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Microcalorimetry in the screening of discovery compounds and in the investigation of novel drug delivery systems ☆

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Abstract

Calorimetry has for some time been proposed as a rapid method for determination of bioactivity. This paper describes the background to this application and describes how it has been extended to the study of bioassay techniques via microcalorimetry in the development of structure activity relationships (SARs). That SARs can be developed indicates that it is possible to guide drug synthetic strategy through the results of microcalorimetric investigations, and this approach is explored here. In an extension of this approach it is argued that microcalorimetry is well suited to the examination of novel drug delivery systems, allowing investigation of the capacity of drug delivery molecules to release the drug in the presence of a target organism.

Keywords: Bioassay; Dendrimer; Drug delivery; Microcalorimetry

In a previous paper [1], the microcalorimetric technique was suggested as enabling overviews of drug/microbial cell interactions which may (i) guide drug design through biologically based group additivity schemes, (ii) reveal mechanistic detail of the nature of these interactions, and (iii) allow enhanced insight into the nature of the biological cell membrane as a lipoid, organised solvent system. It is

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the purpose of this paper to discuss the progress of these studies and to assess the state of microbial/drug studies derived from microcalorimetric investigations.

The background to the proposed application of microcalorimetry is that this technique has frequently been cited as a rapid and sensitive method for the bioassay of antimicrobials upon interaction with sensitive organisms [2–5], and recent reviews [1,6,7] have considered the wider aspects of this technique. In particular, it has been demonstrated that it is possible to use the microcalorimetrically determined bioassay data in the form of the conventional log(dose)–response curve in an essentially fundamental manner [8–12]. Determination of the regression parameters for an observed linear log(dose)–response line permits [1] identification of the value of log(dose) when the response is zero. This intercept value is identified as $\log(\text{dose})_{max}$, i.e. the logarithm of the maximum dose of the antimicrobial that can be applied without eliciting a response. (Naturally this parameter has no literal physical meaning, but it does, of course, allow a scaling and contribution term). This parameter was shown to be linear with respect to the number of carbon atoms in the side chain of homologous series of drugs [8–10] and of *o*-, *m*- and *p*-alkoxyphenols and the *m*-hydroxybenzoates. This form of presentation therefore constitutes a structure activity relationship (SAR) and also conforms to a linear free energy relationship (LFER). In principle, therefore, the regression coefficients of this linear relationship represent group additivity terms: i.e. the contribution to biological activity for the constituent groups of the active drug molecule can be calculated. As was noted earlier [1], the generality of this relationship has as yet received only limited exploration.

This same publication [1] noted that the study of the antifungal activity of some (non-homologous) derivatives of cardanol [11] allowed correlation of the biological activity of these compounds, as determined through their $\log(\text{dose})_{max}$ values, with log *P*, the logarithm of the partition coefficient for transfer of the drugs from water to octan-1-ol. This relationship suggests that, for these drugs, the controlling feature in their mode of action is the transfer of the drug from water into the lipoid solvent of the biological membrane; that is, the partition process is the controlling factor. Thus, whatever the ultimate site of action of the drug within the cytoplasm, it would appear that the solvent properties of the lipid membrane are dominant in the mechanism and kinetics of the interaction process. Interestingly, the plot of $\log(\text{dose})_{max}$ versus log *P* for the cardanols discriminated between two differently substituted groups of molecules, i.e. there was clear indication of the sensitivity of the target organism (*Saccharomyces cerevisiae*) towards these groups of molecules and hence to the role of the membrane in the mode of action. Thus there has been some small progress in studies related to item (iii) in the above list. Progress is, however, rather limited and still does not permit a firm conclusion about the general relevance of this approach to group additivity schemes.

It is in the area of item (i) in the above list that some early results are now available. The approach adopted in our laboratory has been not to synthesise new drug substances but to improve the delivery of drug to target organisms through, initially, improving the solubility of some very sparingly soluble materials. The design strategy required for synthesis of these novel, solubilising drug delivery systems is that it should be possible to improve the delivery of known antimicrobials by (a) forming soluble

"complexes" of drugs with carrier molecules through covalent bonding, and (b) synthesising molecules to carry drugs into aqueous solution through some form of looser association. It is naturally a requirement that, whatever the nature of the complexes so formed, they should be sufficiently strong to persist in a stable form in aqueous solution and that, in the presence of the target organism, they must be weak enough to permit delivery of the drug to the organism. It is in respect of testing new materials that microcalorimetry has revealed its power to guide syntheses and, importantly, to reveal new forms of SARs.

A previous study [12] showed that an SAR could be established for the interaction of some Schiff base compounds with *Saccharomyces cerevisiae*. However, the compounds studied were relatively insoluble in aqueous solution, and a small quantity of methanol was present in the assay system to permit adequate drug concentrations to be achieved. Thus, although the SAR was revealed, there was concern over the impact methanol had on the experimental results (none could be detected experimentally: that the results indicated that solution properties were important raises concern). Polyethylene glycols (PEG) are known to be soluble in water, they are available over a wide range of molar masses and, importantly, they are currently widely used in drug delivery, usually for improving solubility. They are, therefore, free of toxicological problems. Following the preparation of amine teminated PEGs, these were reacted to produce terminal Schiff base compounds [13]. The range of polymers used was from $M_r = 2000$ to 10 000. Each newly synthesised compound was soluble in water (i.e. no methanol was required) and was induced to interact with *Saccharomyces cerevisiae* in a standard microcalorimetric bioassay experiment. All the new compounds yielded linear log(dose)–response lines from which it is possible to derive values of $\log(\text{dose})_{max}$. A plot of these values for $\log(\text{dose})_{max}$ versus the M_r for the PEGs is shown in Fig. 1. This reveals that there is a linear, but invariant, relationship for PEGs of $M_r < 8000$. The biological response for the PEG of $M_r = 10\,000$ is significantly higher than that of the other compounds. Hydrolysis of the Schiff base–PEG complex [13] results in the formation of a free amine terminated PEG. Interaction of this amine terminated PEG with the target organism indicates that these compounds destabilise the yeast membrane, so permitting, we suggest, readier access of the active Schiff base compound. This, we believe, is the first demonstration of a synthetic strategy which has been guided by rapid (each microcalorimetric experiment takes ≈ 40 minutes) determination of the bioactivity of each new compound.

The second approach is to synthesise new carrier molecules which can incorporate water-insoluble drugs, through weak association, into hydrophobic pockets. Novel dendrimeric molecules have been synthesised [14,15]. Molecules of this type which have molar masses of ≈ 2000 and which are soluble in water have also been synthesised [16]. These latter molecules have been used to solubilise some highly insoluble antimicrobials. For example, it has been possible to increase the solubility of iodine from 0.0013 mol dm^{-3} to more than 6 mol dm^{-3} . Microcalorimetric study of the bioactivity of complexes prepared with this water-soluble dendrimer shows that the active compound retains its biological activity. Figs. 2 and 3 show the calorimetric results of interaction with iodine and with 3,5-diiodo-4-hydroxy-

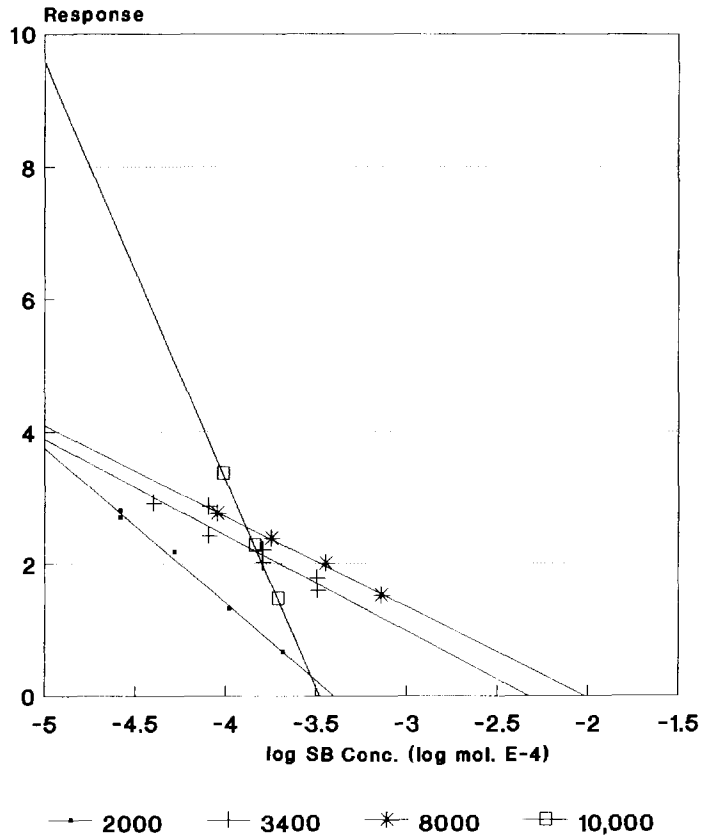

Fig. 1. Plot of $\log(\text{dose})_{max}$ vs. M_r for the PEG Schiff base compounds (for definitions see text). The data are presented as direct experimental output (original) and on the basis of correction for the extent of conversion of the amine terminated PEG to the Schiff base compound (corrected). The form of the relationship is unaffected by the correction procedure applied.

benzoic acid. Both figures indicate a graded response towards the antimicrobial and towards "kill" concentrations. As yet there has not been an exhaustive study of the effects of drug concentration upon the microcalorimetric response of the target organism. However, there is no reason to suppose that there will be no development of a formal relationship.

A recent development from this laboratory [17] permits the calculation of both thermodynamic and kinetic parameters from calorimetric output without the necessity of prior assumptions about initial reactant concentration, reaction order, enthalpy of reaction, etc. As the figures reveal, the interaction of the antimicrobials with target organisms results in a graded response with respect to concentration and, for each concentration, a time related response. Thus, analysis of these data via the methods proposed [17] will permit the derivation of thermodynamic and kinetic data for microbial cell/drug interaction and hence lead to discussions of the

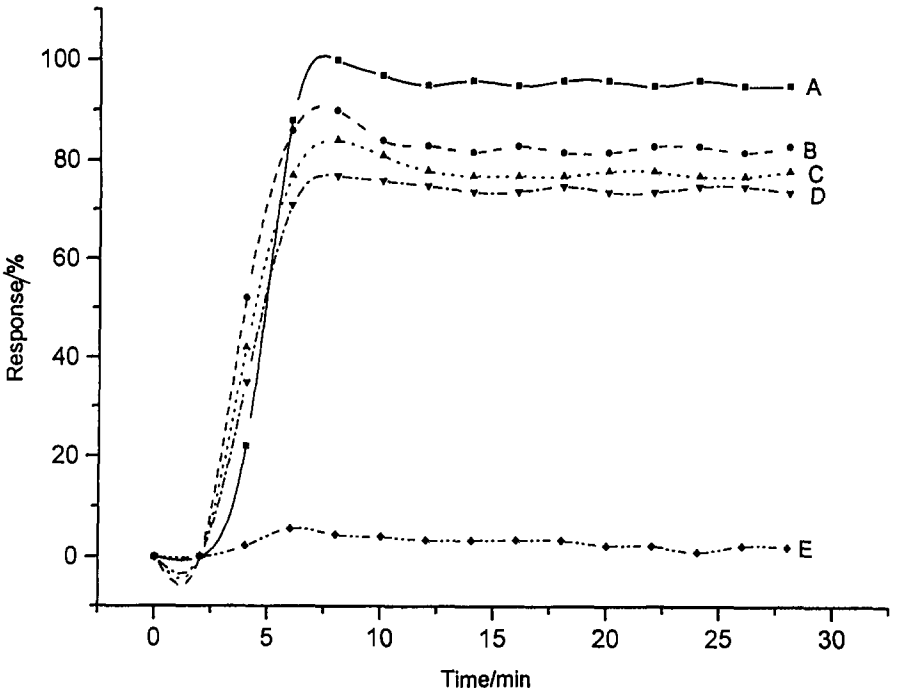

Fig. 2. Microcalorimetric outputs observed for interaction of a dendrimer/iodine material with *Saccharomyces cerevisiae*: A, control; B, 0.5 mg cm^{-3}, C, 1 mg cm^{-3}; D, 1.5 mg cm^{-3}; E, 2 mg cm^{-3}. Note the graded response toward concentration and that the "kill" is achieved at the highest concentration applied. Note, too, that the experimental bioassay time required is only ≈ 30 minutes.

mechanism of action: this is in addition to the established outcomes of bioassay and SAR development.

Conclusions

The objectives outlined in the previous paper [1] and repeated here have been met for both types of complexes. Again, however, although this result is encouraging it is only introductory information; as previously, the generality of usefulness of microcalorimetry in pursuit of the nominated objectives remains to be firmly established. The results do give confidence to continue the studies. It is particularly important to have demonstrated the capacity for using microcalorimetry to control synthetic strategy and to explore the "in use" relative values of the association constants for carrier/drug complexes between aqueous solution and upon interaction with a target organism. The extension to mechanisms of interaction is, we believe, particularly important.

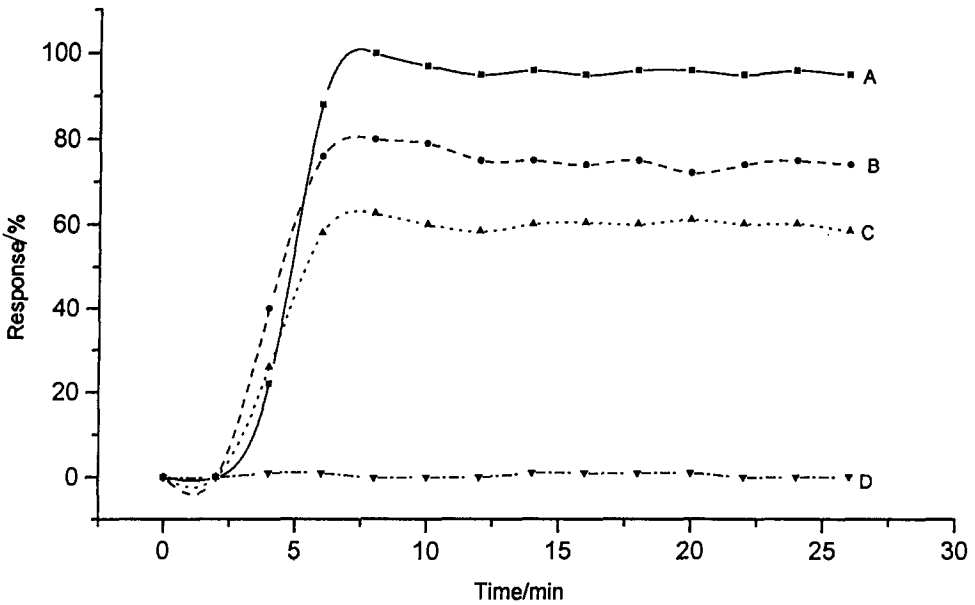

Fig. 3. Microcalorimetric outputs observed for interaction of a dendrimer/3,5-diiodo-4-hydroxybenzoic acid material with *Saccharomyces cerevisiae*: A, control; B, 0.1 mg cm^{-3}; C, 0.2 mg cm^{-3}; D, 0.4 mg cm^{-3}. Note the graded response and that the "kill" is achieved at the highest concentration applied. Note, too, that the experimental bioassay time required is only ≈ 30 minutes.

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