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ADSORPTION AND EXCLUSION CHARACTERISTICS OF NITROCELLULOSE WITH REFERENCE TO A MICROCOLUMN CLEAN-UP TECHNIQUE FOR THE DETECTION OF PROPELLANTS TRACES IN FIREARMS DISCHARGE RESIDUES

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SUMMARY

A study has been made of the adsorption and exclusion characteristics of nitrocelluloses on 12 representative supports under conditions relevant to the trace analysis of nitrocelluloses by size-exclusion chromatography with amperometric detection at a mercury cathode. It was found, in particular, that in acetonitrile nitrocelluloses are largely excluded from Porapak T, whereas they are adsorbed onto this support from mixtures of acetonitrile with diethyl ether. This provides the basis of a described microcolumn clean-up technique that enables nanogram amounts of nitrocellulose in contaminated firearms propellants residues to be characterized.

INTRODUCTION

In forensic science work it is frequently of importance to determine whether a surface, *e.g.* of skin or clothing, has been in the vicinity of a discharging firearm. Although firearms propellants are composed largely of organic materials, most routinely-applied detection techniques are based on inorganic residues derived from the ammunition's primer. Where microprobe analysis techniques are used the results are highly selective[1], but they are time-consuming to obtain and give poor success rates under some circumstances.

Several authors have given examples now of the detection in various ways of traces originating in the organic components[2–9]. Many of the techniques employed are relatively fast and can be used, *e.g.*, for the preliminary sorting of samples for closer attention, for the multisampling of large surface areas when information on the distribution of discharge residues is required, and for the provision of specific evidence to supplement and complement inorganic analyses. Much of this work has been concerned with the low-molecular-mass components (nitroglycerine, diphenylamine, and 2,4-dinitrotoluene). However, the major component of firearms propellants is nitrocellulose, and in the case of single-base propellants this usually is the only active component. Nitrocellulose may be detected by means of the nitrite it liberates on treatment with alkali[10], and the reaction can be used to detect nitrocel-

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lulose in discharge residues in various samples after thin-layer chromatography[2,4] or *in situ*[7]. To some extent, the thin-layer chromatography application enables different nitrocelluloses to be separated[2,11]. However, the detection limits are relatively high, in the region of several hundred nanograms[4,11].

Recently, a new technique based on size-exclusion chromatography with reductive mode electrochemical detection has been introduced for the identification of nitrocelluloses[12]. This makes possible detection limits well below 1 ng, mainly because nitrocelluloses can be reduced very selectively at the mercury electrode held at potentials around 0 V *vs.* Ag/AgCl. The chromatograms, which enable the high molecular mass distributions typical of propellant nitrocelluloses to be distinguished, can be completed within 2 min. The sensitivity is limited largely by the degree of overlap of the low-molecular-mass region of the chromatograms with the totally permeating contaminants[12]. This is aggravated in heavily soiled samples, *e.g.* handswabs, where the totally permeating material may also be associated with a prolonged tail due to adsorption effects. The overlap may be reduced by the use of longer columns, but this results in a considerable dilution of the already broad peaks formed by these highly disperse polymers. A more effective technique is to use a microcolumn clean-up procedure similar to the procedure for monomeric compounds[9]. This removes the bulk of the contaminants and enables nitrocelluloses to be concentrated considerably, if necessary, as the following describes.

EXPERIMENTAL

Materials

The industrial grade nitrocelluloses were kindly provided by the Imperial Chemical Industries Organics Division, U.K., and the propellant grade sample by the Materials Quality Assurance Establishment, Ministry of Defence, U.K. The mass-average relative molecular mass and the nitrogen content specified for the propellant grade sample are 247 000 and 12.6% respectively. For the other samples the molecular mass averages are 140 000, 75 000 and 19 500, and the nitrogen contents are in the range 11.8–12.2%.

The adsorbents are included in Table I. Each was extracted with acetonitrile (HPLC S grade, Rathburn) before use.

Size-exclusion chromatography

Full details have been given before[12]. The only significant modification here is in the reduced column length. In brief, the separations were made on 150 × 4.5 mm columns of LiChrospher Si 300, 5 μm (Merck), run at ambient temperatures in deoxygenated acetonitrile–water (100:5, v/v) containing 0.01 *M* tetramethylammonium perchlorate, with a flow-rate of 1.5 ml min^{-1}. The detector was a pendent mercury drop electrode[14] held at 0 V *vs.* Ag/AgCl.

It should be noted that the pipework carrying the effluent to the electrochemical cell must be electrically insulated from the rest of the chromatograph[15]. In the present case the stainless-steel pipework (0.5 mm O.D., 0.25 mm I.D.) passing into the column end-fitting was sleeved with 1.6 mm O.D. PTFE tubing; within the end-fitting the entrance to the pipe butted up to a thin (*ca.* 0.3 mm) wafer of PTFE cut from 1.6 mm O.D., 0.25 mm I.D. tubing; and the joint was made up with a PTFE

ferrule. This minimal contact with (oxygen-permeable) PTFE ensured a minimal background current (±0.2 nA) from the detector whilst a satisfactory level of insulation was maintained.

Comparison of adsorbents, microcolumn technique

The apparatus was as previously described[9] with the sample loop of an injection valve serving as an eluent reservoir for the microcolumns, which in these experiments were of 40 × 0.6 mm PTFE tubes slurry-packed with a suspension of the adsorbent in acetonitrile. On to each column was injected 1 μg of nitrocellulose in 10 μl of acetonitrile. The column was eluted with 50 μl of acetonitrile at 0.5 μl s^{-1}, and the concentration of the nitrocellulose in the effluent was determined by the size-exclusion chromatography technique.

Liquid chromatography on Porapak T

Porapak T (Table I) was slurried in acetonitrile–diethyl ether (1:1, v/v) and pumped into a 150 × 4.5 mm column at pressures below 35 bar. The column was substituted for the Si 300 column in the size-exclusion system, which was operated with unmodified conditions except the eluent contained varied amounts of diethyl ether (Aristar, BDH; the pyrogallol stabilizer was not removed), and the flow-rate was set at 0.5 ml min^{-1}. The measured parameters of the column equilibrated with the acetonitrile eluent were: mass of packing, 0.823 g; void + internal pore volume, 1.79 ml; total internal volume, 2.39 ml (calculated, 2.38 ml). The dead volume external to the column was 15 μl.

Handswabs

These were of cotton wool or acrylic fibre (100–200 mg) moistened with ethanol. The used swab was placed in a centrifugal microfilter (made from a disposable centrifuge tube carrying a stainless-steel frit[16]), dried in a stream of air pulled through by a vacuum pump, and then treated with 100 μl of acetonitrile. After 1 h the extract was removed at the centrifuge and the extraction immediately repeated with a further aliquot of acetonitrile. The extracts were combined.

Microcolumn clean-up procedure

The columns were of Porapak T (Table I) slurry-packed in acetonitrile into 40 × 0.6 mm PTFE tubes. Otherwise the apparatus and details already mentioned apply. As the columns are very readily and inexpensively made[9], a new one was used for each experiment.

The sample in acetonitrile, *e.g.* 100 μl, was diluted with a 1.5-volume ratio of diethyl ether, centrifuged for the removal of any apparent precipitate, and pumped at 1 μl s^{-1} through a column previously equilibrated with the same solvent mixture. (The effluent may be processed for monomeric components of explosives, which are unretained in this solvent.) The column was rinsed with 200 μl of further solvent mixture, and then eluted at 0.5 μl s^{-1} with acetonitrile. The initial 10 μl of the effluent (displaced ether-containing solvent) was rejected. Most of the nitrocellulose appeared in the following 10–20 μl.

TABLE I

RECOVERIES OF NITROCELLULOSE IN ACETONITRILE FROM VARIOUS ADSORBENTS

	Adsorbent	*Source*	*Particle size (μm)*	*Chemical type**	*Recovery*** *(%)*
1	Spherisorb-ODS	Phase Separations	10	Octadecylsilylsilica	73.7
2	Porapak R	Waters	75-100	Polyvinylpyrrolidone	65.5
3	Porapak S	Waters	75-100	Polyvinylpyridine	48.8
4	Porapak T	Waters	75-100	Poly(ethylene glycol dimethylacrylate)	92.7
5	Amberlite XAD-4	Rohm & Haas	< 100	Styrene–divinylbenzene copolymer	76.4
6	Tenax GC	Akzo Research Labs.	170-250	Poly(2,6-diphenyl-*p*-phenylene oxide)	91.2
7	Chromosorb 104	Johns-Manville	125-150	Acrylonitrile–divinylbenzene copolymer	84.0
8	Partisil 20	Whatman	20	Silica	73.7
9	Polyamide	Macherey Nagel	50-160	Polycaprolactam	< 1
10	Charcoal	BDH	70-150	Carbon	< 1
11	LiChrosorb NH_2	Merck	10	Aminoalkylsilica	< 1
12	Spherisorb alumina	Phase Separations	10	Alumina	< 1

* Taken from suppliers information.

** Means of replicated determinations; standard error, 3.6 (entries 1–8). Further details are given in the text.

RESULTS AND DISCUSSION

Adsorption and exclusion characteristics

Because acetonitrile is the preferred solvent for the chromatography and amperometric detection of nitrocelluloses[12], the product of a clean-up technique must be obtained in acetonitrile, and any adsorbent should be inactive in this solvent. In attempts to determine distribution coefficients, where a nitrocellulose in a fixed volume of acetonitrile was mixed continuously with the adsorbent of interest, it was found that equilibrium could not be attained even after several hours. Therefore, adsorbents were compared only arbitrarily by means of a microcolumn technique (Experimental). This yielded satisfactorily reproducible results, and showed *e.g.* virtually complete adsorption of nitrocelluloses on charcoal whereas substantial amounts remained in the solvent phase in the initial distribution experiments.

The results for a propellant grade nitrocellulose and a representative collection of adsorbents are given in Table I. The recoveries are calculated from peak height measurements on the size-exclusion chromatograms (Experimental). Except in two cases, where the chromatograms were distorted in the region of the total permeation volume, peak area measurements gave closely agreeing results. Wherever substantial amounts of nitrocellulose were eluted, no significant variation from the original sample occurred in the position of the peak maximum *i.e.* no significant molecular mass-selective retention was apparent. Evidently, from Table I, amongst the adsorbents the retention is weakest on Porapak T and on Tenax GC, although some slight adsorption does occur.

The recoveries in Table I are from experiments made with approximately five column-volumes of eluent, in the Porapak T case. When restricted volumes of eluent are used, a size exclusion process becomes apparent. On the other hand, if diethyl ether is added to the eluent a strong adsorption occurs. For a given eluent mixture, the extent of adsorption varies between the nitrocelluloses examined, but with a volume ratio of diethyl ether to acetonitrile of 1.5 the adsorption is complete. These effects are illustrated in Fig. 1, which shows chromatograms on a 15 cm × 4.5 mm Porapak T column of different nitrocelluloses (A–D, in decreasing molecular mass average) in the specified slightly aqueous acetonitrile eluent, and in the same eluent to which a 0.85-volume ratio of diethyl ether has been added (respectively, upper and lower of each pair of chromatograms). The time to total permeation, indicated by the broken lines, is calculated from the measured mass of eluent contained in the column and the dead volume external to the column (Experimental). Clearly, in the absence of diethyl ether the higher molecular mass material, as in A, is almost entirely excluded; permeation and adsorption increase from B to D. In the presence of the ether, which was shown by flow injection experiments not to affect significantly the response of the detector, much of all of the samples is retained on the column. Even so, an exclusion peak persists prominently in A. But despite the steric constraint on the adsorption that is evident here, none of any sample can be eluted when the ether ratio approaches the 1.5-region.

Similar effects were encountered with some of the other adsorbents listed (Table I), but not with the Tenax, which excludes the possibility that the ether was causing precipitation and, hence, the removal of the nitrocelluloses simply by filtration.

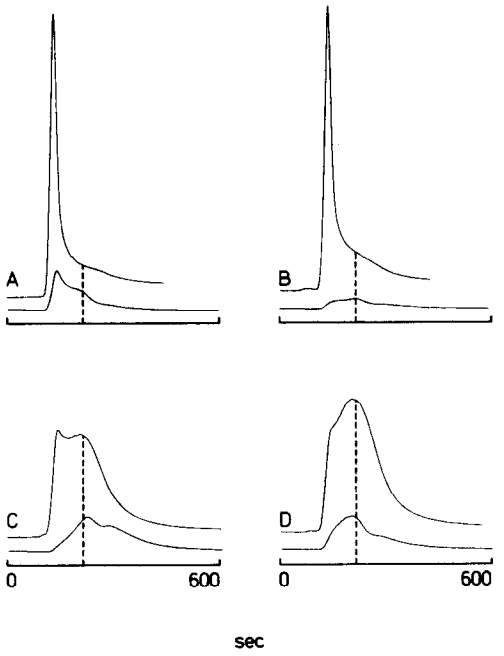

Fig. 1. Liquid chromatograms on a Porapak T column (15 cm × 4.5 mm) of nitrocelluloses with mass-average relative molecular mass values of 247 000 (A), 140 000 (B), 75 000 (C), and 19 500 (D). The upper chromatogram of each pair was run in deoxygenated acetonitrile–water (100:5, v/v), containing 0.01 *M* tetramethylammonium perchlorate, with a flow-rate of 0.5 ml min^{-1}. The lower chromatogram was run in the same eluent diluted with a 0.85-volume ratio of diethyl ether. The broken lines indicate the total permeation volume.

Application

The above results are applied in the described clean-up technique, which enables much of the unwanted contamination characteristic of the samples of interest to be eliminated. Thus, Fig. 2A shows the size-exclusion chromatogram, before clean-up, from the bulked extract of some cotton wool swabs of hands that were heavily soiled but free from any firearms residue contamination. The chromatogram is swamped by material giving a massive offscale peak (possibly mainly due to quinones and peroxidized lipids). After application of the clean-up technique, little of this material remained (B). Chromatograms C–E show the results after the addition to the extract, prior to clean-up, of a propellant grade nitrocellulose in amounts representing 10, 40, and 100 ng/swab. The retention times, listed in the caption of Fig. 2, of the resulting partly excluded peaks are in good agreement with that of the original sample, the chromatogram of which was not distinguishable from the last chromatogram shown (E). The respective recoveries are 28, 63, and 62%, the first-mentioned result representing the region of the practicable detection limit in this sample. Increased recoveries result if the volume of the acetonitrile elution of the column is increased, but at a cost of increased elution of lower molecular mass materials. The variable retention of these causes the variation seen in the total permeation peaks of Fig. 2.

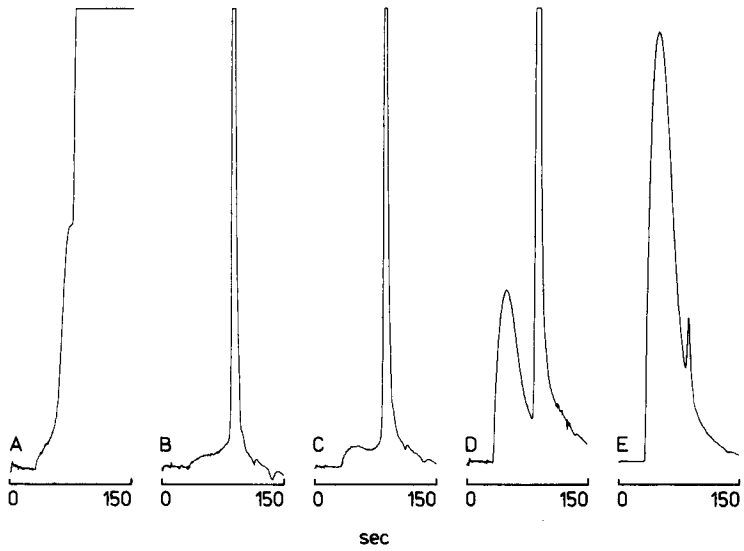

Fig. 2. Size-exclusion chromatograms of the bulked extract of several handswabs (no previous firearms contact) before clean-up (A), after clean-up (B), and cleaned-up after the addition of the equivalent of 10, 40, and 100 ng/swab of propellant grade nitrocellulose. The chromatograms have been scaled to represent the same amount (approximately half) of a swab in each case. The retention times of the partly excluded peaks in C, D, and E, and also of the original nitrocellulose (not illustrated, identical in form to E) were 45.6, 48.8, 47.6, and 47.9 s respectively.

The contamination illustrated in Fig. 2 is relatively severe. In cleaned-up swab extracts from nine other people employed in manual work the high-molecular-mass region of every chromatogram was completely vacant, in contrast to Fig. 2B. Under such circumstances the presence of nitrocelluloses in the region of 1 ng/swab would be readily detected. In a tenth swab, from a painter and decorator, a prominent peak occurred near the total permeation limit. This was entirely differentiated in retention time from the nitrocelluloses of interest, but small amounts of them, *e.g.* < 10 ng, would have been obscured by the overlapping part of the peak.

In Fig. 3 are shown chromatograms given by the cleaned-up extracts of swabs collected from an uncontaminated hand (A) and from the hands of three other people shortly after they had discharged 1–3 rounds from a revolver. The swabs were of an acrylic fibre that partly dissolved in the acetonitrile extractant and formed a copious precipitate (removed by centrifugation) when the diethyl ether was added. Even so, the peak due the propellant residue is clearly demonstrated in each instance where the weapon has been discharged (B–D) and increases in parallel to the number of rounds fired. Traces of nitrocellulose left on a spent shell case gave, after extraction into acetonitrile, the chromatogram shown in Fig. 3E, the peak retention time of which corresponds to those from the handswab material (caption, Fig. 3).

Because of the variation in the coulometric efficiency of the reduction of nitrocelluloses, *e.g.* due to variations in their nitration level and molecular mass average[12], and because both of these quantities may be lowered to an unknown extent in discharge residues, any estimate of the amount of nitrocellulose present is likely

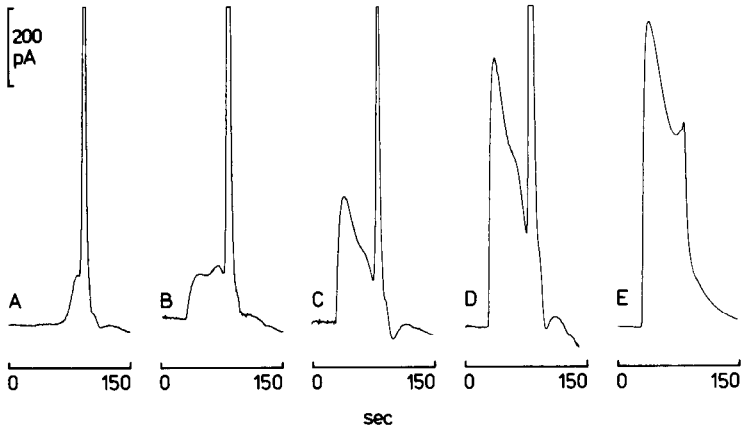

Fig. 3. Size-exclusion chromatograms of cleaned-up extracts of handswabs collected shortly after the discharge of 0 (A), 1 (B), 2 (C) and 3 (D) rounds from a Smith and Wesson .357 revolver. An extract of a spent shell case is shown also (E). The respective retention times of the high molecular mass peak in chromatograms B to E were 44.2, 41.2, 39.2, and 39.4 s.

to be only a minimum. In the present instance, based on unused propellant nitrocellulose, the peaks in Fig. 3B–D, correspond to 10–40 ng/swab. In view of the solubility of the sampling medium used here, these samples too represent a severe test of the technique.

CONCLUSION

Evidently, the results provide a basis on which nitrocelluloses may be characterized in actual samples at sensitivity levels approaching those of other explosives and propellants components[5,9], and essentially by the same techniques. The same chromatographic detector can be used for all of the components, and most of the other equipment is in common also. Thus, the transfer and persistence of firearms residues may now be readily studied selectively and sensitively in terms of both of the major precursors.

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