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Invited Review

Observations on the biopharmaceutical importance of chain length in chemically related compounds

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Summary

The physicial properties of compounds which have a common parent structure, and differ only by the sequential addition of a methylene group to an alkyl side chain might be expected to demonstrate a linear variation in behaviour as a function of the length of the alkyl carbon chain. Although this is indeed so in many instances, there are many examples in the literature in which a break in the pattern of the behaviour of the product is observed at a chain length of five carbons (± 1). In this paper, examples are highlighted in which this is true for solid-state properties (melting point, wettability), solubility, liquid properties, partition and biological response. NMR data demonstrate that certain substituent alkyl chains are essentially rigid until a chain length of 5 carbons is exceeded, after which the freedom of movement is increased; this is used as a basis to explain the observed behaviour.

Introduction

Numerous studies on disparate subjects testify to a significant change in properties of compounds as the length of the alkyl chain is increased through a chain length of 5 (± 1) carbon atoms. We are not aware of any attempts to collate information on this phenomenon. The purpose of this work is not to provide a comprehensive review of all reports of quantitative structure activity relationships, but to present evidence from a variety of different areas as examples of how chain length may affect: (i) solid-state properties, (ii) solubility, (iii) liquid properties, (iv) partition, and (v) biological response. Much of the data that will be discussed will be taken from work of our own research groups, thereby providing continuity of examples. We do, however, acknowledge that this behaviour is not a uniform occurrence, and there are examples of linear relationships between physical properties and carbon chain length of homologous compounds; such linear systems will not be discussed.

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Solid-State Properties

Wettability

Little work has been presented on the variation of wettability in homologous series of powdered solids. It is acknowledged that chemical structure will influence the wettability of a powder, i.e. a variation in the proportion of hydrophobic and hydrophilic groups will be reflected in the wettability. Forster et al. (1991), have reported on the change in wettability of a series of alkyl *p*-hydroxybenzoates by water (Fig. 1); the samples were aged untreated powders, thus the effects of energy changes due to physical treatments (Buckton et al., 1988) should be minimised. The methyl derivative was clearly an exceptional member of the group (as might be expected), having a low contact angle. The ethyl to pentyl derivatives had essentially identical contact angles, this indicates that the surface composition of the powder does not change as the chain length is increased from 2 to 5; this may be due to the rigidity of the short alkyl chain. However, the contact angles for the hexyl and heptyl derivatives were larger. It can be assumed (see later) that after the chain exceeds five carbon units, there is freedom for it to rotate about carbon/carbon bonds giving rise to different conformations during crystallisation, and thus the opportunity for the ring and part of the side chain to co-exist at the surface of the crystal.

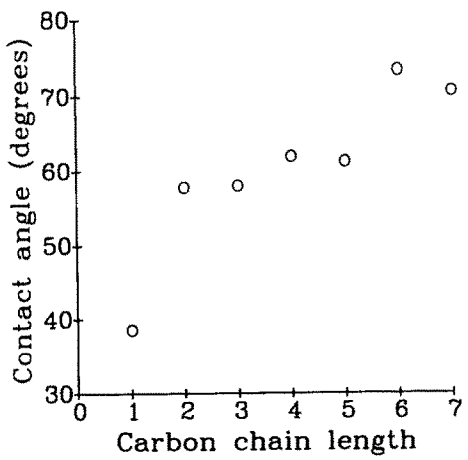

Fig. 1. Contact angles of water on alkyl-*p*-hydroxybenzoates (Reproduced from Forster et al., 1991).

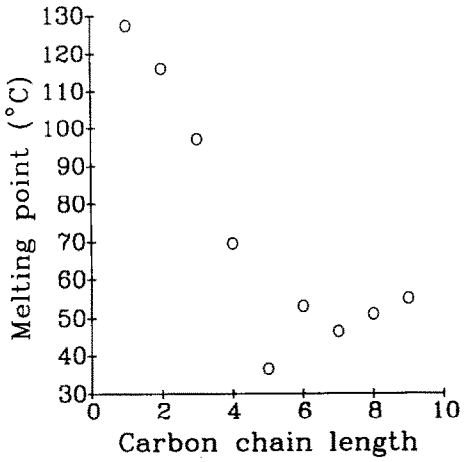

Fig. 2. The melting points of the alkyl-*p*-hydroxybenzoates (Reproduced from Forster et al., 1991).

Melting points

Melting points are often quoted as a crude method of predicting solubility, on the basis that for a solid to dissolve there must be a breaking of solid/solid bonds. There must also be a breaking of liquid/liquid bonds to form a cavity, and the subsequent formation of bonds between the solute and the solvent, following the removal of a molecule from the solid phase and accommodation in the liquid cavity. The success of melting points as a predictor of solubility will depend on the relative magnitudes of the solute/solute, the solvent/solvent and the solute/solvent interaction energies.

Numerous workers have reported the change of melting points in a homologous series. Examples include the *p*-aminobenzoates (Yalkowsky et al., 1972) and the *p*-hydroxybenzoates (Forster et al., 1991). Fig. 2 shows the variation of melting point as a function of alkyl chain length for the alkyl *p*-hydroxybenzoates, the trend of which is similar to that observed with the alkyl *p*-aminobenzoates (see Yalkowsky et al., 1972). The melting points decrease as the chain lengths increase from 1 to 5 carbon units; when the chain length exceeds five carbons, the melting points rise. The chain lengths 1 to 5 show an ordered (nearly linear) decrease in melting point, but the melting point of the longer chains, is neither linear, nor does it show an odd/even variation (this is particularly true for

the *p*-aminobenzoates). The concept of odd/even variation in homologous alkyl chains will be discussed below, but is believed to be related to the orientation of the methyl group at the end of the chain; the orientation will alternate due to the 'zig-zag' structure of alkyl chains. Yalkowsky et al. (1972) designated the butyl derivative as the position at which the properties changed, the *p*-hydroxybenzoates show a more clear break at the pentyl derivative.

Solubility

On the basis of the melting point data, the solubility of the alkyl *p*-aminobenzoates and the *p*-hydroxybenzoates should rise to a maximum at a chain length of five, and then fall. The solubilities of the *p*-aminobenzoates in hexane and in silicone oil follow this predicted trend (Yalkowsky et al., 1972). In both these cases the semi-logarithmic plots yield a straight line relationship for chain lengths of 1 to 4, then an oscillating decrease, following an odd/even variation. For the *p*-hydroxybenzoates, a similar trend was observed, however, the methyl derivative was anomalous, and the odd/even variation for the higher chain lengths was not obvious (Forster et al., 1991). For each of these systems there was a very clear break in the solubility plot at a chain length of five carbons. The fact that the solubilities correlated reasonably well with the melting points, implies that the disruption of the crystal lattice is a dominant factor in the solution process, and that the accommodation of the solute molecule in the solvent is not a limiting factor. Thus, the structure of the crystal lattice is influenced by the chain length in such a way as to cause a change in properties at a five carbon chain. Yalkowsky et al. (1972) presented X-ray diffraction data to demonstrate that the crystal packing altered at around the butyl derivative.

The aqueous solubilities of both benzoate series plotted as a function of carbon chain length (Yalkowsky et al., 1972; Forster et al., 1991), were not of the shape that would be predicted by the melting points. In both cases, the semi-logarithmic plots revealed a linear decrease in aqueous solubility from methyl to butyl (and potentially pentyl for the *p*-hydroxybenzoates), followed by a break. The longer chain *p*-aminobenzoates formed a second linear region, of steeper gradient than the short chain members. The longer chain *p*-hydroxybenzoates produced a line of shallower gradient than the short chain members, and although there were few data points on this line, there was a possible indication of odd/even variation. Since the aqueous solubilities are not related to the solid melting points, it must be the accommodation of the solute molecules in the solvent cavities that is the limiting factor in the solution process. Therefore, the break in the behaviour of these series at the butyl/pentyl derivative relates to the structure of the liberated molecule, not to the crystal lattice. This demonstrates that the influence of chain length can be expected to remain after liberation from the solid state, and breaks in properties at the butyl/pentyl derivative can be expected in solution phase properties and in biological response.

The solution thermodynamics of the *p*-hydroxybenzoates have been investigated in water, 95% ethanol/water mix, octan-1-ol and hexane (Beezer et al., 1991). In each case the Gibbs free energy of solution showed a break at a chain length of about 5 carbon units.

The observations on a break in chain length are not just applicable to the two benzoate series. The solubilities of the *m*-alkoxy phenols (liquids at ambient conditions) have been investigated, and these can be used as a further example of such behaviour. The calorimetrically determined enthalpies of solution of the *m*-alkoxy phenols demonstrate that the methyl derivative has anomalous behaviour, and that ethyl to hexyl fit to a straight line of enthalpy of solution as a function of carbon chain length (Fig. 3), after the hexyl derivative, the enthalpies of solution decrease (Beezer et al., 1983). Further studies have described the solubility of the *o*-alkoxy phenols in water and water/alcohol systems (Beezer et al., 1987a). In this study the solubility of the methoxy- to propoxy- *o*-alkoxy phenol derivatives was measured in mixtures of alcohol/water ranging from methanol to octanol. The solubility of the *o*-methoxy phenol was about 190 mM in water, and mixes of standard molar

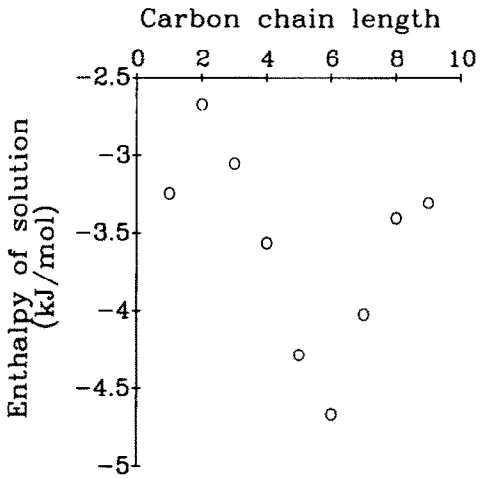

Fig. 3. The enthalpies of solution of *m*-alkoxy phenols (drawn from data in Beezer et al., 1983).

concentrations of ethanol/water, propanol/water, butanol/water and pentanol/water; the solubility in higher alcohol/water mixtures showed a linear decrease with increasing chain length of the alcohol. The *o*-ethoxy phenol had a solubility of approx. 60 mM in the water/lower alcohol mixtures, and then exhibited a linear decline for alcohol chain lengths greater than 6. A similar trend was reported for the *o*-propoxy phenol.

The Gibbs free energies of hydration for aliphatic alcohols have been discussed by Beezer and Hunter (1983), highlighting the possible existence of odd/even oscillations in the increments on a plot of free energy as a function of number of carbons, up to pentan-1-ol; for the longer chain alcohols, increments were more uniform, once again demonstrating a break in properties at a chain length of five.

Liquid/Liquid Transfer and Partitioning

The enthalpies of transfer of the methoxy to butoxy derivatives of the *o*-, *m*- and *p*-alkoxyphenols between octanol and water, and isotonic solution and *Escherichia coli* have been determined (Beezer et al., 1987b). The enthalpies of transfer between octanol and water were significantly different to those between the isotonic solution and the bacterial cells, indicating that octan-1-ol is not necessarily a good model for a biological membrane. The ortho- and para-alkoxyphenols showed a break in the enthalpies of transfer at the butoxy derivative, in both solvent systems (Beezer et al., 1987b), as do the enthalpies of transfer of the methoxy- to pentoxy- *m*-alkoxyphenols from water to cells (Beezer et al., 1987c).

Biological Activity

Interaction with esterases

Hofstee (1952, 1954, 1958) described the action of selected esterases against a homologous series of substrates.

The behaviour of two different pancreatic esterases towards a homologous series of *n*-fatty acid esters (Hofstee, 1952) was such that the maximum reaction rate was attained at an aliphatic chain length of 5 (for esterase I) and 7 carbons (for esterase II). Throughout the entire series (three to nine carbons) there was an obvious odd/even variation in the plot of number of carbons as a function of maximum reaction rate. When the activity of cholinesterase was investigated in human blood serum, with sodium salts of the *n*-fatty acid esters of *m*-hydroxybenzoic acid (Hofstee, 1958), the esterolytic activity peaked at a substrate chain length of five carbons, with clear odd/even variation in response either side of this maximum. The level of activity was increased in proportion to the calcium ion concentration, but always attained maximum activity at C_5.

Hofstee (1954) investigated the behaviour of horse liver esterase towards the same series of *n*-fatty acid esters. In this instance, the first order reaction rate constant increased by a factor of 2.5 for each additional methylene group, but beyond a chain length of six carbons, the subsequent linear increase was of the order of a factor of 2 for each additional methylene group, thus a distinct break in properties occurred at a substrate chain length of six.

In vitro assessment of biological response

The effect of *o*- and *p*-alkoxyphenols on bacterial cells has been investigated microcalorimetrically by Beezer et al. (1987d). The dose re-

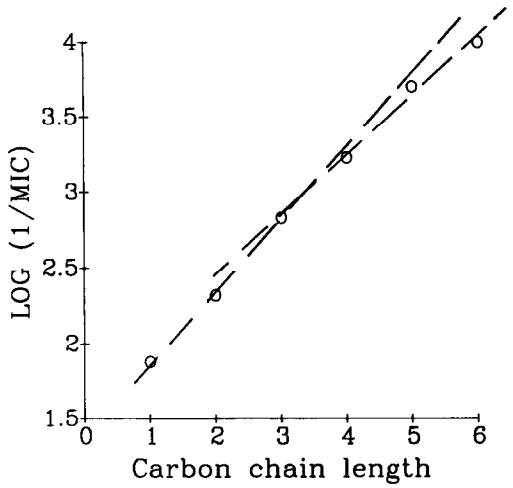

Fig. 4. An example of minor deviations in structure activity trends associated with the bacteriostatic action of a homologous series of 4-*n*-alkylphenols (drawn from data in Witham, 1983).

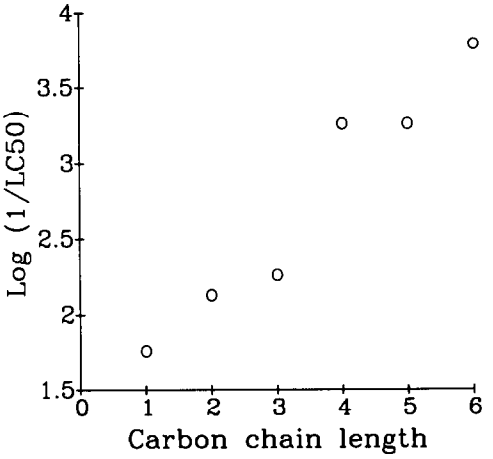

Fig. 5. The effect of alkyl chain length on the membrane disrupting properties of 4-*n*-alkylphenols, as demonstrated by pentose leakage (LC_{50} = concentration causing 50% leakage of intracellular pentose) (drawn from data in Witham, 1983).

sponse curves for bacterial kill had a much steeper gradient for the *o*- and *p*-butoxyphenols, compared to the other members (C_1–C_5).

In studies investigating the mechanism of antibacterial action of a homologous series of 4-*n*-alkyl phenols against *E. coli,* Denyer et al. (1980) and Witham (1983) observed minor deviations in structure-activity trends arising at the butyl/pentyl interface (e.g. Fig. 4). A close similarity in the chain length related behaviour was seen between bacteriostatic activity (minimum inhibitory concentration, MIC), alkylphenol-bacterium binding at the MIC, and the antimicrobial events of proton translocation and potassium leakage. The common reliance on chain length for all these phenomena implied a relationship anticipated from the selective membrane permeabilising action of these agents at bacteriostatic concentrations (Hugo and Bowen, 1973).

It might be expected that the observed minor discontinuities would be more clearly revealed if bactericidal events were to be considered since these reflect extensive drug induced damage. In this context, gross membrane disruption, as determined by pentose leakage following sustained contact (Denyer and Hugo, 1991), shows a marked discontinuity at *n*-butylphenol (Fig. 5). Extensive leakage is likely to be closely associated with cell death (Denyer and Hugo, 1991). Significantly, therefore, the rate of *E. coli* kill (Decimal reduction time, *D* value) for the six alkylphenols at their MIC in buffer showed a similar break point (Fig. 6).

The principal cellular target for phenolic agents is believed to be the cytoplasmic membrane (Denyer, 1990). It is likely that discontinuities associated with carbon chain length reflect altered interactions with the phospholipid bilayer.

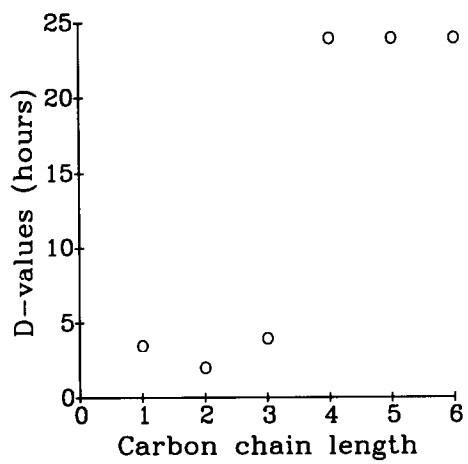

Fig. 6. *D* values for alkyl phenols against *E. coli* suspended in Hepes buffer (drawn from data in Witham, 1983).

General Discussion

In the solubility, partitioning and transfer data discussed above, we have been dealing with the variation in a Gibbs function (e.g. the Gibbs function of transfer, $\Delta_{trans}G = -RT \ln P$, where R is the gas constant, T is the absolute temperature and P is the partition coefficient) with carbon number. It would be interesting to know whether enthalpy and/or entropy terms that constitute ΔG (since $\Delta G = \Delta H - T\Delta S$) also show the same kind of variation with carbon number. These data would shed light on the reasons for the observed changes in properties at the carbon chain length of 5 (± 1). For example, Nilsson (1986) has shown that if the enthalpy of solution at infinite dilution in water of a series of *n*-alkan-1-ols is plotted as a function of carbon number, a distinct break occurs at carbon number 5, with the values of $\Delta_{sol}H_m$ remaining constant from C_6–C_{10}.

Work by Bratt et al. (1990) and Gillies et al. (1990) using NMR relaxation techniques to study internal motions in micellar and related systems has shown that, for alkyl chains, internal motions become more unrestricted along the chain. For a series of *n*-octyl compounds examination of the internal correlation times revealed that carbons 5, 6, 7 and 8 had similar values for all compounds studied. This suggests, in these compounds at least, that the hydrocarbon chain becomes significantly more flexible from carbon number 5 onwards. From this it is possible to propose an explanation for the observed break in solution phase properties at carbon number 5. If we take a hypothetical series of compounds which have the same 'parent' chemical structure, and which differ only in the length of the pendant alkyl chain, then for short alkyl chain lengths the solution phase properties (for example) will be dictated by the parent structure and one might intuitively expect the equilibrium solubility of these compounds, in any given solvent, to vary in a linear fashion with the carbon chain length of the alkyl group. This would only be valid if all the CH_2 groups in the alkyl chain were physically equivalent. The NMR data suggest that this is not the case, and that in structural, and hence entropic, terms, there will be a break in the linearity upon the addition of further methylene groups after carbon 5, because there is much greater flexibility introduced into the alkyl chain after this point. It now becomes clear that from C_2 to C_5 the alkyl chain may be regarded as 'rigid', and therefore will impose a minimum free energy conformation on the neighbouring solvent molecules. For alkyl chain lengths of five or more carbons, it will be the solvent that will be able to dictate the minimum free energy conformation on the alkyl chain of the solute, and it is possibly this change in the degree to which each species controls the overall structure of the solution that accounts for the break in the observed solution phase properties at carbon 5, for some series of compounds as illustrated by the examples cited here.

Naturally, the observed behaviour of solute-solvent systems is, therefore, dependent upon the balance of hydrophobic and hydrophilic forces. Abraham (1984) has summarised data for a wide series of compounds which indicate, that for many systems, this balance may result in apparently linear relationships between thermodynamic functions and chain length. It is, however, precisely for those systems where linearity does not exist that interest arises.

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