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DRUG BIOASSAY, SYNERGIC INTERACTIONS IN DRUG COMBINATIONS, THERMODYNAMICS AND BIOLOGICALLY BASED STRUCTURE-ACTIVITY RELATIONSHIPS. A SYNTHESIS *

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Microcalorimetry has been used extensively to investigate drug/microbial cell interactions [1-4] however, inspection of the published data reveals that the majority of reports are of a purely qualitative character. This is surprising given the speed, sensitivity, precision and accuracy of the microcalorimetric method. In a study [5] of the influence of cryo-preservation of microbial species used in various bioassay techniques such as plate, tube, microcalorimetry was also evaluated. The performance characteristics in all methods were shown to be improved through use of liquid nitrogen stored inocula: those for the microcalorimetric technique being [5] reproducibility ±0.6% (in association with liquid nitrogen stored standard solutions), ±0.1 unit cm^{-3} (for nystatin).

And, whilst there have been several microcalorimetric investigations of the effects of combinations of metabolic modifiers [see refs 1-4] there has not been any study yet reported of a comprehensive quantitative nature. This again is surprising given the microcalorimetric performance characteristics outlined above. Moreover the quantitative interpretation of more classically determined data on synergic interactions in drug combinations is difficult and somewhat subjective [6]. In a series of papers [7] Kjeldsen et al. have reported quantitative data for the interaction of 3 antibiotics (neomycin, bacitracin and polymyxin B) separately upon interaction with their target organisms (Bordatella bronchiseptica, Bacillus pumilus, Micrococcus luteus). In addition binary and ternary combinations of these antibiotics were examined at two concentrations upon interaction with these organisms and also with Escherichia coli. The results revealed that, at least in the in vitro experiment, minimum inhibitory concentrations (MICs) were not a good guide to the concentration ratios which would lead to the most effective response. In experiments such as these microcalorimetry has a great advantage over more conventional bioassay procedures since the experimental record, dq/dt vs t, reveals not only thermal data but also kinetic data.

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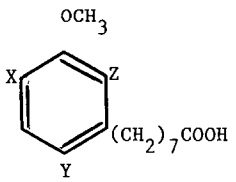
The judgement of what constitutes best response is therefore quantitative in respect of time to organism death and/or reduction in metabolic activity. These data have been taken as the stimulus to extend such studies into a truly quantitative investigation [6] of the proportions of drugs to be prepared to maximise response in the interaction of the classic synergic drug combination of trimethoprim and sulphamethoxazole upon interaction with Escherichia coli (NCTC 1048). The surprising outcome of these studies is that the proportion, in this in vitro experiment, to maximize the developed synergic index is quite different from that presented in commercial preparations. The detailed accounts of these experiments will appear elsewhere [6,8]. However it has been possible to develop a synergy index which has been explored over the whole range of drug combinations from 100% trimethoprim to 100% sulphamethoxazole. The index may act, therefore, as a guide to the proportions to be achieved in serum in the clinical situation. The kinetic information also contained in the power-time curves (i.e. dq/dt vst) is revealing of the sequence of individual reaction steps involved in the mechanism.

However it has also been shown [9-11] recently that the microcalorimetrically determined bioassay data when presented as a conventional linear log(dose)-response curve has a more fundamental significance. Determination of the regression parameters for the linear log(dose)-response line permits the identification of the value of log(dose) when response = 0. The intercept is identified as $\log(\text{dose})_{\text{max.}}$ i.e. the maximum value of the logarithm of the dose which can be added without eliciting a response. In investigating homologous series of drugs (namely o-, m-, p-alkoxyphenols and m-hydroxybenzoates) it has been observed [9-11] that $\log(\text{dose})_{\text{max.}}$ is linear with respect to the number of carbon atoms in the side-chain i.e. it is a Linear Free Energy Relationship [LFER; 12]. The generality of this biologically based group additivity scheme has, as yet, received only a limited exploration. The establishment of such a group additivity scheme on a wide and firm basis would eliminate the need to infer the relationship between chemical structure and biological response through partition coefficient measurements [13]. The method for exploration of the relationship between Biological Response and chemical structure in straightforward in the case of homologous series. However resort to some other LFER basis is necessary in relating the Biological Response ($\log(\text{dose})_{\text{max.}}$ in these microcalorimetric studies) to the chemical structure of the drug.

Adopting a similar approach to that outlined above de Morais et al. [14] have studied the antifungal activity of a series of derivatives of the cashewnut component shell liquid cardanol. The studied compounds were [15]



a) X = Y = Z = H
b) X = Z = H; Y = Cl
c) X = Y = Z = Cl
d) X = Z = H; Y = Br
e) Z = Y = H; X = NO_2
f) X = Z = H; Y = NO_2

In this case however it is necessary to correlate the biological activity of the compounds with log P, the logarithm of the calculated [13] octanol/water partition coefficient. The regression coefficients for this plot (which relates to compounds a, b, d and f i.e. those studies quantitatively) are intercept = 5.9820; slope = -1.2460; r^2 = 0.9954. It is encouraging to note that this study [14] extends the previous studies [9-11] from interactions with bacterial cells to interaction with yeast cells. Moreover the existence of this biological LFER correlated to log P suggests that partitioning of these antifungal compounds into the wall/membrane is a critical feature of the mechanism of action. This conclusion is reinforced by consideration of the Hammett σ values [12] for these compounds. The σ values fall in the order H < Cl, Br < NO_2 i.e. these values do not correlate at all with $\log(\text{dose})_{max.}$.

The correlation outlined above for the relationship between $\log(\text{dose})_{max.}$ and log P, the octanol/water partition coefficient implies that partitioning of drugs into octanol is a process which mimics the partitioning of drugs from an aqueous phase into the lipoid solvent which is the microbial membrane. There have been many solvents proposed [13] as adequate mimics of the microbial membrane solvent system but there has not been a wide ranging evaluation of the appropriateness of such bulk solvent systems. Measurement of log P is, of course, an indirect measurement of the Gibbs function for the transfer of solute from water to the selected non-aqueous solvent; $\Delta_{trs}G = -RT\ln P$. The Gibbs function for transfer is, of course, factorable into the separate enthalpy and entropy contributions:

$$\Delta_{trs}G = \Delta_{trs}H - \Delta_{trs}S.$$

Of these two the easier to measure is the enthalpy of transfer. Direct microcalorimetric measurement of enthalpies of solution in water $\Delta_{soln,w}H$ and in an immiscible organic solvent, $\Delta_{soln,o}H$ allows calculation of the desired function as

$$\Delta_{trs}H = \Delta_{soln,o}H - \Delta_{soln,w}H.$$

Details of such measurements [16] in a range of solvents are presented in Table 1.

Table 4. Values of $\Delta_{trans}H$ (kJ mol^{-1}) for transfer of m-alkoxyphenols from water to cells, octan-1-ol, heptane and propylene carbonate

Solute	Cells	Octan-1-ol	Heptane	Propylene carbonate
m-Methoxy	-0.22	-8.03 ± 0.19	20.9 ± 0.6	23.2 ± 0.3
m-Ethoxy	-1.1	-6.95 ± 0.15	19.3 ± 0.9	23.4 ± 0.4
m-Propoxy	-2.02	-6.94 ± 0.14	16.0 ± 0.5	23.9 ± 0.4
m-Butoxy	-4.06	-	13.9 ± 0.5	23.4 ± 0.4
m-Pentoxy	-5.14	-	12.0 ± 0.4	23.2 ± 0.3

Here it is apparent that, certainly in enthalpic terms, none of the solvents investigated resembles the properties of the membrane lipid solvent system of Escherichia coli.

A scheme to evaluate solvent systems for their utility in partitioning experiments has recently been proposed [17]. This scheme exploits the thermodynamic basis of the Collander equation [18]. The parameter which results from the thermodynamic analysis of the Collander equation is believed [17] to be related to the quantity of water present in the non-aqueous solvent system. This theory has only, as yet, been tested with partitioning data for solute transfer to complex organised solvent systems such as multi-lamellar vesicles i.e. no biological cells have been examined. Such investigations are in progress in the author's laboratory.

In summary, therefore, the microcalorimetric technique is, it is believed, going to permit an overview of drug/microbial cell interactions which may (i) guide drug design through the biologically based group additivity schemes, (ii) reveal mechanistic detail of the nature of these interactions, and (iii) allow more insight into the nature of the biological cell membrane as a lipoid, organised solvent system. The technique, therefore, is the basis of a synthesis of both applied and fundamental enquiries into drug/cell interactions. It is, of course, the ubiquity of the enthalpy changes which accompany all reactions that lies at the heart of this synthesis. Indeed the existence of a common and easily measured reaction parameter with which to evaluate enormously complex, heterogeneous systems has presented biophysical chemists with both simplicity and challenge in the interpretation of experimental data.

REFERENCES

1. A. E. Beezer, ed., Biological Microcalorimetry, Academic Press, London, 1980.
2. A. E. Beezer, B. Z. Chowdhry and E. J. Greenhow, Analysis of drugs by power conduction calorimetry and thermometric titrimetry. Talanta 30 (1983) 209-265.
3. B. F. Perry, Microcalorimetry in antibiotic bioassay in: N. D. Jesperson (Ed.) thermal Analysis, Part B, Volume XII of Comprehensive Analytical Chemistry, Elsevier Amsterdam, 1982.
4. R. J. Miles, A. E. Beezer and B. F. Perry, Growth and Metabolism of Yeasts in: A. M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, Wright, Bristol, 1987.
5. R. F. Cosgrove, A. E. Beezer and R. J. Miles. The application of cryobiology to the microbiological assay of nystatin. J. Pharm. Pharmacol. 31 (1979) 83-87.
6. M. Shafiq, Ph.D. thesis, University of London, 1990.

7. N. Kjeldsen, A. E. Beezer and R. J. Miles, Flow microcalorimetric assay of antibiotics I. Polymyxin B sulphate and its combinations with neomycin sulphate and zinc bacitracin on interaction with Bordatella bronchiseptica (NCTC 8344). J. Pharm. Biomed. Anal. 7 (1989) 851-857.; II. Neomycin sulphate and its combinations with polymyxin B sulphate and zinc bacitracin on interaction with Bacillus pumulus (NCTC 8241). J. Pharm. Biomed. Anal. 7 (1989) 859-864.; III. Zinc bacitracin and its combinations with polymyxin B sulphate and neomycin sulphate on interaction with Micrococcus lutens. J. Pharm. Biomed. Anal. 7 (1989) 865-869.; IV. Polymyxin B sulphate, neomycin sulphate, zinc bacitracin and their combinations with Escherichia coli suspended in buffer plus glucose medium. J. Pharm. Biomed. Anal. 7 (1989) 871-875.
8. M. Shafiq, A. E. Beezer and R. J. Miles, manuscript in preparation.
9. A. E. Beezer, P. L. O. Volpe, C. A. Gooch, W. H. Hunter and R. J. Miles, Quantitative structure - activity relationships: microcalorimetric determination of a group additivity scheme for biological response. Int. J. Pharm., 29 (1986) 237-242.
10. A. E. Beezer, M. C. P. Lima, C. A. Gooch, W. H. Hunter and B. V. Smith, Quantitative structure - activity relationships: extension of a scheme for group additivity in biological response to o-, m- and p-alkoxyphenols on interaction with E. coli., Int. J. Pharm., 38 (1987) 251-254.
11. A. E. Beezer, G. G. Fox, C. A. Gooch, W. H. Hunter, R. J. Miles and B. V. Smith, Microcalorimetric studies of the interaction of m-hydroxybenzoates with E. coli. and with S. aureus. Demonstration of a Collander relationship for biological response. Int. J. Pharm., 45 (1988) 153-155.
12. C. D. Johnson, The Hammett Equation. Cambridge, 1973.
13. C. Hansch and A. J. Leo, Substituent constants for correlation analysis in chemistry. Wiley, New York, 1979.
14. S. M. de Morais, A. E. Beezer, L. J. Ashby and R. Bolton, Examination of biological group additivity schemes in QSARs through study of some natural products: derivatives of the cashewnut component shell liquid cardanol - interaction with Sacch. cerevisiae. Int. J. Pharm., submitted for publication.
15. S. M. de Morais and R. Bolton, ^{13}C and ^{1}H-nmr spectroscopic studies of natural products. 1,8-aryloctanoic acids. J. Chem. Soc. Perkin II, submitted for publication.
16. A. E. Beezer, P. L. O. Volpe, R. J. Miles and W. H. Hunter, Microcalorimetric measurement of the enthalpies of transfer of a series of m-alkoxyphenols from isotonic solution to Escherichia coli. J. Chem. Soc. Faraday I. 82 (1986) 2929-2932.
17. A. E. Beezer, C. A. Gooch, W. H. Hunter and P. L. O. Volpe, A thermodynamic analysis of the Collander equation and establishment of a reference solvent for use in partitioning studies. J. Pharm. Pharmacol. 39 (1987) 774-780.
18. H. Kubinyi, The Collander Equation, Prog. Drug. Res. 23 (1979) 97-136.