ations may be more useful for particular aspects of such
e, oxygen diffusion rates (Saini, 1976) will be more sensitive
rement of air capacity. Determination of the volume of coarser
an 120 μm, is useful in studies of root extension, and shear or
is useful in relation to cultivations and poaching. Saturated
aulic conductivity may also be helpful in some situations.

Acknowledgements

colleagues in the Soil Survey who sampled the soils studied,
l and Mrs. V. F. Wright for the water retention measurements
is. Mr. C. L. Bascomb supplied the mechanical and chemical
. Thomson prepared the diagrams.

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An automatic micro-injection system and its use in the microcalorimetry of cation-exchange sorp

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Herts

Reprinted from Laboratory Practi

Abstract

Details are given of a remote-control device which can deliver known microlitre volumes of solutions simultaneously to the 'control' and 'reaction' cells in the LKB calorimeter, and can do so accurately, automatically and at preset intervals. The device incorporates Hamilton microsyringes. The facility for injection at preset intervals is designed to allow the heat change after each addition of reacting solution to be recorded completely before the next addition, the interval for a reaction being predetermined in a pilot scale experiment.

A method is described for measuring differential enthalpies of potassium-calcium exchange in soils and clays using an LKB microcalorimeter incorporating this device. This achieves a considerable economy in time over the method of deriving the information from thermodynamic treatments of cation-exchange isotherms. The degree of cation-saturation is interpolated from a parallel 'isotherm' experiment on a macroscale in which several consecutive additions in the 'calorimeter' experiment are telescoped into single steps.

THE energetic heterogeneity of distribution of potassium and calcium ions in the soil exchange complex can be determined by measuring the differential enthalpy of exchange as a function of cationic composition so that the free energy parameter can be divided into its enthalpy and entropy components. In micaceous and related interstratified minerals alone, surface charge density, the Fe^{2+}/Fe^{3+} distribution in the octahedral layer and the particle size of the minerals are some of the factors influencing such energetic heterogeneity. Previous work on soils using exchange isotherms suggested that groups of homoenergetic sites exist that selectively sorb K (Talibudeen 1972). Microcalorimetry offers a more precise method for obtaining such information, although to measure enthalpies of ion-exchange reactions at solid-solution interfaces, good mixing is essential (Harter and Kilcullen, 1976). We describe a modification of the usual technique of mixing dry powders with reacting solutions to enable the 'tumbling' action of the LKB microcalorimeter to achieve this.

To investigate all the sites involved in cation-exchange more quickly than is possible with normal procedures, a continuous addition and recording procedure is desirable. An automated device is described for delivering microlitre volumes of reactant solution to the LKB microcalorimeter that enables a full isotherm to be measured in a few days with little manual attention. This device is developed from the original version of Dr. R. C. Woledge (see Acknowledgements).

The automatic cell injection system

The cell injection system is designed to dispense precise volumes of solutions repetitively at constant temperature into the two reaction cells of the calorimeter. It is attached to the cylindrical case inside the constant temperature cabinet and is an electrically-driven and controlled twin microsyringe unit (Figure 1). The authors can make available on request, detailed constructional drawings of the complete injection system.

The microsyringe unit (Figures 1 and 2)

The output shaft of a miniature reversible electric motor (8), fixed to the main block (2) is coupled 'in line' to the thimble (9) of a micrometer head. The piston shafts (10A) of two precision microsyringes (10) are attached to an arm (3) fixed to the barrel (9A) of the micrometer. The syringe barrels (10) are held rigidly in the main block (2) by their neck flanges (11). A double cam (5,5A) is fixed on to the micrometer spindle (9B) and operates two microswitches (MS1, MS2) fixed to the endplate (2A) of the main block.

This assembly, fixed to a base-plate mount (1), is attached to the cylindrical drum casing diametrically opposite the cell access cover and is counter-balanced by weights fixed to the rim of the drum. Teflon capillary tubing, connected to the two syringes, is fed through holes in the end of the drum casing, the insulation and the heat sink. The tubing is fed into the reaction cells through their respective caps (Figure 3).

Basic operation of the microsyringe u
The motor unit (8) is energised
'automatic' sequence on the cont
thimble (9) at 3 r.p.m. The thimbl
laterally by a ball-bearing fitted in
micrometer barrel (9A), and thus t
piston shafts (10A), move 0.635
thimble (9). This dispenses 5.29 μl c
cells through the capillary tubing. Th
microswitch (MS1), ensures one c
motor-micrometer unit. The other
(MS2) control the automatic-timer ze

Each syringe piston travels 19.7 mm
(i.e. 31 'shots' of 5.29 μl). The sy
reversing the rotation of the motor-
control unit. Limit switches (MS3, MS
of the arm (3) on 'inject' and 'refill' ope
and integral delivery volumes can be
microsyringes of various capacities.)

The electrical control unit

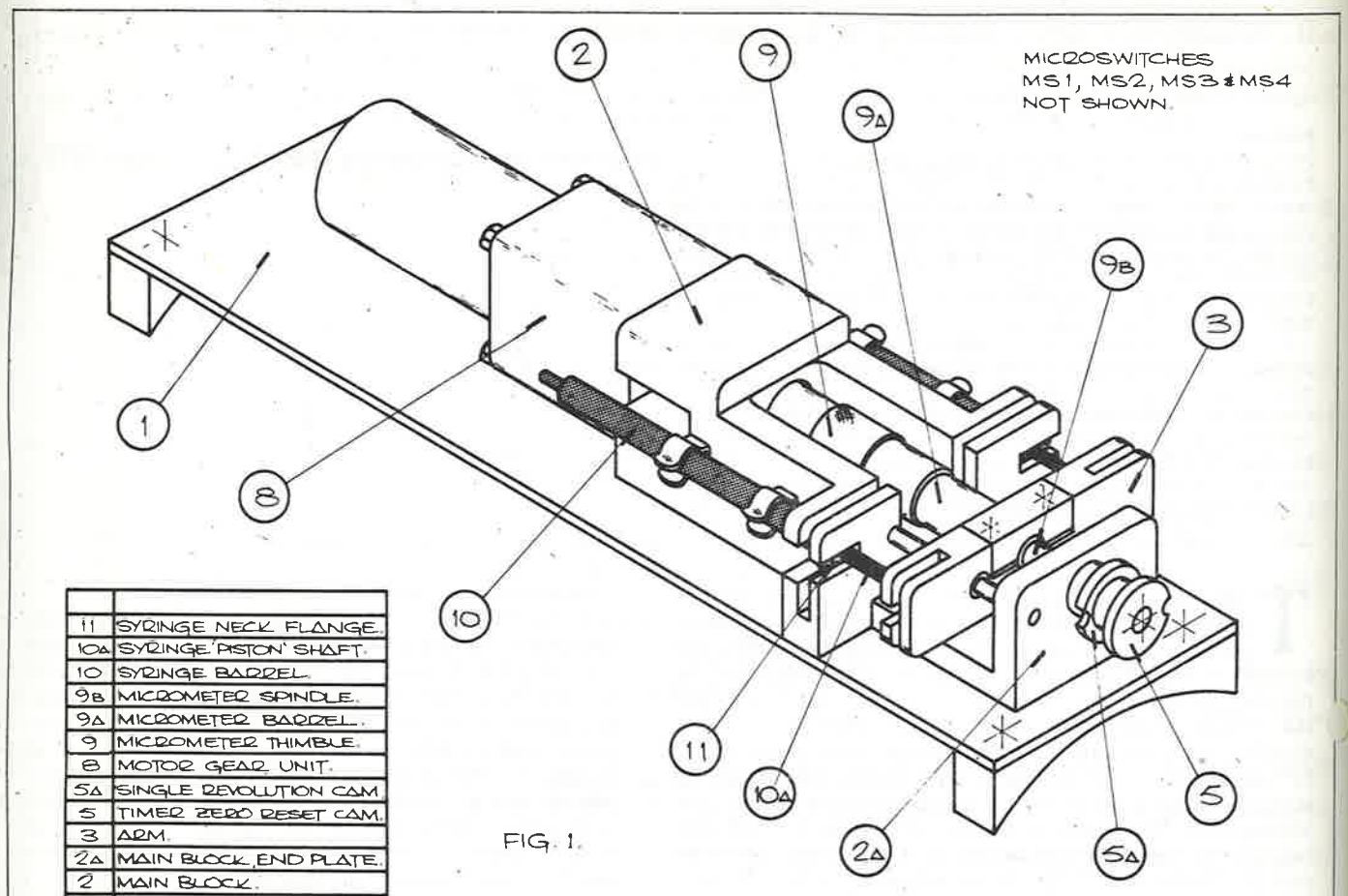
This unit incorporates: (i) a manual 'single
start, with an automatic stop after each dis
to prevent any overtravel of the arm
discharging the syringes and a visual ind
limits; (iii) a by-pass switch to stop the rota
any desired position for easy access to the m
a variable timer to enable repetitive dispen
start; (v) a fully automated time-sequence o
'shots' are dispensed and 'mixed' in the reac
intervals to allow for recording the full he
from each 'shot'.

Basic operation of the electrical control unit (Fi

On pressing motor-start button A, relay RL1, brief
hold-on delay, starts the microsyringe motor. MS1
then keeps the motor running until the micro and
cam 5A has completed one revolution. Cam 5 ns off
microswitch MS1, stopping the microsyrin and
'shunting' the now open-circuit supply. This a very
efficient regenerative braking system to thus
preventing the motor shaft from 'over-running ensuring
that equal volumes of solution are dispensed. Th switches
MS3, MS4 also operate lamp indicators on the fro el.

This part of the circuit is linked with the *man* fill' and
'inject' facility and comprises a *double-pole cen* off toggle
switch with associated relays RL3, *RL4. Manual ope* ion of the
syringes in either mode activates relay RL2 which emporarily
isolates the motor from the normal 'single dispense' or
'automatic' supply circuitry.

In the automatic 'timed dispense' mode, timer RL5 is energised and an initial cycle accomplished by the 'manual start' procedure. During this period, cam 5 on the micrometer spindle operates microswitch MS2 and resets the timer to zero. After a



11	SYRINGE NECK FLANGE.
10A	SYRINGE 'PISTON' SHAFT.
10	SYRINGE BARREL.
9B	MICROMETER SPINDLE.
9A	MICROMETER BARREL.
9	MICROMETER THIMBLE.
8	MOTOR GEAR UNIT.
5A	SINGLE REVOLUTION CAM
5	TIMER ZERO RESET CAM.
3	ARM.
2A	MAIN BLOCK, END PLATE.
2	MAIN BLOCK.
1	BASE PLATE & MOUNT.

FIG. 1.

Figure 1. The microsyringe unit.

preset period, the timer operates again energising manual start relay RL1 and the process continues until the syringes are nearly empty, when limit switch MS4 is activated.

The 'mixing' start button in the control circuitry of the LKB microcalorimeter (LKB circuit diagrams, Figure 7) rotates the calorimeter drum through 420° and ensures that before and after a mixing cycle, the drum cover rests in a vertical position for access to the reaction cells. Because the microsyringe unit is diametrically opposite the cell-access cover on the drum, the normal rotation cycle is stopped in a suitable position for access to it by inserting a single-pole on-off switch B ('drum-stop' switch) in the 240 V feed line to the calorimeter rotation motor. The drum can be started with the 'mixing' start button when this switch is on, and stopped when off. Normal rotation can be resumed by putting this switch on.

Switches are also provided on the microsyringe control unit front panel for 'auto-drum-rotate' and 'auto-record' for use with a 'completely automatic' injection cycle.

Calorimetric measurements for K-Ca exchange in soils

Two soils of contrasting texture and mineralogy were used in these preliminary experiments (Table I). The Harwell soil contains a larger proportion of small particles (especially clay particles less than 2 μm), a much higher negative charge, and twice as much of the non-expanding or slightly expanding micaceous minerals. The content of highly expanding smectites is similar in the two soils but the Harwell soil contains about 10 per cent (w/w) of a zeolite, clinoptilolite, of high specific negative charge in which K-Ca exchange is a time-dependent process between 0-30 per cent K saturation (Deist and Talibudeen, 1967).

The soil was fully Ca-saturated with 0.1 *M* $CaCl_2$, then washed free of Cl^- with water and absolute alcohol, and dried at about 40°C. About 3 g of this Ca-soil was mixed into a smooth paste with 5 g of 0.05 *M* $CaCl_2$. With a Pasteur pipette enough of this paste to contain 50 microequivalents of negative charge was transferred to the small (rear) compartment of the reaction cell in the LKB microcalorimeter (system 2107). The exact amount was determined by weight.

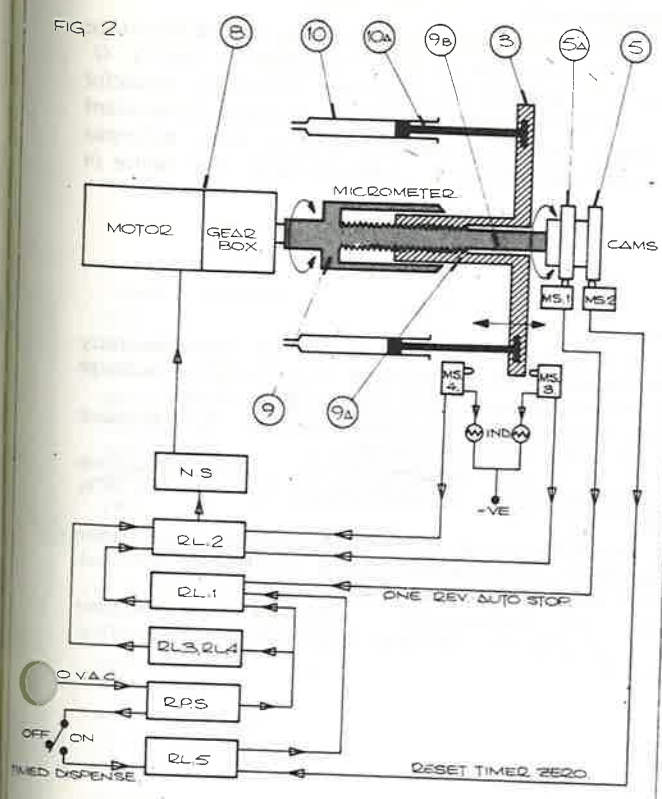
We syringed 2.50 ml of 0.05 *M* $CaCl_2$ into the large (front) compartment of the reaction cell and the reference cell. The Hamilton syringes in the injection system were filled with 0.5 *M* KC1, eliminating leaks and air-bubbles. The calorimeter was then closed and allowed to attain thermal equilibrium over the weekend (3 days).

Table I. *Textural, mineralogical and charge characteristics of Saxmundham and Harwell soils, expressed per gram of dry soil.*

	Saxmundham	*Harwell*
Clay + silt (0–20 μm)		
Weight g	0.460	0.750
Total charge μe	177	284
Kaolinite		
Weight g	0.053	Trace
negative charge μe	4.6	0.0
Micas		
Weight g	0.064	0.119
negative charge μe	19.7	37.8
Smectites		
Weight g	0.195	0.224
negative charge μe	153	178
Zeolite		
Weight g	0	0.091
negative charge μe	0	69.0

Before starting the exchange enthalpy measurements, the cell contents were mixed to check that the soil was thoroughly wetted, so that the heat of wetting would not affect the measurements. The KC1 solution was then injected during two minutes in five 'shots' of 5.29 μl each, totalling 13.25 μequiv. K per measurement and the mixing cycles repeated over 15 to 30 minutes until no more heat change was recorded. The equilibriation time for completing any detectable heat change after each addition was less than thirty minutes. For the Harwell soil, however, this meant that only partial equilibrium was attained between 0-30 per cent K saturation because earlier work had shown that complete equilibrium with the zeolite component

FIG. 2



RL 1.	RELAY 1. START & AUTO (SINGLE DISPENSE)
RL 2.	RELAY 2. MODE CHANGEOVER.
RL 3 & 4.	RELAY 3 & 4. MANUAL FILL & INJECT.
RL 5	RELAY 5. FEGRANTI ZN1034E TIMER.
RPS.	REGULATED POWER SUPPLY. 5v & 12v.
N.S.	NOISE SUPPRESSOR.
IND.	INDICATOR LAMPS.
MS1,2,3 & 4	MICRO SWITCHES.

Figure 2. A block diagram of the automatic dispenser.

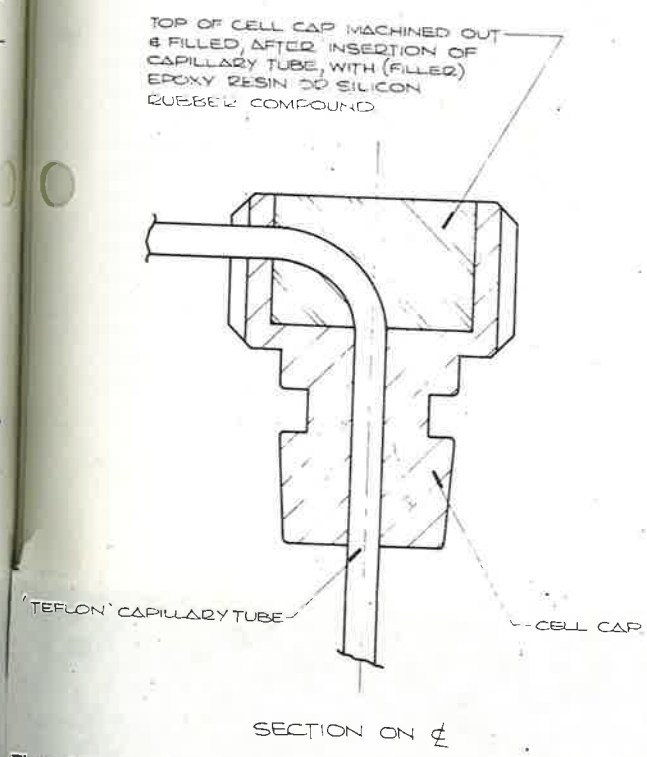


Figure 3. Section through the teflon cap of the microcalorimeter cell after machining and inserting PTFE tubing.

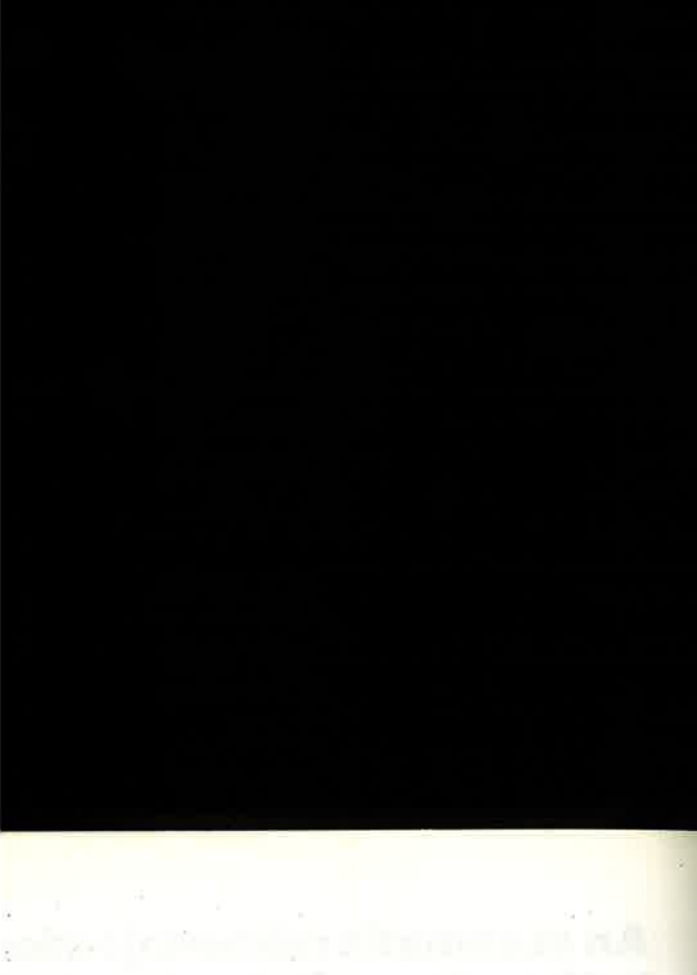


Figure 1. The microsyringe unit.

compartment of the reaction cell and the reference cell. The Hamilton syringes in the injection system were filled with 0.5 *M* KCl, eliminating leaks and air-bubbles. The calorimeter was then closed and allowed to attain thermal equilibrium over the weekend (3 days).

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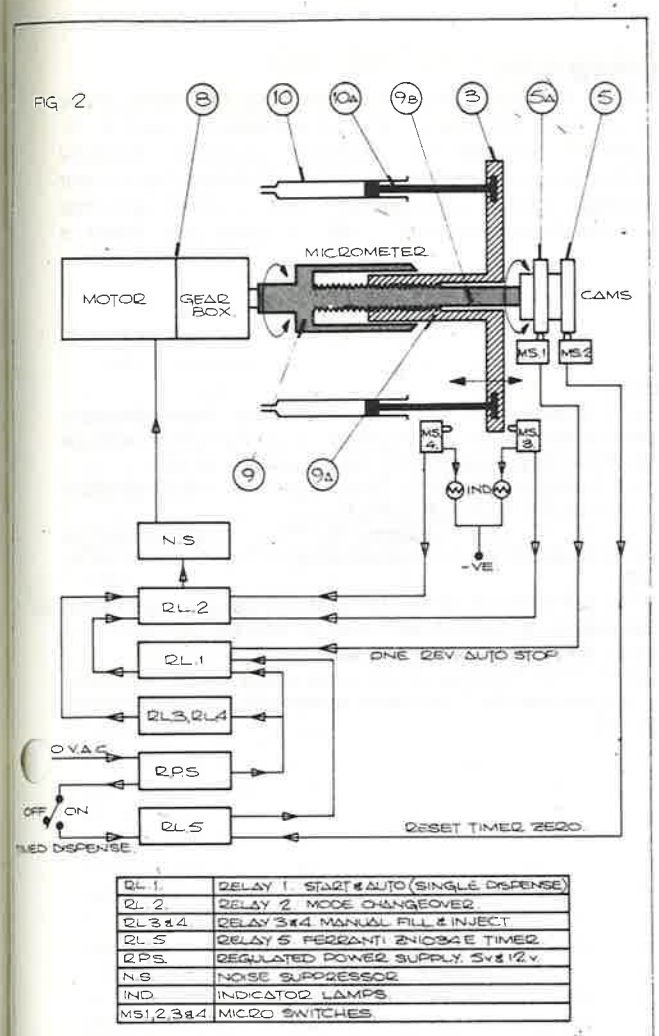
Before starting the exchange enthalpy measurements, the cell contents were mixed to check that the soil was thoroughly wetted, so that the heat of wetting would not affect the measurements. The KCl solution was then injected during two minutes in five 'shots' of 5.29 μl each, totalling 13.25 μequiv. K per measurement and the mixing cycles repeated over 15 to 30 minutes until no more heat change was recorded. The equilibriation time for completing any detectable heat change after each addition was less than thirty minutes. For the Harwell soil, however, this meant that only partial equilibrium was attained between 0-30 per cent K saturation because earlier work had shown that complete equilibrium with the zeolite component



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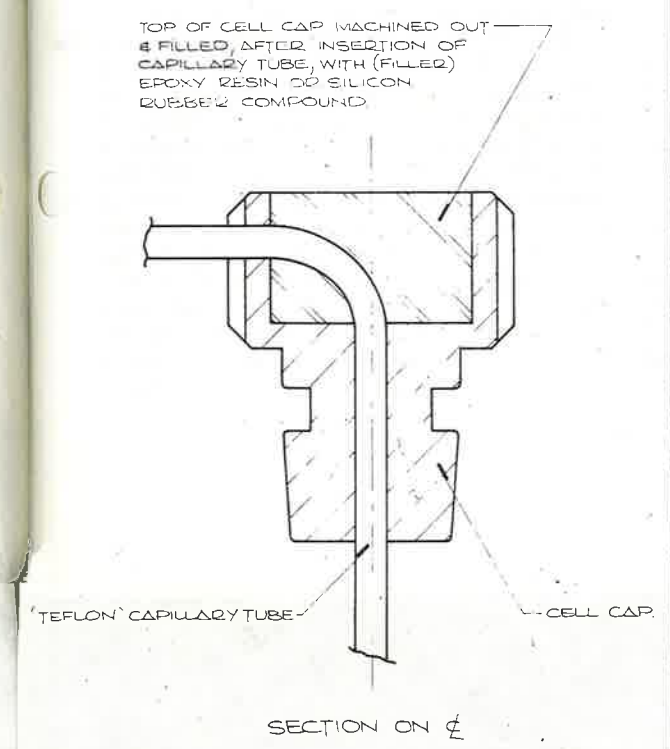

Figure 3. Section through the teflon cap of the microcalorimeter cell after machining and inserting PTFE tubing.

was attained only after 16 hours (Deist and Talibudeen 1967). In these initially semi-automatic operations, 1.058 ml (530 μequiv.) KCl solution was added in a run lasting five days so that the 125 μl syringes had to be refilled periodically. We estimate that fully automatic operation (except refilling of the syringes) would reduce this time to two to three days.

The extent of exchange at each measurement point was determined in a parallel exchange isotherm experiment. Soil weights and solution volumes were four times greater than in the calorimeter run for easier handling and more precise determinations of equilibrium concentrations and fractional K-saturations of the soil. Several 'measurement' points in the calorimeter run were telescoped into single points in the isotherm experiment, but the 'mixing' and 'equilibration' times were the same in both runs, which were carried out at 30°C.

Results and discussion

The exchange reaction is:

$$C_o(KCl) + C'_o(CaCl_2) + w(H_2O) + [(\tfrac{1}{2}Ca(soil) + w_i\,(H_2O)] \rightarrow$$
$$[K_x.\tfrac{1}{2}Ca_{1-x}\,(soil) + w_f(H_2O)] + [w(H_2O) + w_i(H_2O) - w_f(H_2O)]$$
$$+ (c_o - x)KCl + (c'_o + \tfrac{1}{2}x)\,(CaCl_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where $x(=N_K)$ is the K saturation per equivalent of negative charge on the soil, c_o the equivalents of KCl and c_o the equivalents of $CaCl_2$ in w moles of water initially, and $w_i(H_2O)$ and $w_f(H_2O)$ the initial and final number of moles of H_2O combined with the soil.

The experimental procedure used was designed to minimise differences in the heats of mixing, ΔH_m, of KCl and $CaCl_2$ solutions between the 'reaction' and 'reference' cells. In any case, the total molarity did not exceed 0.18 when the actual heat of mixing in each cell would be small compared with the other heat changes occurring in the soil: solution reaction (Barrer, Rees and Ward, 1963).

The only other terms necessary for correcting the experimental heat of partial exchange, ΔH_x, to the corresponding standard value, ΔH°_x, result from (1) the difference in the apparent molar heats of the two chloride salts in solution at equilibrium after 'x' equivalents exchange has occurred, and (2) the non-ideality of the KCl solution initially.

In these preliminary experiments, these corrections were assumed to be small because the chloride concentration changes from 0.05 *M* initially to 0.18 *M* finally (when $x = N_K = 0.6$ approximately). So the experimental heats of exchange were considered to be not significantly different from the corresponding standard functions. The latter represent the algebraic sum of the differences between molar heat contents of [($\frac{1}{2}Ca_{1-x}$ + K_x): soil] and ($\frac{1}{2}$Ca:soil) and between that of x equivalents KCl and ($c'_o + \frac{1}{2}x$) equivalents $CaCl_2$ at infinite dilution. The former difference contains terms for the enthalpies of x equivalents of K-Ca exchange and of the corresponding changes in cationic hydration.

The relationship between the (experimental) differential heat $d(\Delta H_x)$ and K saturation (Figure 4) shows that for the Saxmundham soil two groups of sites are distinguishable and for the Harwell soil, three groups, although we have not yet succeeded in measuring the $d(\Delta H_x)$: % K saturation relationship for the complete isotherm. The standard heats of exchange $\Delta H^\circ_{(Ca\,K)}$ were obtained by extrapolating ΔH°_x to x = 1, the fitted curve giving values of −6.5 and −10.0 kJ equiv.$^{-1}$: for the Saxmundham (Goulding and Talibudeen 1977) and Harwell (Deist and Talibudeen, 1967) soils respectively. When compared with ΔH° values of −6.2 and −24 kJ equiv.$^{-1}$, we conclude that in the Harwell soil, where at least in part a rate-controlling process is involved in the cation exchange process, the standard enthalpy of exchange is considerably underestimated. This indicates the need to modify the equilibrium procedure during the microcalorimetric measurements without losing sensitivity.

We recognise that this analysis of our preliminary measurements can only be regarded as tentative but the results illustrate the contribution such measurements can make to the identification of soil components active in the adsorption of water, cations, anions and organic molecules, when comparing

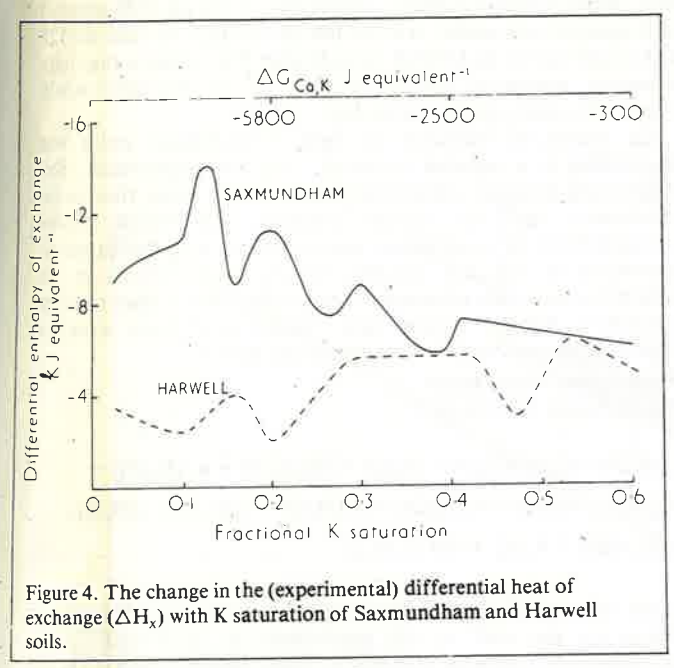

Figure 4. The change in the (experimental) differential heat of exchange (ΔH_x) with K saturation of Saxmundham and Harwell soils.

various soils, provided adequate allowance is made experimentally for any kinetics of exchange at the solid-solution interface.

Acknowledgements

We thank T. M. Woodcock for constructing the microsyringe unit and fitting it into the LKB Microcalorimeter, and A. G. Hobbs for its detailed constructional drawings. Grateful acknowledgement is extended to Dr. R. C. Woledge, Department of Physiology, University College, London, for a generous description and demonstration of his micropipetting device in 1975.

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Reprinted from MICROBIOLOGY 1978

On the Diversity of Nitrifiers i

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Our knowledge of the distribution of autotrophic nitrifying bacteria in nature in different environments is very meager. One cannot avoid referring to Winogradsky's outstanding contribution to the study of nitrification. In a review of the subject that he wrote in collaboration with his daughter, Hélène, they (6) noted that nitrosomonads proliferate in rich, well-manured soils, biological filters, or activated sludge; *Nitrosocystis* spp. occur in much poorer soils, especially forest soils to the exclusion of other forms; and *Nitrosospira* spp. occur in virgin soils. This brief summary of his views provides a background to my comments.

In a search which started around 1960 for better or more quickly growing nitrifiers than *Nitrosomonas europaea*, I have isolated 42 ammonia-oxidizing bacteria in pure culture and 20 or so *Nitrobacter* strains, mostly from soil. Of the 42 isolates, 17 were *Nitrosolobus multiformis* strains, 9 were *Nitrosomonas* spp., 11 were *Nitrosospira* strains, 1 was a nonmotile *Nitrosovibrio* sp., and 4 were unidentified. Except for two nitrosomonads from sewage, all the above were isolated from soils collected from South America, Greenland, Spitzbergen, West and East Africa, Bangladesh, Sri Lanka, Italy, Yugoslavia, and Great Britain. The only *Nitrosomonas* sp. found in a Rothamsted soil was from the farmyard manure plot of Broadbalk field, the same plot from which *N. europaea* was previously isolated by Meiklejohn (2). *Nitrosomonas* strains were isolated from soil irrigated with liquid sewage near Milan and from a farm soil that received much cow manure near Mymensingh in Bangladesh. But *Nitrosomonas* spp. were also obtained from woodland soil near Skofja Loka in Slovenia and from a tea soil in Sri Lanka. Thus the occurrence of *Nitrosomonas* is not exclusively associated with sewage, animal manure, or soils treated therewith. Moreover, Sims and Collins (3) found *Nitrosomonas* frequently in many Australian desert soils. The *Nitrosomonas* isolates were not all *N. europaea*; one strain from sewage was similar to a strain isolated by Watson and Mandel (5) from Chicago sewage, logically from obtained from ically a typic dark colonies described by W *coccoides*.

Nitrosolobu eral fields in which were a forest soils. O ious farm soil quently for three yielded from two of t lated. Viable obtained fro soils stored that these or *solobus multi* from red late desh, from w slavia, from tea soil in Sri soil in Engl urine had bee

There is gr species and n nitrifiers of a ologically ve differences b and *Nitrosol* I have found certain nitri Since Winog *Nitrosospira* by, for exam Argentina a who has des cies *Nitroso* pure *Nitroso* gen soils, th virgin soils. obtained fro Weed Resea near Oxford

This past