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Centrifugal counter-current chromatography, a promising means of measuring partition coefficients

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SUMMARY

Centrifugal counter-current chromatography (CCCC) was examined as a means of measuring lipophilicity values. Using an aqueous buffer as the stationary phase and 1-octanol as the eluent, seventeen solutes of widely different structure and of log D_{oct} [log (distribution coefficient) at pH 7.4] spanning 2.5 units were examined. A very good correlation was found with literature log P [log (partition coefficient) of neutral species] values measured by the classical shake-flask method. The efficiency and precision of the CCCC method makes it a promising technique for measuring lipophilicity.

INTRODUCTION

Lipophilicity is an important molecular property of drugs and other xenobiotics often correlated with their biological activity. A number of experimental techniques have been developed to simulate partition processes in biological systems and to measure lipophilicity. The best known is the shake-flask (SF) method, which remains the standard system for measuring the lipophilicity of chemical compounds. In this system, two poorly miscible liquid phases (water and an organic solvent) are stirred gently until an equilibrium is reached; 1-octanol is universally accepted as the reference organic solvent[1–3], but other solvents are very useful, for example in comparing the hydrogen-bonding ability of series of solutes[4–6]. However, despite its value, the SF method suffers from a number of practical limitations due to various perturbing factors such as time consumption, solute stability or volatility, solute impurities, formation of microemulsions and concentration and salt effects, etc., as cogently discussed by Dearden and Bresnen[7].

Solid–liquid partition chromatography has been used as an alternative means

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for measuring lipophilicity for many years. In particular, chromatographic retention parameters obtained by reversed-phase high-performance liquid chromatography (RP–HPLC) have become increasingly popular in replacing the 1-octanol–water partition coefficient in quantitative structure–activity relationship (QSAR) studies[8,9]. Unfortunately, the presence of a solid support with a non-negligible proportion of residual silanol groups on the surface of the alkyl-bonded phase dramatically influences the partitioning process of polar basic compounds owing to an additional adsorption mechanism; the use of a masking agent becomes necessary[10–12], but this introduces an additional variable in the experimental conditions.

Recently, a new method[13–15] called centrifugal partition chromatography (CPC), high-speed counter-current chromatography (HS-CCC) or centrifugal counter-current chromatography (CCCC) has found its first applications in determining lipophilicity. This is a liquid–liquid chromatographic method in which two nonmiscible solvents are used as the stationary and mobile phase, respectively. A centrifugal force maintains the stationary phase, while the mobile phase is pumped through the system. Using a Sanki CPC model[16], the method has demonstrated its potential for measuring partition coefficients[13,14,17]; limitations are the high cost of the equipment, its restricted availability and a number of technical difficulties. Another apparatus is available, namely the Ito multi-layer coil separator–extractor[15,18–20], which is cheaper and easy to obtain. To the best of our knowledge, this equipment has never been used to determine partition coefficients. Our preliminary investigations showed a narrow range of measurable partition coefficients, a problem that can be partly overcome by varying the relative volumes of the two phases in the apparatus using two distinct pumps to fill the coil.

EXPERIMENTAL

Chemicals

β-Pyridylalkanols were synthetized as described[21]. All other solutes were commercially available; 1-octanol (purum) was purchased from Fluka (Buchs, Switzerland).

Apparatus

Measurements were performed at room temperature using an Ito Multi-layer coil separator–extractor (P.C, Potomac, MD, U.S.A.) equipped either with preparative coil No. 10 (I.D. 2.6 mm, volume 370 ml) or with analytical coil No. 14, which we shortened to about one third of the initial length (I.D. 1.6 mm, volume 107 ml). The speed of rotation was controlled with a d.c. motor speed control/basic speed range ASH-600 (Bodine Electric, Chicago, IL, U.S.A.). For commuting between "head" and "tail" ends of the coil, an SRV-4 four-way valve (Pharmacia, Uppsala, Sweden) was installed. The samples were injected through a Lobar six-port valve injector (Merck, Darmstadt, F.R.G.) with the 2.5-ml loop mounted. The samples were detected at 254 nm (morphine at 287 nm) using a Uvikon 725 UV detector (Kontro, Zurich, Switzerland) equipped with a QS 1.000 80-μl UV cell. The chromatograms were recorded with a Model 3392A integrator (Hewlett-Packard, Meyrin, Switzerland). The coil was filled using two T414 LC pumps (Kontron), which were joined with a metallic T-piece. All connecting tubes were Lobar No. 15455 (Merck). A more detailed description of the apparatus was published by Slacanin *et al.*[22].

Procedures

Preparation of phases. The organic and aqueous phases were mutually saturated by stirring for 1–2 h and then separated in a decantation funnel.

Preparation of samples. Concentrated or saturated solutions of each solute were prepared in the mobile phase (*i.e.*, water-saturated octanol).

Filling of the coil. To allow the volume ratio of the two phases to be chosen at will, the technique of coil filling was modified by using two pumps, one for each phase. While the system was in rotation, the coil was first completely filled with the mobile phase and then a desired volume of the stationary phase was added to the column by displacing the mobile phase. This allowed good control of the volume ratio of the two phases in the coil (as checked repeatedly by emptying the coil), and no exclusion of stationary phase was observed once the mobile phase had been pumped through the coil during measurement of retention volumes. It was found useless to empty and wash the coil between days; the pump and the motor were stopped overnight, and it was sufficient the next day to set the coil in rotation, wait for 15–30 s and then start pumping the mobile phase.

Although the distribution of the stationary phase in the coil may not be totally uniform, this cannot affect the distribution equilibrium and hence the results, provided that the interface area is not decreased[15].

Sample injections. These were done manually without stopping either the eluent flow or rotation. The usual volume injected was 0.5–1.0 ml (loop capacity 2.5 ml), making it possible to inject simultaneously three or four compounds, provided that they were of sufficiently different lipophilicity and did not interfere with each other's retention.

Determination of column dead time. In the normal operating mode used (the apolar solvent being the mobile phase), anthracene ($\log P = 4.5$) was taken as the non-retained compound whose duration of passage was the column dead time, t_0.

Reuse of solvents. The mobile phase was immediately recycled, provided that it did not contain any solute; contaminated mobile phase was distilled and saturated with the aqueous phase before reuse, thus saving significant volumes of solvent.

Washing of the coil. This was performed with methanol; nitrogen was then used to dry the coil.

Operating conditions

The temperature throughout was 21 ± 1°C. The mobile phase was 1-octanol and the aqueous stationary phase was a 0.01 *M* solution of 3-morpholinopropanesulphonate at pH 7.4; both phases were mutually saturated. Three different sets of conditions were used. When the preparative coil was used with a volume ratio of mobile and stationary phases of *ca.* 15:1, the flow-rate of the eluent was measured as 7.45 ml/min (setting 8 ml/min) and the speed of rotation was 800 rpm. When the preparative coil was used with a volume ratio of mobile and stationary phases of *ca.* 1:2, the flow-rate of the eluent was 4.95 ml/min (setting 5 ml/min) and the speed of rotation was again 800 rpm. When the analytical coil was used, a volume ratio of mobile and stationary phases of *ca.* 3:2 was chosen, the flow-rate of eluent was 3.8 ml/min (setting 4 ml/min) and the speed of rotation was 1000–1200 rpm (no precise scale on the apparatus).

The octanol–water distribution coefficient of a given solute (apparent partition

coefficient at the pH of study, here 7.4) was calculated according to the equation[23].

$$\log D^{ITO} = \log [V_s/(V_r - V_0)] \quad (1)$$

where D^{ITO} is the octanol–water distribution coefficient, V_0 the dead volume (mobile phase volume, calculated as V_0 = flow · t_0), V_s is the volume of the stationary phase (calculated as total volume minus V_0) and V_r the retention volume of the solute (calculated as V_r = flow · t_r from the retention time of the solute, t_r).

As some of the solutes examined were partially or completely ionized at pH 7.4, the distribution coefficient at pH 7.4 (also called the apparent partition coefficient, and designated here as log D^{ITO} or log D^{SF}, respectively) was distinguished from the "true" partition coefficient of the neutral species (log P). Log D and log P are identical for non-ionizable compounds, whereas for ionizable compounds log P was calculated by correcting log D for ionization as described by Rekker[24].

RESULTS AND DISCUSSION

Preparative coil, volume ratio of mobile and stationary phases ca. 15:1

In this system, the mean dead time t_0 was 46.55 ± 0.26 min (anthracene as the non-retained solute; average of eight measurements over four days). The small standard deviation indicates the stability and the reproducibility of the equipment. The dead volume V_0 was calculated to be 346 ml and the volume of the stationary phase V_s was 24 ml, *i.e.*, a volume ratio close to 15:1.

Twelve solutes with published log P values covering a range of almost 2 units were examined in this sytem. The solutes were also chosen for their structural differences (acids, bases, phenols, heterocyclic derivatives, etc.). Their t_r values are reported in Table I; the derived distribution coefficients (as log D^{ITO} values) and calculated log P^{ITO} values are also given. These values are the means of two or three injections, the deviations always being smaller than 0.01 on the log D scale.

Preparative coil, volume ratio of mobile and stationary phases ca. 1:2

In this system, the mean dead time t_0 was 25.50 ± 0.38 min (anthracene as the non-retained solute; average of four measurements over two days). The dead volume V_0 was calculated to be 126 ml and the volume of the stationary phase V_s was 244 ml, *i.e.*, a volume ratio close to 1:2.

Eight solutes were examined, their published log P values ranging from −0.1 to 1.1. Their t_r values are reported in Table I together with log D^{ITO} (deviations <0.01) and log P^{ITO} values. Note that three solutes (Nos. 12, 13 and 16) were examined in both systems, the differences in the log D^{ITO} values being 0.32, 0.08 and 0.11, respectively.

Analytical coil, volume ratio of mobile and stationary phase ca. 3:2

In this system, the mean dead time t_0 was 18.00 ± 0.20 min (anthracene as the non-retained solute; average of six measurements over four days). The dead volume V_0 was calculated to be 68 ml and the volume of the stationary phase V_s was 39 ml, *i.e.*, a volume ratio close to 3:2.

The ten solutes examined had published log P values ranging from −0.8 to 2.7.

TABLE I

OCTANOL–WATER PARTITION COEFFICIENTS MEASURED BY CCCC USING A PREPARATIVE COIL

No.	*Solute*	t_r *(min)*	*Log* D^{ITO} [a]	*Log* D^{SF} [b]	*Log* P^{ITO} [c]	*Log* P^{SF} [d]
1	4-Iodobenzoic acid	53.77[e]	−0.36		3.00	3.02
2	4-Bromobenzoic acid	61.19[e]	−0.66		2.74	2.86
3	4-Chlorobenzoic acid	64.54[e]	−0.75		2.66	2.65
4	4-Hydroxybenzoic acid	105.5[e]	−1.26		1.56	1.58
5	Benzyl alcohol	28.42[f]	1.22	1.10	1.22	1.10
6	Benzylamine	67.50[e]	−0.82		1.15	1.09
7	4-Fluorobenzamide	30.89[f]	0.96	0.91	0.96	0.91
8	2-Bromobenzamide	35.00[f]	0.71	0.73	0.71	0.73
9	Benzamide	36.56[f]	0.65	0.64	0.65	0.64
10	β-Pyridylpropanol	38.00[f]	0.59	0.60	0.59	0.60
11	Sulpiride	98.44[e]	−1.21	−1.15	0.52	0.58
12	2-Aminopyridine	47.04[e]	0.73		0.83	0.49
		44.81[f]	0.41		0.51	0.49
13	3-Aminophenol	48.33[e]	0.23	0.17	0.23	0.17
		60.00[f]	0.15	0.17	0.15	0.17
14	Sulphamerazine	57.32[e]	−0.53	−0.12[g]	0.07	0.14
15	Sulphathiazole	54.82[e]	−0.42	−0.41[g]	0.04	0.05
16	β-Pyridylmethanol	50.97[e]	−0.15	−0.02	−0.15	−0.02
		79.21[f]	−0.04	−0.02	−0.04	−0.02
17	Sulphanilamide	69.35[e]	−0.85	−0.89[g]	−0.85	−0.72

[a] Log (distribution coefficient) (apparent partition coefficient) measured by CCCC at pH 7.4.

[b] Log (distribution coefficient) (apparent partition coefficient) measured by the shake-flask method at pH 7.4 (literature data[25]).

[c] Log (partition coefficient), corrected for ionization when applicable, measured by CCCC.

[d] Log (partition coefficient), corrected for ionization when applicable, measured by the shake-flask method (literature data[25]).

[e] Measured by CCCC at a phase ratio of about 15:1.

[f] Measured by CCCC at a phase ratio of about 1:2.

[g] Experimental value at pH 7.5.

TABLE II

OCTANOL–WATER PARTITION COEFFICIENTS MEASURED BY CCCC USING AN ANALYTICAL COIL

No.	*Solute*	t_r *(min)*	*Log* D^{ITO} [a]	*Log* D^{SF} [b]	*Log* P^{ITO} [c]	*Log* P^{SF} [d]
1	Sulphanilamide	76.50	−0.76	−0.89	−0.76	−0.72
2	Morphine	29.00	−0.03	−0.12	0.84	0.76
3	β-Pyridylmethanol	30.10	−0.08	−0.02	−0.08	−0.02
4	Sulphathiazole	42.66	−0.38	−0.43	0.08	0.05
5	Sulphamerazine	46.00	−0.44	−0.12	0.16	0.14
6	3-Aminophenol	25.59	0.13	0.17	0.13	0.17
7	Sulpiride	120.0	−1.00	−1.15	0.73	0.58
8	β-Pyridylpropanol	20.43	0.61	0.60	0.61	0.60
9	Benzylamine	76.75	−0.76		1.17	1.09
10	4-Chlorobenzoic acid	69.50	−0.70		2.71	2.65

[a–d] See Table I.

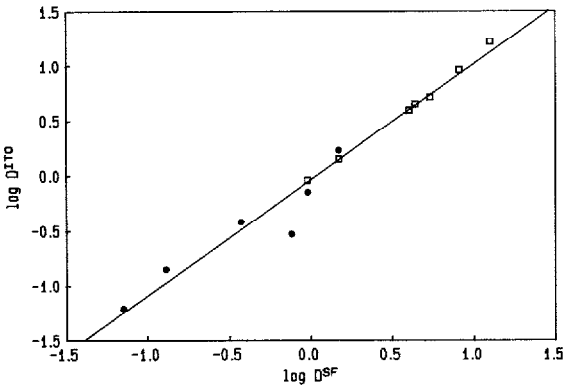

Fig. 1. Correlation of log D values (apparent partition coefficient at pH 7.4) measured by CCCC (Ito apparatus equipped with preparative coil) with literature values obtained by the shake-flask (SF) method. The line corresponds to eqn. 2. The symbols ● and □ correspond to values measured at phase ratios of 15:1 and 1:2, respectively.

Their retention times (t_r), log D^{ITO} (deviations <0.01) and log P^{ITO} values are reported in Table II.

Comparison of distribution and partition coefficients measured by the CCCC method with literature values

Preparative coil. A correlation was first sought between log D^{ITO} values and log D values measured by the shake-flask method and reported in the literature (*i.e.*, log D^{SF} values, see Table I), yielding the equation

$$\log D^{ITO} = 1.055(\pm 0.122) \log D^{SF} - 0.036(\pm 0.081) \quad (2)$$
$$n = 13;\ r^2 = 0.970;\ s = 0.130;\ F = 364$$

where n is the number of observations, r the correlation coefficient, s the standard deviation and F the Fisher's test parameter.

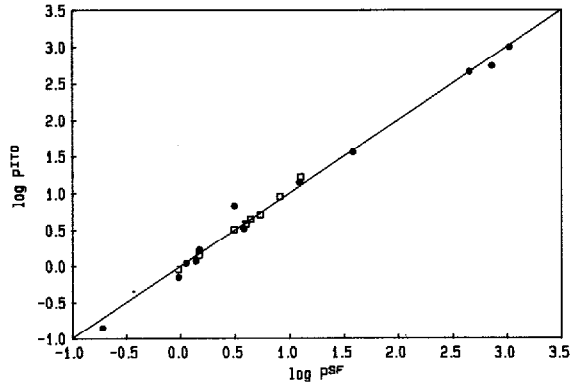

Fig. 2. Correlation of log P values ("true" partition coefficient corrected for ionization) determined by CCCC (Ito apparatus equipped with preparative coil) with literature values obtained by the shake-flask (SF) method. The line corresponds to eqn. 3. Symbols as in Fig. 1.

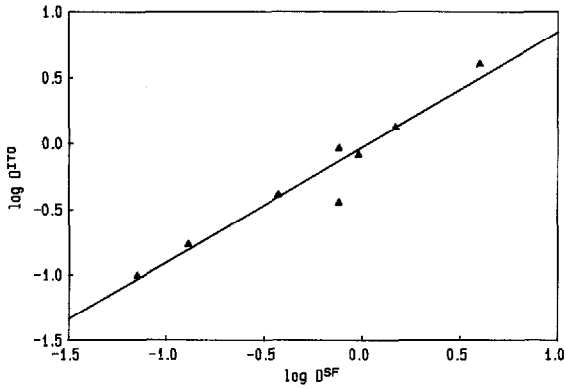

Fig. 3. Correlation of log D values (apparent partition coeffient at pH 7.4) measured by CCCC (Ito apparatus equipped with analytical coil) with literature values obtained by the shake-flask (SF) method. The line corresponds to eqn. 4.

In a second step, the correlation between log P^{ITO} and log P^{SF} (Table I) was calculated, yielding the equation

$$\log P^{ITO} = 1.001(\pm 0.051) \log P^{SF} + 0.001(\pm 0.065) \quad (3)$$
$$n = 20;\ r^2 = 0.990;\ s = 0.105;\ F = 1730$$

Figs. 1 and 2 show these relationships and demonstrate the regular lipophilicity distribution of the seventeen solutes investigated.

Analytical coil. Here, the following equally satisfactory equations were obtained:

$$\log D^{ITO} = 0.875(\pm 0.234) \log D^{SF} - 0.029(\pm 0.136) \quad (4)$$
$$n = 8;\ r^2 = 0.933;\ s = 0.143;\ F = 84$$

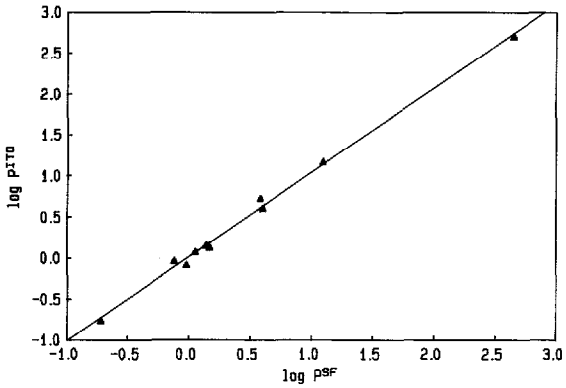

Fig. 4. Correlation of log P values ("true" partition coefficient corrected for ionization) determined by CCCC (Ito apparatus equipped with analytical coil) with literature values obtained by the shake-flask (SF) method. The line corresponds to eqn. 5.

$$\log P^{ITO} = 1.030(\pm 0.054) \log P^{SF} + 0.017(\pm 0.053) \quad (5)$$
$$n = 10;\ r^2 = 0.996;\ s = 0.064;\ F = 1927$$

These relationships are represented in Figs. 3 and 4.

The four equations, but especially eqns. 3 and 5, are characterized by good correlation coefficients, indicating that the CCCC method indeed is an effective means of measuring lipophilicity. Even more interesting is the fact that in the four equations the slope is equal to unity and the intercept is zero, implying that within the lipophilicity range investigated log P values determined by the CCCC method can be equated with those determined by the shake-flask system without any calibration or proportionality factor.

CONCLUSION

This study has established the interest of CCCC as a novel technique for measuring lipophilicity. Using an aqueous buffer as the stationary phase and octanol as the eluent, log P values were found that correlate well with literature values as measured by the shake-flask method. In addition, the very good reproducibility of the CCCC system suggests that its precision must be significantly better than that of the shake-flask method, as shown by subsequent studies in our laboratory (work in progress). Certainly it is less time consuming and thus more efficient, our estimate being that the gain in time is 4–6-fold.

The compounds investigated span a log D range of 2.5 units (Fig. 1) and a log P range of almost 4 units (Fig. 2). This is still too limited a range to render the CCCC method generally applicable, but work is now in progress to alleviate this limitation, one possibility worth exploring being the reversed elution mode (organic solvent as stationary phase). The use of coils with different internal diameters and volumes could yield additional advantages, allowing retention times and amounts injected to be closer to those in high-performance liquid chromatography.

SYMBOLS AND ABBREVIATIONS

$\log D_{oct}$	log (octanol–water distribution coefficient) at a given pH
$\log D^{SF}$, $\log D^{ITO}$	log (distribution coefficient), solvent system not specified, measured by the shake-flask method or with the Ito equipment, respectively
$\log P$	log (partition coefficient) of neutral species, solvent system not specified
$\log P^{SF}$, $\log P^{ITO}$	log (partition coefficient), solvent system not specified, measured by the shake-flask method or with the Ito equipment, respectively
t_0	column dead time
t_r	retention time of the solute
V_0	dead volume of the column (volume of the mobile phase)
V_r	retention volume of the solute
V_s	volume of the stationary phase
CCCC	centrifugal counter-current chromatography
SF	shake-flask method

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REFERENCES

1 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
2 T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, 86 (1964) 5175.
3 J. C. Dearden and J. H. O'Hara, *Eur. J. Med. Chem.*, 13 (1978) 415.
4 P. Seiler, *Eur. J. Med. Chem.*, 9 (1974) 473.
5 T. Fujita, T. Nishioka and M. Nakajima, *J. Med. Chem.*, 20 (1977) 1071.
6 M. Gryllaki, H. van de Waterbeemd, B. Testa, N. El Tayar, J. M. Mayer and P. A. Carrupt, *Int. J. Pharm.*, 51 (1989) 95.
7 J. C. Dearden and G. M. Bresnen, *Quant. Struct.–Act. Relat.*, 7 (1988) 133.
8 Th. Braumann, *J. Chromatogr.*, 373 (1987) 191.
9 N. El Tayar, G. J. Kilpatrick, H. van de Waterbeemd, B. Testa, P. Jenner and C. D. Marsden, *Eur. J. Med. Chem.*, 23 (1988) 173.
10 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
11 E. Bayer and A. Paulus, *J. Chromatogr.*, 400 (1987) 1.
12 N. El Tayar, A. Tsantili-Kakoulidou, T. Roethlisberger, B. Testa and J. Gal, *J. Chromatogr.*, 439 (1988) 237.
13 A. Berthod and D. W. Armstrong, *J. Liq. Chromatogr.*, 11 (1988) 547.
14 A. Berthod and D. W. Armstrong, *J. Liq. Chromatogr.*, 11 (1988) 567.
15 Y. Ito, *CRC Crit. Rev. Anal. Chem.*, 17 (1986) 65.
16 W. Murayama, T. Kobayashi, Y. Kosuge, H. Yano, Y. Nunogaki and K. Nunogaki, *J. Chromatogr.*, 239 (1982) 643.
17 N. El Tayar, A. Marston, A. Bechalany, K. Hostettmann and B. Testa, *J. Chromatogr.*, 469 (1989) 91.
18 Y. Ito, *J. Chromatogr.*, 188 (1980) 33.
19 Y. Ito, *J. Chromatogr.*, 188 (1980) 43.
20 Y. Ito and W. D. Conway, *J. Chromatogr.*, 301 (1984) 405.
21 J. M. Mayer and B. Testa. *Helv. Chim. Acta*, 65 (1982) 1868.
22 I. Slacanin, A. Marston and K. Hostettmann, *J. Chromatogr.*, 482 (1989) 234.
23 A. J. P. Martin and R. L. M. Synger, *Biochem. J.*, 35 (1941) 1358.
24 R. F. Rekker, *The Hydrophobic Fragmental Constant, its Derivation and Application—A Means of Characterizing Membrane Systems*, Elsevier, Amsterdam, 1977.
25 C. Hansch and A. Leo, *Log P and Parameter Database: a Tool for the Quantitative Prediction of Bioactivity*, Pomona College Medicinal Chemistry Project, Comtex Scientific, New York, 1983.