

## Method for concentrating polynuclear aromatic hydrocarbons in cigarette smoke condensate

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The primary tumour-initiating activity of cigarette smoke condensate in animals occurs in the neutral fraction, which contains trace amounts of carcinogenic polynuclear aromatic hydrocarbons (PAH). However, the amounts of PAH present are too small to account for the observed activity.<sup>1</sup> This discrepancy may be due to synergistic interactions of PAH or to the biological contribution of unidentified compounds not structurally related to the PAH. A major obstacle in differentiating between these effects is the lack of satisfactory preparatory methods for separating or greatly concentrating the trace amounts of PAH from the bulk of the constituents. Improved methods would facilitate compositional studies on non-polynuclear compounds and biological tests on combinations of isolated PAH or enriched fractions thereof.

Although most attempts to achieve a high degree of separation or enrichment of the PAH in condensate have failed, a recent report has described removal of 98.6 per cent of PAH accompanied by small (but unstated) amounts of non-polynuclear material from a sample of condensate using high voltage column electrophoresis in one experiment.<sup>2</sup> This method has significant promise but suffers from some disadvantages, including the need for specialised equipment and the hazard of routine operation. Also, the effects of the high potential on the structures of the non-polynuclear material are unknown. The method reported below is not subject to these shortcomings and may serve many purposes, including use as an adjunct to column electrophoresis.

Smoke condensate (1 kg) is separated by solvent partition into neutral and other fractions as previously described.<sup>3</sup> The neutral fraction (245 g) is separated by chromatography on activated silicic acid (4000 g) and eluted successively with light petroleum (20 l.), 25 per cent benzene in light petroleum (32 l.), benzene (28 l.), ether (18.5 l.) and methanol (10 l.). The residues from the first three eluates are individually partitioned between cyclohexane (512 ml.) and dimethyl sulphoxide (5 × 104 ml.) (DMSO). Repre-

sentative findings on the resulting weights and benzo[a]pyrene (BAP) levels are shown in Table I. BAP was determined by a previously described method using perylene to correct for losses.<sup>4</sup> More than 97 per cent of the BAP occurs in one fraction and the concentration is increased from less than 1 ppm (in smoke condensate) to 105 ppm.

**Table I**

*Distribution of weight and benzo[a]pyrene (BAP) after silicic acid chromatography and solvent partitioning of neutrals from smoke condensate*

Fraction <sup>a</sup>	Weight (%)		BAP	
	Of neutrals	Of condensate	µg <sup>b</sup>	ppm
P.E. eluate				
CH-soluble	7.2	1.8	0	—
DMSO-soluble	1.4	0.34	2.5	0.74
25% Benzene in P.E. eluate				
CH-soluble	8.2	2.0	0	—
DMSO-soluble	2.3	0.57	596	105
Benzene eluate				
CH-soluble	16	4.0	0	—
DMSO-soluble	4.9	1.2	18	1.5
Ether eluate	52	13	—	—
Methanol	6.3	1.5	—	—

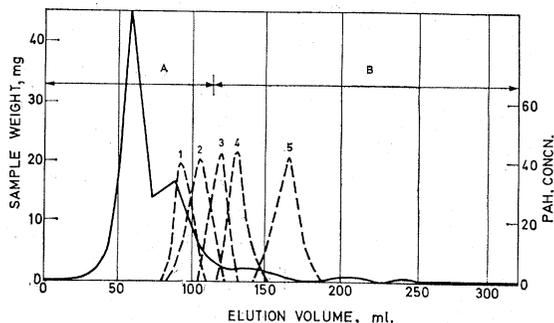
<sup>a</sup>P.E. = light petroleum, CH = cyclohexane, DMSO = dimethyl sulphoxide

<sup>b</sup>From fractionation of 1 kg of smoke condensate containing 0.62 ppm BAP

The DMSO-soluble material from the 25 per cent benzene eluate is then separated on cross-linked polystyrene by lipophilic gel filtration. Fractionation of PAH on cross-linked dextran has been reported<sup>5</sup> and later used in an analytical method for determining anthracene and pyrene in smoke condensate;<sup>6</sup> however, the use of lipophilic gels has not been studied in the present problem. A portion (0.095 g) of the DMSO-soluble material is dissolved in acetone and added to a column (2.2 × 27.2 cm) of Bio-Beads S - X2\* (*M.* exclusion limit: 2,700) (30.0 g) previously

\*Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

swelled in acetone. Elution is performed with the same solvent. Representative elution patterns of the sample and certain pure PAH known to be in condensate† are shown in the Figure. The curves for the PAH are given in arbitrary



Separation of smoke condensate fraction and four polynuclear aromatic hydrocarbons (PAH) on Bio-Beads S-X2 in acetone. ——— = fraction (95 mg total), - - - - = PAH. 1 = anthracene (100 µg), 2 = pyrene (100 µg), 3 = dibenz[a,h]anthracene (20 µg), 4 = benzo[a]pyrene (20 µg), 5 = dibenzo[a,i]pyrene (20 µg). See text for limitations on evaluation.

units of concentration based on relative intensities at the respective maxima in the excitation spectra. For improved visual representation in the Figure the relative intensities of anthracene and pyrene are increased several fold to compensate for the relatively low absorptivities of these compounds.

To confirm that BAP in the DMSO-soluble material elutes similarly to the pure hydrocarbon, BAP analyses were performed on two fractions (1-105 ml. and 106-315 ml.) obtained by three replicate separations of the material. No BAP was found in the first fraction and the second contained BAP equivalent to 613 µg per kg of condensate.

These findings show that PAH can be separated easily from the bulk of the sample weight. The PAH with larger ring numbers (which include the carcinogenic members) elute after anthracene and pyrene by an adsorptive mechanism in a manner similar to PAH behaviour on silicic acid<sup>7</sup>

†Anthracene, pyrene and PAH of less than 5 rings may be present in the light petroleum eluate from silicic acid chromatography.<sup>6</sup>

and cross-linked dextran using a hydrophilic solvent.<sup>5</sup> Whether or not the bulk of the DMSO-soluble material separates by gel permeation, adsorption or a combination thereof is not known.

The conditions described above in the gel permeation separation may be scaled up at least fifteen fold with no change in elution patterns. Fractions may be combined in any way to obtain eluates with different proportions of weight to PAH and different combinations of PAH for compositional and biological studies. For example, combining to form Fractions A and B (Figure) gives the data shown in Table II. This compares with published reports of enrichments of 750 ppm BAP in fractions representing 0.15 per cent of a condensate obtained by successive chromatographic separations on silicic acid followed by partitioning between cyclohexane and nitromethane.<sup>1</sup>

Table II  
Distribution of weight and BAP in fractions from chromatography on cross-linked polystyrene (Figure)  
Weight (%)

Fraction	Weight (%)		BAP	ppm BAP
	Of sample	Of condensate		
A	95.3	0.54	0.4	0.44
B	4.7	0.027	99.6	2200

Separations on cross-linked dextran (Sephadex LH-20) using tetrahydrofuran and on other cross-linked polystyrenes (Bio-Beads S-X2, exclusion limit, 1000 and Bio-Beads SM-2, exclusion limit, 14,000) in acetone have also been investigated. Generally, the DMSO-soluble fraction and PAH tend to elute less sharply and more overlapping occurs with these gels.

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