THIN-LAYER ELECTROPHORESIS*

PART III. STUDIES ON THE DISSIPATION OF HEAT

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Previous studies have indicated that a load of 0.021 W/cm² is tolerable for electrophoresis with thin-layer plates of cellulose[2] or kieselguhr G[3] for periods of up to 15 min. A lower load is recommended for longer electrophoretic runs[2,3]. Despite a build up of heat, the above loads may be exceeded, for example, the electrophoresis of food dyes on kieselguhr G has been found to be satisfactory for 2 h at an initial load of 0.026 W/cm² and for 15 min at the greater initial load of 0.041 W/cm² (ref. 3). However, these increased loads bring about a quicker breakdown in the electrophoretic process and, unless steps are taken to dissipate the heat that is necessarily produced by the passage of current, there is a rather severe limit to the load that can be used.

It is true that the heat produced can be diminished by diluting the electrolyte thus lowering the current passing, but a common practice, developed initially for paper electrophoresis, is to place the thin-layer plate on a cooling plate through which water is circulated[4-6]. Where extra cooling is required, passage of brine through the cooling plate has been recommended[6], and prior passage of the cooling water through a refrigerated thermostat has also been employed[7].

With the trend for thin-layer electrophoresis to be used for fingerprinting studies on amino acids and peptides, there is the tendency for the thin-layer electrophoretic process to be carried out at higher voltages. The availability of suitable methods of dissipating excessive heat is, therefore, a matter of prime importance, and it is for this reason that the present investigation on the dissipation of heat during thin-layer electrophoresis has been carried out.

EXPERIMENTAL

The thin layers (250 μ thick) were prepared from a slurry of 30 g of the appropriate support material in 60 ml water with a Shandon "Unoplan" Leveller and Spreader and dried at 105°.

The electrolyte solution was 0.05 *M* aqueous borax (pH 9.2).

The Shandon Constant Voltage Power Pack was used for the experiments in conjunction with a specially constructed horizontal electrophoresis tank made of Perspex and provided with a specially thin bottom (1.5 mm thickness) to the bridge compartment to facilitate the conduction of heat.

* For Part II of this series, see ref. 1.

For the experiments in which cooling was employed the electrophoresis tank rested on a prototype 4 × 6 (15 A module) couple "Frigistor" thermoelectric stage cooler (20 × 12.5 cm) through which tap water was circulated. When in operation the cooler was connected to a "Frigistor" 1503 D.C. Power Supply of maximum output 15 A and 3 V.

A few experiments were conducted with the glass plate supporting the thin-layer resting directly on the "Frigistor" stage cooler by using an electrophoresis tank from which the bottom of the bridge compartment had been removed. On these occasions the stage of the cooler was electrically insulated by painting with a solution of polystyrene in chloroform.

The thin-layer plates were saturated with electrolyte by capillary action in the manner previously described[3] and electrophoresis conducted in the usual way at different constant voltages and with different degrees of cooling by the "Frigistor" cooler.

RESULTS AND DISCUSSION

The dissipation of heat during electrophoresis using thin-layer material as support has been studied at initial loads within the range 0.03 to 0.18 W/cm^2. Using 0.05 *M* borax as electrolyte and thin layers of kieselguhr G of 250 μ thick, these loads correspond to applied voltages ranging from 500 to 1000 V for a 20 cm long plate, that is, 25 to 50 V/cm. Inspection of Table I shows that the loads and conditions employed are among the most severe of those previously reported in the literature.

TABLE I

SUMMARY OF LOADS AND CONDITIONS EMPLOYED (OR RECOMMENDED) FOR THIN-LAYER ELECTROPHORESIS

Initial load (W/cm²)	*Voltage (V/cm)*	*Current (mA/cm)*	*Duration*	*Substrate*	*Method of cooling*	*Ref.*
0.014	23	0.63	1 h	Silica gel G	None	8
0.022	22	0.98	1 h	Silica gel G	None	8
0.0125–0.0333	10–13.7	1.25–2.5	1.5–2 h	Plaster of Paris	None	9
0.021	—	—	15 min	Cellulose	None	2
0.073*	49	1.5	1 h	Silica gel G	Water	5
—	46	—	5 min	Kieselguhr	Water	4
—	45	—	2 min	Kieselguhr	None	4
0.076	47.5	1.6	40 min	Silica gel H	Refrigerator at —1°	7
—	20	—	1.5 h	Silica gel G	None	10
—	20	—	1.5 h	Kieselguhr	None	10
0.026	20	1.30	2 h	Kieselguhr G	None	3
0.041	25	1.65	15 min	Kieselguhr G	None	3
0.120**	40	3.0	30 min	Kieselguhr G	Water at 6.5°	11
0.088	40	2.2	28 min	Kieselguhr G	"Frigistor"	Present work
0.160	50	3.2	7 min	Kieselguhr G	"Frigistor"	Present work

* This figure relates to separations quoted by RITSCHARD[6] but this author also claims that water provided sufficient cooling for loads within the range 0.071–0.200 W/cm^2.

** Using Desaga Thin-Layer Electrophoresis Tank with Shandon Constant Voltage Power Pack.

Experiments with bridge of electrophoresis tank resting on "Frigistor" stage cooler

As stated above, the procedure adopted for the electrophoresis was that normally employed, except for the use of the "Frigistor" stage cooler for exercising different degrees of cooling. Typical results obtained in these circumstances are summarised in Table II for thin layers of kieselguhr G using 0.05 *M* borax as electrolyte. Similar trends were also observed for thin layers of silica gel G and alumina G.

It is clear from Table II that use of the "Frigistor" stage cooler appreciably extends the length of time during which electrophoresis can be successfully carried out at the loads studied. At the lower applied voltages, moderate cooling output by the "Frigistor" stage cooler is adequate for dissipating the heat produced. However, at the higher applied voltages, a cooling output approaching the maximum by the cooler serves only to extend for a short time the period for which electrophoresis can be carried out with 5 cm wide plates before the electrophoresis current rises to the maximum of 25 mA permitted by the Shandon Constant Voltage Power Pack.

It is, of course, possible that the cooler is potentially capable of controlling the heat produced at higher loads. However, since a likely application of the present

TABLE II

EFFECT OF VARIOUS DEGREES OF COOLING BY "FRIGISTOR" UNIT IN DISSIPATING HEAT PRODUCED DURING ELECTROPHORESIS AT CONSTANT VOLTAGE

Support: kieselguhr G (250 μ thick); electrolyte: 0.05 *M* borax; plate dimension: 20 × 5 cm.

Voltage (V)	*Initial load (W/cm^2)*	*Load after 60 min unless otherwise stated (W/cm^2)*	*"Frigistor" setting (A)*	*Temperature change on surface of a glass plate resting on the bridge compartment (°C)**
500	0.031	0.030	13	4–6
500	0.035	0.042	10	4–8
500	0.037	0.048	5	8–12
500	0.042	0.120**	—	20–30
600	0.038	0.044	13	2–7
600	0.050	0.114 (90 min)	10	4–12
600	0.055	0.138** (75 min)	5	8–15
600	0.060	0.144** (20 min)	—	20–27
700	0.056	0.081	13	4–10
700	0.070	0.172** (30 min)	10	3–10
700	0.077	0.172** (15 min)	5	7–14
700	0.084	0.172** (7 min)	—	21–24
800	0.088	0.208** (28 min)	13	2–7***
800	0.096	0.200** (15 min)	10	4–12
800	0.108	0.196** (5 min)	5	9–10
800	0.116	0.200** (4 min)	—	20–22
1000	0.160	0.260** (7 min)	13	1–2
1000	0.180	0.250** (1.5 min)	—	20–21

* Recorded by covering the thermometer bulb with wet filter paper and resting on the glass plate.

** These runs were terminated because the current carried by the 5 cm wide plates approached the measuring capacity of the power pack.

*** Temperature of thin layer at end of experiment was 17°. This was recorded by covering thermometer bulb with wet filter paper and resting on the thin layer.

work is to two-dimensional electrophoretic/chromatographic studies using 20 × 20 cm plates, a power pack with a current output of at least 100 mA will be required, particularly where electrolytes of the conducting power of 0.05 *M* borax are used. An output of this order approaches those of power packs used by RITSCHARD[5] and STEGEMANN AND LERCH[7].

Experiment with the thin-layer plate resting directly on "Frigistor" stage cooler

When the bridge compartment of the electrophoresis tank rests on the "Frigistor" stage cooler there are several heat insulating layers between the thin layer and the cooling surface of the "Frigistor" itself. The effect of two of these insulating layers, namely, an air layer and the layer of Perspex forming the bottom of the bridge of the electrophoresis tank, is removed by carrying out electrophoresis with the thin-layer plate resting directly on the "Frigistor" stage cooler. This has the effect of greatly improving the cooling performance during electrophoresis as is shown by the typical results quoted in Table III for experiments using applied voltages of 700 and 800 V.

TABLE III

COOLING PERFORMANCE OF "FRIGISTOR" UNIT ILLUSTRATING EFFECT OF REMOVING BOTTOM OF BRIDGE COMPARTMENT OF ELECTROPHORESIS TANK

Support: kieselguhr G (250 μ thick); electrolyte: 0.05 *M* borax; output of "Frigistor": 10 A; plate dimension: 20 × 5 cm.

Voltage (V)	*Load (W/cm²)*			
	Bottom of bridge compartment in position		*Bottom of bridge compartment removed*	
	Initial	*After time stated*	*Initial*	*After time stated*
700	0.070	0.172* (30 min)	0.070	0.161 (150 min)
				0.154 (180 min)
800	0.096	0.200* (15 min)	0.088	0.200* (75 min)

* These runs were terminated because the current carried by the 5 cm wide plates approached the maximum measuring capacity of the power pack.

CONCLUSION

The "Frigistor" stage cooler is able to usefully extend the length of time for which electrophoresis on thin layers at applied voltages of 40 to 50 V/cm can be carried out. The results indicate that a power pack of high current measuring capacity is desirable together with a "Frigistor" stage cooler of greater cooling capacity than that used in the present investigation.

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SUMMARY

The application of the "Frigistor" thermoelectric cooling device as a means of dissipating the heat produced during thin-layer electrophoresis has been studied at applied voltages within the range 25 to 50 V/cm for thin layers of kieselguhr G, silica gel G, and alumina G and using 0.05 *M* borax as electrolyte. The detailed results for kieselguhr G are presented and discussed and clearly indicate that the "Frigistor" stage cooler is able to usefully extend the length of time for which electrophoresis under the stated conditions can be carried out.

REFERENCES

1 W. J. CRIDDLE, G. J. MOODY AND J. D. R. THOMAS, *J. Chromatog.*, 18 (1965) 530.
2 N. NYBOM, *Physiol. Plantarum*, 17 (1964) 434.
3 W. J. CRIDDLE, G. J. MOODY AND J. D. R. THOMAS, *Proc. Soc. Anal. Chem. Conf., Nottingham*, Heffer, Cambridge, 1965, p. 135.
4 A. MOGHISSI, *Anal. Chim. Acta*, 30 (1964) 91.
5 W. J. RITSCHARD, *J. Chromatog.*, 16 (1964) 327.
6 *Thin Layer Electrophoresis*, Desaga and Camlab (Glass) Ltd., Technical Publication, 1964.
7 H. STEGEMANN AND B. LERCH, *Anal. Biochem.*, 9 (1964) 417.
C. G. HONEGGER, *Helv. Chim. Acta*, 44 (1961) 173.
9 F. DOBICI AND G. GRASSINI, *J. Chromatog.*, 10 (1963) 98.
10 G. PASTUSKA AND H. TRINKS, *Chemiker-Ztg.*, 85 (1961) 535.
11 W. J. CRIDDLE, G. J. MOODY AND J. D. R. THOMAS, unpublished work.