only in the study of macromolecules, but also for small molecules. The details of the results presented here are expected to be published elsewhere.

ACKNOWLEDGEMENT

We thank Prof. G. N. Ramachandran for suggesting this problem.

We wish to acknowledge the financial support from the Department of Science and Technology (India).

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ELECTROPHORESIS OF ^{32}P-LABELLED OLIGONUCLEOTIDES ON THIN-LAYER DEAE-CELLULOSE FOR RAPID SEPARATION IN FINGERPRINTING

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THE two-dimensional fractionation procedure for radioactive oligonucleotides, developed by Sanger *et al.*[1] is now widely used in fingerprinting and sequencing of nucleic acids. In this procedure separation in the first dimension is by electrophoresis on a cellulose acetate membrane strip at high voltage and in the second dimension on a DEAE-cellulose paper. Brownlee and Sanger[2] have employed chromatography on thin-layer DEAE-cellulose for fractionation of large oligonucleotides, especially deoxy-oligonucleotides. Grohmann and Sinsheimer[3] have noted certain advantages in carrying out the first dimension electrophoresis on thin-layer cellulose instead of cellulose acetate. We have devised a simple procedure for carrying out electrophoresis in the second dimension on a thin-layer DEAE-cellulose plate. In this method voltages of the order of 50–60 per cm can be applied without appreciable heating effect and the fractionation completed in a few hours.

EXPERIMENTAL

Mycobacterium smegmatis, used in these studies, was from Microbiology and Cell Biology Laboratory of this Institute. DEAE-cellulose (0·85 meq/g) and cellulose powder (microcrystalline) were purchased from Centron Research Laboratory, Bombay. RNase T_1 and RNase A were from Sigma Chemical Company, St. Louis. Carrier free ^{32}P-orthophosphate was from Bhabha Atomic Research Centre, Bombay. All other reagents were of analytical grade.

Preparation of ^{32}P-labelled oligonucleotides

^{32}P-labelled total RNA was isolated from *Mycobacterium smegmatis* grown with radioactive phosphate in low phosphate medium[4,5]. 5S RNA was separated by polyacrylamide gel-electrophoresis and digested with RNase T_1 or RNase A according to the procedure of Sanger and Brownlee[6].

Preparation of thin-layer DEAE-cellulose plates

DEAE-cellulose and microcrystalline cellulose in the required proportions were thoroughly mixed with water (6 ml per g of mixture) in a Waring Blendor and the slurry was applied to glass plates using a Desaga spreader with thickness setting at 0·5 mm. The plates were dried at 60–80° C.

Determination of base composition

The radioactive spots, as revealed by autoradiography, were scraped from the thin layer plates, eluted with 30% triethylammonium carbonate[2] and digested with 0·2 M NaOH. The mononucleotides were separated by electrophoresis at pH 3·5 on Whatman No. 1 paper using cold nucleotides as markers, and counted in a liquid scintillation counter.

Procedure

RNase T_1 or RNase A digest of ^{32}P-labelled 5S RNA was subjected to electrophoresis in the first dimension on a cellulose acetate membrane strip and the oligonucleotides were transferred to the thin-layer DEAE-cellulose plate as described by Brownlee and Sanger[2]. After washing with alcohol the plate was sprayed with a buffer, consisting of pyridine (0·5%), acetic acid (5%) and EDTA (0·005 M), the pH being 1·9. A wet Whatman No. 1 paper was attached to that end of the plate where oligonucleotides were transferred from the cellulose acetate paper. The plate was placed in the tank (Fig. 1) and subjected to electrophoresis.

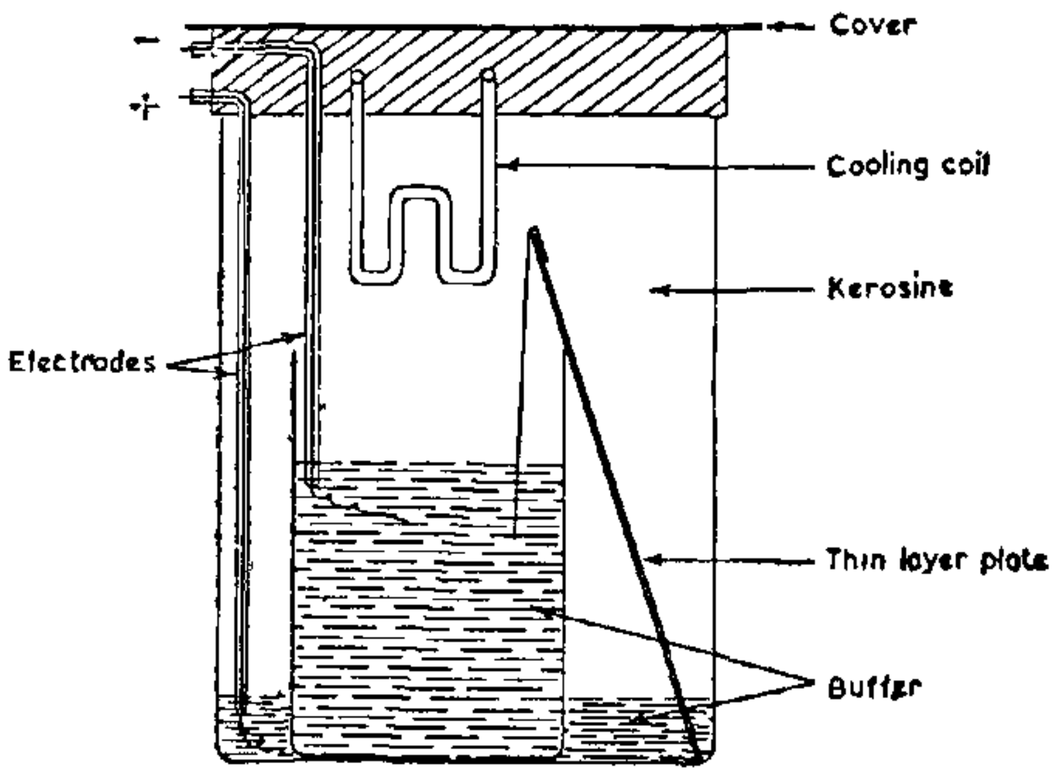

FIG. 1. Electrophoresis apparatus. The chromatographic tanks used were of dimension 25 × 30 × 40 cm and 10 × 25 × 25 cm. The buffer containing a mixture of 2·5% formic acid, 8·7% acetic acid and 0·005 M EDTA (pH 1·9) was added to a height of 3 cm in the larger tank and 16 cm in the smaller tank. Kerosene was added to fill the larger tank. Tap water was circulated though the cooling coils.

The apparatus was made out of two chromatographic tanks of unequal dimensions. The smaller tank formed the cathode compartment and the larger one the anode compartment. The space above the buffer in the two tanks was filled with kerosene which acted as the coolant. A wooden frame fixed to the top of the larger tank served as a convenient support for the electrodes and the cooling coils. The electrodes were of platinum wire. The smaller tank acted as a support for keeping the thin layer plate in a slightly slanting position and the Whatman No. 1 paper conducted the current.

Results and Discussion

With the setup described above a voltage of 50–60 per cm could be applied without appreciable heating effect and fractionation completed in 1 to 2 hr. Good resolution of the oligonucleotides, as revealed by autoradiography, was observed. A typical fingerprint of 5S RNA of *Mycobacterium smegmatis* is shown in Fig. 2. The radioactive spots were scraped out from the plate and they were analysed for their base composition as described under experimental. The base compositions of the fragments obtained by digestion with RNase T_1 are shown in Table I. These results were

TABLE I

Base composition of RNase T_1 fragments of Mycobacterium smegmatis

Spot No.	Base Composition	Spot No.	Base Composition
1	G	13	$(C_2U)G$
2	G ! (cyclic)	14	$(CA_2)G$
3	GC	15	AAAG
4	AG	16	(CAU) G
5	UG	17	$(CAU_2)G$
6	CCG	18	$(C_3A_2)G$
7	(AC) G	19	$(C_2A_2U_2)G$
8	AAG	20	$(C_5AU_2)G$
9	(AU) G	21	$(C_4A_3U_2)G$
10	(AU) G	22	$(C_4A_2U_3)G$
11	CCCG	23	pGp
12	$(C_2A)G$	24	A_2C

The radioactive spots were analysed as described under Experimental. The ratio of bases is expressed relative to the counts for G. The sequences of the nucleotides within brackets are not known.

highly reproducible. Some of the faint spots also were analysed. Two spots thus analysed were (CA)G and (UC)G (not shown in Table I). Movement of the various oligonucleotides on thin layer was somewhat similar to that observed by Sanger and Brownlee on DEAE-cellulose paper[6]. For example, the relative positions of spots 1, 2, 3, 6 and 11 in Fig. 2 which represent G_p, cyclic G_p, C_pG_p, $C_pC_pG_p$ and $C_pG_pC_pG_p$ are identical to those on DEAE-cellulose paper. Similar regularities with respect to other oligonucleotides were observed both in RNase T_1 (Fig. 2) and RNase A digests (not shown). Spots 9 and 10 in the RNase T_1 digest had the same base composition. It is evident that one of them must have the sequence AUG and the other UAG. Such separation of isomers have been observed on DEAE-cellulose paper[6]. Being smaller, fingerprints of tRNAs, in general, can be expected to be simpler and the separation of oligonucleotides better than that for 5S RNA shown in Fig. 2.

Separation was better when nucleotides moved down the plate than when they moved up.

In other words, for good resolution oligonucleotides must be applied on the top of the plate (*see* Fig. 1). Time for electrophoresis depended to some extent on the relative proportion of DEAE-cellulose in the thin-layer mixture; lower amounts of DEAE-cellulose in the mixture decreased the time for separation. Optimum resolution was, however, obtained with DEAE-cellulose to cellulose powder in the ratio 1 : 2, in about 2 hr. It usually takes about 16 hr for fractionation on DEAE-cellulose paper. Comparison of the base composition of various oligonucleotides showed that the 5S RNA of *Mycobacterium smegmatis* was different from that of other bacteria[7].

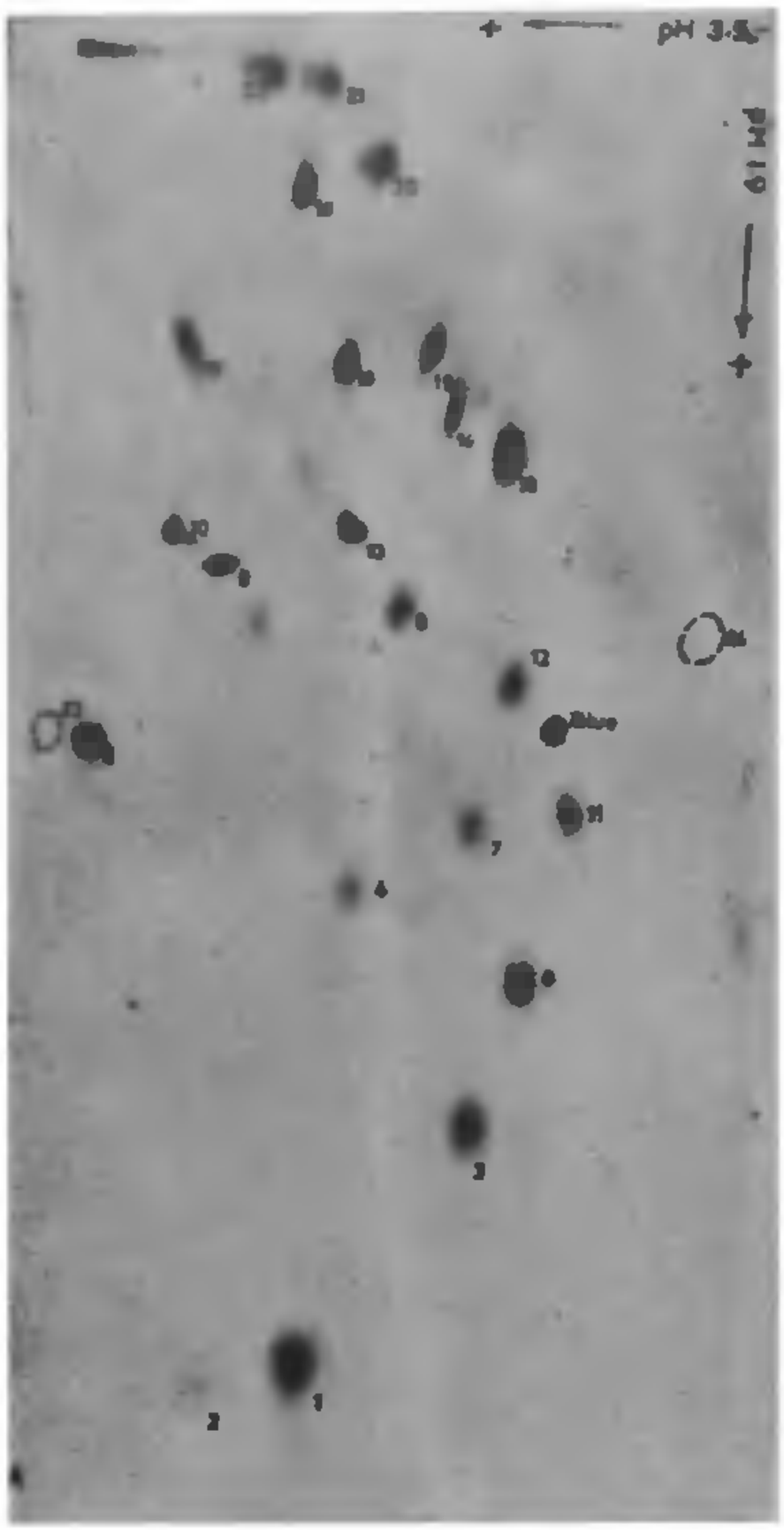

FIG. 2. Autoradiograph of RNase T_1 digestion products of 5S RNA. Thin layer DEAE-cellulose was prepared by coating a 20 × 40 cm glass plate with a mixture of 2 g of DEAE-cellulose and 4 g of microcrystalline cellulose blended with 36 ml of distilled water. Electrophoresis was carried as described in the section on experimental. A voltage of 4,000 for 2 hr was applied. Current gradually decreased from 35 mA to 20 mA.

The method has certain obvious advantages. As the time for the second dimension separation is only 1–2 hr, electrophoresis in both directions can be completed on the same day and fingerprinting time reduced considerably. The saving in time is advantageous when dealing with ^{32}P label which has a short half life. The ratio of DEAE-cellulose to cellulose powder may be varied to get an altered resolution if desired. Larger fragments are expected to separate better by decreasing the proportion of DEAE-cellulose in the thin layer mixture. This method is thus more advantageous in fingerprinting high molecular weight RNAs. It is more convenient to handle DEAE-cellulose plates than DEAE-cellulose paper as the latter is very fragile. Besides the apparatus can be easily assembled in any laboratory.

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