AQ because its retention time is 1.7 min, whereas that of AQ is 4.2 min with a standard deviation of 0.09 min under the chromatographic conditions used. A typical HPLC trace is shown in Fig. 2. The AQ peak is sharp and well separed from the matrix (DMF).

3.1. Range

Analysis of standard solutions of AQ in DMF showed that the absorbance of AQ is essentially a linear function of concentration between 1 and 500 μg ml^{-1}, but deviated slightly above this range.

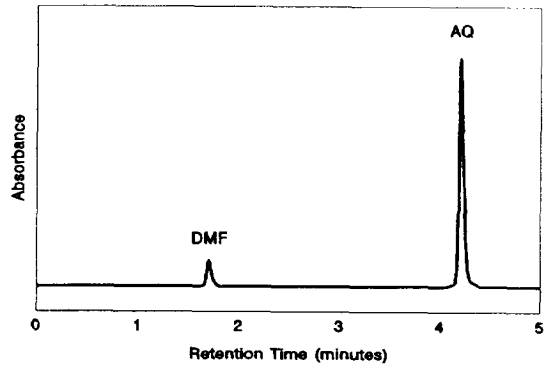

Fig. 2. Chromatogram of 50 μg ml^{-1} of AQ concentration in DMF. Operating conditions: detection wavelength, 254 nm; flow-rate, 1.5 ml min^{-1}; temperature, 40°C; eluent, methanol-water (82:18 v/v); and volume of sample injected, 10 μl.

3.2. Calibration

A calibration graph of peak area versus the concentration of AQ in the injected aliquot of DMF (10 μl) covering the range 1–500 μg ml^{-1} was plotted. There is a very strong relationship between the two variables. In order to quantify this relationship, the data were fitted by a least-squares regression equation of the form $y = a + bx$:

$$y = 0.0185 + 0.03511x \qquad r^2 = 0.998 \qquad (2)$$

where y is the peak area in cm^2 and x is the AQ concentration in DMF solution in μg ml^{-1}. The standard deviations of the slope and intercept are $s_b = 9.2 \cdot 10^{-5}$ and $s_a = 0.0217$, respectively. The standard error is 0.0511 and the number of data points $n = 10$. The 95% confidence interval for the true slope is $b \pm ts_b$:

$$0.03511 \pm (2.306 \cdot 9.2 \cdot 10^{-5}) = 0.03511 \pm 0.00021$$

(i.e. 0.03490–0.03532 cm^2 ml μg^{-1})

The 95% confidence interval for the true intercept is $a \pm ts_a$:

$$0.01849 \pm (2.306 \cdot 0.02170) = 0.01849 \pm 0.05004$$

(i.e. 0.06853 to −0.03156 cm^2)

The confidence interval for the true intercept extends from −0.03156 to 0.06853 cm^2 ml μg^{-1}, which includes zero. Therefore, it would be reasonable to fit a line which passed through the origin of the form $y = bx$. The equation of the best line through the original is

$$y = 0.03516x \qquad r^2 = 0.999 \qquad (3)$$

This equation is not very different from the best line through the origin ($y = 0.01848 + 0.03511x$) over the range of concentrations used in the calibration. The standard deviation of the slope is $s_b = 6.8 \cdot 10^{-5}$, the standard error is 0.0503 and $n = 10$. The 95% confidence interval for the true slope is $b \pm ts_b$:

$0.03516 \pm (2.306 \cdot 6.8 \cdot 10^{-5}) = 0.03516 \pm 0.0015$

(i.e. 0.03501–0.03531 cm^2 ml μg^{-1})

However, the confidence interval for the true slope extends from 0.03490 to 0.03501 cm^2 ml μg^{-1} for the line through the origin, whereas the confidence interval for the true slope of the other line was much wider (0.0349–0.03532 cm^2 ml μg^{-1}). By assuming that the true line passes throught the origin, one obtains a better estimate of the true slope, provided that the assumption is justified.

3.3. Confidence intervals for the true concentration

For an unknown sample that gives an peak area y, the true concentration and its confidence interval is given by

$$\frac{y}{b} \pm \frac{ts_b}{b}\sqrt{1+\left(\frac{y}{b}\right)^2} \quad (4)$$

For the sample corresponding to a peak area of 1.80 cm^2, its concentration in the 95% confidence interval is 51.19 ± 0.04 μg ml^{-1}. That is, the true concentration of the unknown sample lies between 51.15 and 51.23 μg ml^{-1}. In this way, by substituting several values of y in Eq. 4 different confidence intervals are obtained, which are shown in Table 1. Note that these intervals become wider as y increases.

3.4. Limits of detection and quantification

To obtain the limit of detection the following equation must be applied:

$$\text{Limit} = \frac{Ks_{bl}}{b} \quad (5)$$

where s_{bl} is the standard deviation (0.00117) for ten blank readings, b is the slope of the calibration line through the origin (0.03516 cm^2 ml μg^{-1}) and K is equal to 3 for the limit of quantification and $K = 10$ for the limit of detection [13]. Applying Eq. 5, one obtains 0.1 and 0.4 μg ml^{-1} for the limits of detection and quantification of AQ in DMF, respectively.

Table 1
95% confidence intervals for ture concentration of AQ in DMF solutions

Peak area, y (cm^2)	95% Confidence interval (CI) for true concentration x	
	x ($\mu g\,ml^{-1}$)	CI
0.00	0.00 ± 0.004	−0.004 to 0.004
0.16	4.55 ± 0.01	4.54 to 4.56
0.37	10.52 ± 0.02	10.50 to 10.54
1.80	51.20 ± 0.05	51.15 to 51.25
3.50	99.54 ± 0.22	99.32 to 99.76
7.07	201.08 ± 0.43	200.51 to 201.51
10.52	299.20 ± 0.87	298.33 to 300.07
14.17	403.01 ± 1.30	401.71 to 404.31
17.50	497.72 ± 1.75	495.97 to 499.47

3.5. Accuracy and precision

To determine the accuracy of the method, a recovery study was carried out. The recovery of the analyte, R, is defined as fraction of analyte added to the sample (fortified sample) prior to analysis, which is measured (recovered) by the method. When the same method is used to analyse both the unfortified and fortified samples R is calculated as follows:

$$R(\%) = \frac{C_F - C_U}{C_A} \cdot 100 \quad (6)$$

where C_F is the concentration of analyte measured in the fortified sample, C_U the concentration of analyte measured in the unfortified sample and C_A the concentration of analyte added to the fortified sample. Note that C_A is a calculated value, not a value measured by the method being used.

Table 2 shows that the recoveries are all near 100%, providing a further example of the excellent accuracy of the analysis by HPLC. The accuracy is excellent at concentrations of AQ between 1 and 500 μg ml^{-1}, where the recovery of AQ rises 99.24%, but it deteriorates significantly above 500 μg ml^{-1}. This limit is caused partly by a departure from linearity of the calibration line due to the limited solubility of AQ in DMF at higher AQ concentrations. On other hand, to validate any analytical method,

Table 2
Recovery for AQ in DMF determined by HPLC

Added ($\mu g\ ml^{-1}$)	Recovery (%)
200	100.30, 99.11, 98.63
250	99.61, 99.32, 98.70
300	98.77, 99.15, 99.58
	Average, A 99.24
	Standard deviation, s 0.50
	R.S.D. = $(s/A)\cdot 100$ (%) 0.50

the relative standard deviation (R.S.D.) should be no more than 2%. In the present case, the R.S.D. is 0.51%, which indicates good accuracy of the HPLC analysis.

3.6. Analysis of white pulping liquor

The concentration of AQ in white pulping liquors may be calculated by using the following equations:

$$C_{AQ\ H_2O} = \frac{V_{DMF} C_{AQ\ DMF}}{V_S}; \quad C_{AQ\ DMF} = \frac{y}{0.03516} \tag{7}$$

where $C_{AQ\ H_2O}$ = concentration of AQ in white pulping liquor ($\mu g\ ml^{-1}$); $C_{AQ\ DMF}$ = concentration of AQ in DMF ($\mu g\ ml^{-1}$); V_S = sample volume of white pulping liquor (10 ml); and V_{DMF} = volume of DMF used in the dissolution stage (50 ml).

The accuracy of the method was evaluated from the percentage recovery of AQ and Na_2AQ from the white pulping liquor samples. As shown in Table 3, the average recovery is 98.80% with a standard deviation of 0.45% and an R.S.D. of 0.45%. These results indicate that the overall analytical procedure has good accuracy.

To test the proposed method, a wide variety of white pulping liquors containing sodium sulfide (0.1–0.3 mol l^{-1}) and sodium hydroxide (0.7–1.7 mol l^{-1}) at different temperatures (140–160°C) were analysed. This study showed that the proposed method can be used to control the

Table 3
Recovery for determination of AQ in samples of white pulping liquor containing 1.1 mol l^{-1} of sodium hydroxide, 0.184 mol l^{-1} of sodium sulfide and 1–500 $\mu g\ ml^{-1}$ of AQ at 150°C

Concentration ($\mu g\ ml^{-1}$)				Recovery (%)
AQ added	AQ[a] found	Na_2AQ[a,b] found	Total found	
5[c] (1)[d]	(n.d.)[e]	4.91 (0.98)	4.90 (0.98)	98.10
50 (10)	(n.d.)	49.75 (9.95)	49.75 (9.95)	99.50
250 (50)	3.30 (0.65)	242.95 (48.60)	246.25 (49.25)	98.50
500 (100)	37.10 (7.40)	454.40 (90.88)	491.50 (98.30)	98.30
1000 (200)	536.95 (107.40)	454.05 (90.80)	991.00 (198.20)	99.10
1500 (300)	1037.85 (207.60)	448.65 (89.70)	1486.50 (297.30)	99.10
2000 (400)	1538.00 (307.60)	444.08 (88.80)	1982.00 (396.40)	99.10
2500 (500)	2046.25 (409.25)	421.25 (84.25)	2467.50 (493.50)	98.70
			Average, A	98.80
			Standard deviation, s	0.45
			R.S.D. = $(s/A)\cdot 100$ (%)	0.45

[a] Average of three injections.
[b] Amount of Na_2AQ expressed as AQ.
[c] Amount of AQ or Na_2AQ in white pulping liquor.
[d] Amount of AQ in DMF solution.
[e] Not detected.

Table 4
Comparison of methods for the determination of AQ in pulping liquors by HPLC

Ref.	Detection limit ($\mu g\ ml^{-1}$)	Calibration range ($\mu g\ ml^{-1}$)	Recovery (%)	Standard deviation (%)
[2]	Low ppm region	2–120	98.0	2.30
[3]	0.1	1–200	98.4	0.70
[4]	Low ppm region	2–40	90.0	–
[5]		50–200	–	0.24
[6]	–	1–9	–	0.50
[7]	0.8	1–320	–	–
This work	0.1	1–500	98.8	0.45

AQ dosage in the manufacture of pulp. The method offers advantages over other methods with regard to the limit detection, range and accuracy, as shown in Table 4.

4. Conclusions

The proposed HPLC method is simple, rapid and precise. It allows the determination of AQ and its reduced form (Na_2AQ) in a few minutes. It can be successfully applied to the determination of quinonoid compounds in pulping liquors with a detection limit in the low ppm region.

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