J. Pharm. Pharmacol. 1981, 33: 65–68
Received June 1, 1980

0022–3573/81/020065–04 $2.50/0

The measurement of phenol coefficients by flow microcalorimetry

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Microcalorimetric bioassay procedures have been applied to the determination of phenol coefficients and dilution coefficients. The derived values are compared with those from a standard AOAC test procedure. The microcalorimetric results indicate close agreement with AOAC values, good reproducibility (±2%), rapidity (30 min per test), potential for automation and the determination of in-use dilutions of disinfectants.

A review (Coates 1977) of the methods currently used to determine the relative efficacies of disinfectants reveals that there is no universally accepted standard technique. In fact the country of manufacture of the disinfectant often dictates the method of assay to be used (Reybrouck 1978).

Up to 1965 in Britain the phenol coefficient type of assay such as the Chick-Martin or Rideal-Walker assay was used. Phenol coefficient tests (Coates 1977) however have three main disadvantages, namely

(1) Test conditions are unrealistic in respect of the test organism (*Salmonella typhi*) and the enforced comparison of the disinfectant with phenol.
(2) The reproducibility is poor.
(3) Application is restricted to phenolic compounds.

The advent of more diverse disinfectant products emphasises the need for a more widely applicable test which should at least give an indication of the strength of disinfectant necessary under practical conditions. The Kelsey-Sykes capacity test (Kelsey & Maurer 1974) was designed for this purpose and can be applied to all chemical types of disinfectant. Even the rather complex Kelsey-Sykes test (1969) the reproducibility of which is considered good, has a ±20% variability (Gibson 1978) whilst with certain other tests ±50% is not uncommon. A further disadvantage of these tests is the length of time taken before results are known (2–3 days).

The reproducibility of tests involving microorganisms has been much improved in recent years by the use of inocula stored in liquid nitrogen (Beezer et al 1976) and by the use of microcalorimetry which has been described as a suitable tool for use in biological assay systems (Spink & Wadsö 19[illegible]; Monk 1978). Microcalorimetric methods measure the enthalpy changes accompanying any physical or chemical process and require careful interpretation of the results since all changes that occur in a reacting system will contribute to the recorded effects (Perry et al 1980). For complex systems however some general reaction property may prove a useful means of recording events; thus microcalorimetric observations on growing organisms can be used to investigate metabolism (Belaich 1980; Lamprecht 1980) identification (Beezer et al 1979; Newell 1980; Perry et al 1980) and to study the effects of metabolic modifiers (for a review see Beezer & Chowdhry 1980). In particular it has been shown (Beezer & Chowdhry 1980) that quantitative bioassays of antibiotics by such methods are more reproducible, more sensitive and faster than conventional agar plate diffusion methods. Perry et al (1980) have reported the determination of phenol and of some commercial disinfectants by recording the effect of adding phenol to a respiring culture of *Escherichia coli* NCTC 8196 which had been recovered from liquid nitrogen storage.

In the course of our work on the oil-water partition of phenols we have used microcalorimetric methods extensively and now report their application to the measurement of phenol coefficients for a range of homologous resorcinol mono-ethers. The results obtained are compared with those obtained from application of the American Association of Official Agricultural Chemists (AOAC) phenol coefficient test method (Official methods of analysis of AOAC 1960).

MATERIALS AND METHODS

Deionized water for making up all solutions had a specific conductance of 1×10^{-6} ohms^{-1} or less, complex media were supplied by Oxoid Ltd. The

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resorcinol monoethers, normal saturated alkyl derivatives up to the octyl ether were prepared as described by Beezer et al (1980). They were repeatedly distilled under reduced pressure and the purity established by gas chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy. All other chemicals were AR grade.

Escherichia coli NCTC 8196 was obtained from the National Collection of Type Cultures (Central Public Health Laboratory, London). The bacterium was maintained on agar slopes containing (g litre^{-1}) in deionized water: nutrient broth, 13, agar, 20. The slopes were stored at 4 °C after incubation for 24 h at 37 °C. The preparation and storage of inocula in liquid nitrogen was as described by Perry et al (1980).

The microcalorimetric measurements were conducted in phosphate buffered glucose solution (pH 7·0) containing (g litre^{-1}) in deionized water: K_2HPO_4, 3·68, KH_2PO_4, 1·32, glucose 1·80, acetone (as required, see later) 100. This medium was also used for the AOAC phenol coefficient tests. The design of the microcalorimeter, its operation and full experimental details have been described previously (Perry et al 1980). The microcalorimeter was thermostatted at 30 °C.

Methods

Acetone (10% v/v) was added to the glucose-phosphate buffer solution to dissolve the n-heptyl and n-octyl resorcinol monoethers. To observe the effect of this on the bactericidal action of the resorcinol monoethers the standard AOAC test was first performed at 30 °C (the temperature of operation of the microcalorimeter) in the glucose-phosphate buffer in the absence of acetone and then repeated with acetone. In both cases the results were read after incubation of the agar plates for 48 and 72 h at 37 °C, the sampling period being extended from the usual 10 min to 1 h to be more strictly comparable with the microcalorimetric results. The bactericidal potency of each derivative was then determined microcalorimetrically at 30 °C. A concentration of phenol which gave a total kill in approximately 45 min was chosen from the results of the AOAC test and taken to be the standard concentration of phenol.

It was found from the power-time curves (previously termed thermogram: IUPAC, IUB, IUPAB draft recommendations) recorded, that the time taken to achieve total kill i.e. return of the power-time curve to the baseline (Fig. 1) might vary by a few minutes, but the peak height attained on each curve was to within ±2% (repeat control and assay power-time curves are illustrated in Fig. 1). The peak

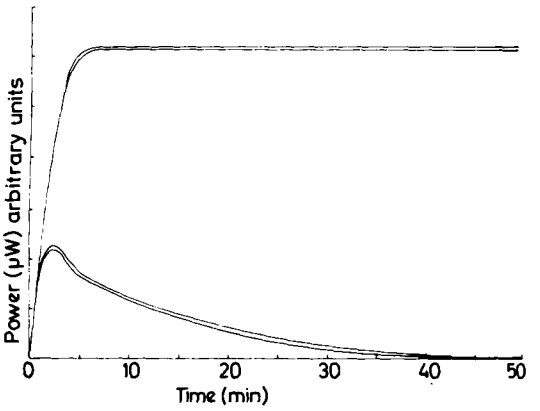

FIG. 1. Power-time curves for repeat control (upper curves) and phenol treated (lower curves) incubations.

height observed with the standard concentration of phenol was taken as the standard response and the concentration of each resorcinol monoether which gave an identical peak height was then determined microcalorimetrically. Dilution coefficients were calculated from the AOAC test results by the standard relationship $n \log C + \log t = a$ constant, where n is the dilution coefficient, C (M) is the concentration of the disinfectant achieving total kill, as defined by the AOAC test, in time t (min).

As the peak height observed on each power-time curve was found to be more reproducible than time to return to the baseline, the log t term in the above equation was replaced by the term log (peak height). Dilution coefficient values were then derived from the equation $n \log C + \log$ (peak height) = a constant.

RESULTS AND DISCUSSION

Fig. 1 shows power-time curves for control and phenol-treated incubations. Table 1 shows the phenol coefficients of the resorcinol monoethers determined by the three techniques described under methods.

The data in Table 1 show that the addition of 10% v/v acetone as cosolvent reduces the concentration of bactericide necessary to give a specified response but does not affect the values of the phenol coefficients.

The results of the AOAC assay and the microcalorimetric assay are in excellent agreement for the resorcinol monoethers up to n-hexyl; thereafter the microcalorimetric results are about 10% lower.

Table 2 shows, for the three procedures used, the derived values of the dilution coefficients. The microcalorimetrically determined values permit a

Table 1. Phenol coefficients of resorcinol monoethers as determined by (A) standard AOAC test in phosphate buffered glucose; (B) standard AOAC test but with 10% v/v acetone added as cosolvent; (C) microcalorimetry. The microcalorimetric results described in this Table and those in Table 2 are the means of at least 4 experiments. The peak heights observed were always within ± 2%.

Phenol test	A	B	C
Phenol	1	1	1
Methyl	1·5	1·4	1·2
Ethyl	3·1	2·9	2·4
n-Propyl	8·0	7·4	7·4
n-Butyl	21	29	25
n-Pentyl	80	74	74
n-Hexyl	200	180	170
n-Heptyl	—	440	380
n-Octyl	—	700	640

standard equation for this homologous series of compounds. It can be seen that the dilution coefficient may account for the small discrepancy between the microcalorimetric and AOAC values for the phenol coefficients in Table 1. This arises since, in the AOAC assay, the dilution coefficient does not greatly influence the results because of the short time interval (10 min) of phenol/organism contact, while the microcalorimetric assay lasts for approximately 45 min. The relative concentrations of bactericides necessary to achieve total kill in approximately 45 min as opposed to 7·5 min depend upon the magnitude of their dilution coefficients. This results in slightly lower microcalorimetrically-determined phenol coefficients, the effect is reduced however, when a series of related compounds with similar phenol coefficients is being examined.

The dilution coefficients (Table 2) from the microcalorimetric assay are lower than those from the AOAC assay probably because of the different conditions of the two tests; similar effects have been noted by Sykes (1965).

Table 2. Dilution coefficients determined from the procedures described in the text and in the heading to Table 1.

Phenol test	A	B	C
Phenol	5	5	—
Methyl	10	10	4
Ethyl	10	10	4
n-Propyl	9	8	4
n-Butyl	9	9	4
n-Pentyl	8	8	4
n-Hexyl	8	8	4
n-Heptyl	—	8	4
n-Octyl	—	8	4

The microcalorimetric assay has several advantages over the conventional AOAC assay and similar methods. It is unaffected by coloured solutions or by the presence of suspended matter provided this will pass through tubing of 1 mm internal diameter. Apart from the marked increase in reproducibility, the microcalorimetric assay is much quicker since only a single determination at a given concentration is needed to measure the peak height and to assay the bactericide. This occupies only 30 min (only the peak height need be observed not the whole power-time curve) from the moment of making up a suitable solution of the bactericide to the calculation of the result.

Smaller concentrations of compounds may be used since one does not need to achieve total kill in 7·5 min but in 45 min, a distinct advantage for poorly soluble compounds. The number of viable cells used in the microcalorimetric assay, approximately 3×10^8 cells ml^{-1} is higher than that used in conventional assay procedures (between 10^7 and 10^8 cells ml^{-1}). However, the concentration of standard phenol is approximately 3-fold smaller in the microcalorimetric assay. Thus the actual dose per cell is, as expected, much less i.e. of the order of the concentration required for practical use. If use-dilutions of bactericides are required then the complete power-time curve may be observed i.e. the time to total kill, and hence use dilutions may be rapidly determined.

The microcalorimetric assay procedure can readily be modified to cope with more involved testing procedures, for example by inclusion of organic material or by mimicking "dirty" practical situations. Therefore, as suggested by Perry et al (1980), this microcalorimetric assay system may well be a reasonable alternative to classical methods. The simplicity and rapidity of the method suggest that the whole procedure could easily be automated to give a printed read-out of results either as phenol coefficients or as series of use-dilution ranges.

Acknowledgements

We acknowledge the award of an SRC CASE Research Studentship in co-operation with The Lilly Research Centre, Windlesham, Surrey (to D.E.S.) and we thank Mr R. P. Lipscombe for his expert technical assistance.

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