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Letter to the Editor

Pitfalls in the choice of isotherms for the calculation of band profiles in preparative chromatography

A reply

Sir,

Czok and Guiochon in the preceding paper [1] have called into question our program (CRAIG4) [2] for the computer simulation of separations by preparative high-performance liquid chromatography (HPLC). Since a substantial body of work reported by us is directly [2–5] or indirectly [6,7][a] based on these computer simulations, the validity of conclusions derived from this work [2–7] is similarly brought into question. Three major points have been raised by Czok and Guiochon:

(1) The approximate isotherm used in CRAIG4 is unnecessary; an exact isotherm can be used with no sacrifice in computation time.

(2) The CRAIG4 isotherm is physically meaningless and yields incorrect predictions of separation.

(3) Conclusions derived from the use of CRAIG4 "... cannot be trusted ...", specifically for the BIOPREP program described in ref. 8.

In the present paper we will respond to each of these issues.

DISCUSSION

(1) Approximate vs. exact isotherms for use in computer simulation

It is stated in ref. 1 that the Craig (or other) models of preparative HPLC require comparable computation time, regardless of whether an exact or our approximate isotherm is used. This was not obvious (to us) at the time our computer simulation studies began in 1984, but the data of Table II of ref. 1 provides a convincing demonstration that this is in fact true. The comparable computation times for simulations based on either approximate or exact isotherms was recently confirmed by Poppe [9], and we now have no reason to doubt that this is the case.

Therefore we agree with ref. 1 that (in view of our *present* knowledge) there was no advantage to our past use of approximate Langmuir isotherms for the computer simulation of preparative HPLC, in place of exact solutions.

[a] The algorithm used for computer simulation in refs. 6, 7 is similar to that criticized in ref. 1 and thus is subject to the same questions.

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(2) Accuracy of computer simulations based on the isotherm of ref. 2

The authors of ref. 1 have speculated on the rationale for our use of the isotherm of ref. 2, since the only justification[a] given in ref. 2 was that "... our algorithm has been found to be accurate to within about $\pm 10\%$ ...". In order to better appreciate the reason for the apparent discrepancy between Fig. 1 of ref. 1 and the latter claim, the basis of our approximate isotherm requires a further discussion (which should have been given in ref. 2).

Origin of the isotherm of ref. 2. The original reason for the development of CRAIG4 was to simulate preparative HPLC under gradient elution conditions [3–5] (its later application to isocratic systems [2] was an afterthought). Since gradient separations involve mobile phases with a broad range of small-sample capacity factors, k' (k_0), a two-solute isotherm was needed that would be applicable for (roughly) $1 < k_0 < 1000$ and a wide range of sample compositions (varying amounts of solutes X and Y). The isotherm of ref. 2 was developed empirically to meet these conditions (see discussion of Appendix), and several hundred comparisons were carried out between the predictions of this isotherm and those for the exact Langmuir isotherm for a two-component system. These comparisons indicated that the isotherm of ref. 2 was indeed accurate within "about $\pm 10\%$", but we also observed somewhat larger errors for $k_0 < 3$.

Upon seeing ref. 1, we re-examined the agreement between the approximate and exact isotherms and confirmed the observations of Czok and Guiochon. The accuracy of our isotherm may be better appreciated by comparing single-solute isotherms for different values of k_0, as in Fig. 1. The isotherm discontinuity which is the basis of the criticism presented in ref. 1 is clearly seen in each of these examples. However, this discontinuity becomes progressively smaller for larger values of k_0. For simulations of preparative HPLC based on gradient elution, it appears to us that the effects of this discontinuity will be small for solutes that are initially well retained ($k_0 > 10$). This is confirmed by the good agreement between experimental and simulated chromatograms for single-solute gradient elution [3].

Accuracy of CRAIG4 simulations. We were aware that the isotherm of ref. 2 is only an approximation of the exact Langmuir isotherm[b]. However our goal in the various studies based on the CRAIG4 program has been described [2] as "... to uncover general (if approximate) relationships for application to preparative HPLC, rather than to present equations for predicting preparative HPLC separations exactly". In this connection it should be recalled that the requirements[c] for Langmuir adsorption in reversed-phase HPLC systems (especially for large samples) are unlikely to be met exactly in practice. An apparent failure of the Langmuir model for a representative two-component sample has also been reported by Katti and Guiochon [12].

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[a] A number of inferential checks on the reliability of this isotherm were also reported; see Table III of ref. 2 and Fig. 2 of ref. 3 as well as related discussion.

[b] Our earlier use of Craig simulations [10,11] employed polynomial equations which fit the Langmuir isotherm more accurately than the isotherm of ref. 2, without any discontinuity.

[c] *I.e.*, 1-for-1 replacement of sorbed solvent molecules by the adsorbing solute molecule, negligible interactions between solute and solvent molecules in the mobile and stationary phases, etc.

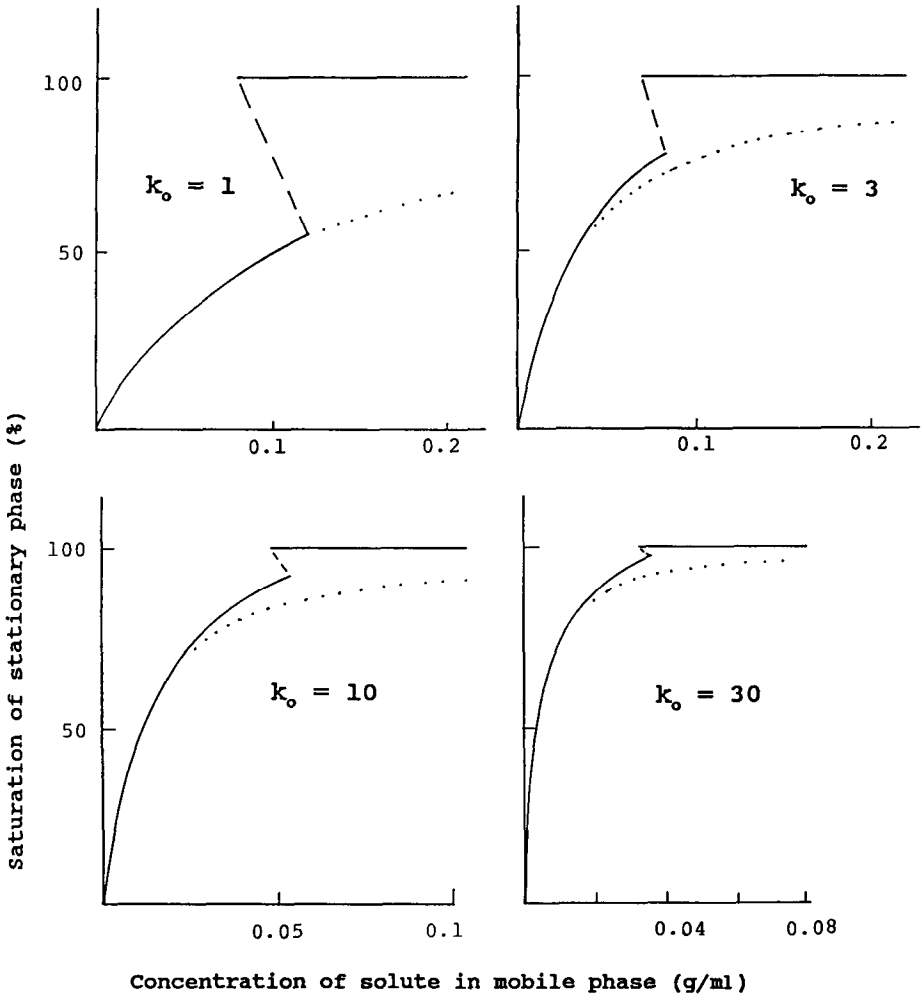

Fig. 1. Approximate (solid lines) *vs.* exact (dotted lines) Langmuir isotherms for a single solute. Conditions of Fig. 1, ref. 1.

Czok and Guiochon [1] stress the erroneous peak shapes that can be obtained via CRAIG4 simulations. Guiochon and co-workers continue to place considerable emphasis on band shape ("displacement" and "tag-along" effects) [13], whereas our recent studies deal solely with production rate as a function of separation conditions —or the relative overlap of two adjacent bands and their resulting purity. At this stage in the development of the theory of preparative HPLC, we feel that band shape *per se* should be of less concern. In this connection we might cite (a) the comment in ref. 1 that "... individual elution band profiles are sensitive to *minor* changes in the isotherms" (computer simulations), and (b) the diversity of band shapes encountered in experimental preparative HPLC separations [14].

Returning to the question of the accuracy (and use) of our predictions of band overlap in preparative HPLC (based on an empirical adjustment of sample size by a factor of 1.8), the data of ref. 1 fail to show that predictions based on CRAIG4 are inadequate for our intentionally approximate treatment of preparative HPLC. The only quantitative comparisons of *overlap* between CRAIG4 simulations and those of Ghodbane and Guiochon [15] (that we are aware of) indicate agreement of $\pm 2\%$ absolute, or $\pm 5\%$ relative —which we judge to be adequate.

Ref. 1 also refers to inaccuracies in the prediction of retention by CRAIG4. Apart from the effect of such retention errors on predictions of band overlap (which from the above discussion appear not to be serious), such discrepancies would not affect any of the conclusions reached by us on the basis of our use of CRAIG4.

Finally, Czok and Guiochon [1] suggest that the BIOPREP program described in ref. 8 "... cannot be trusted ..." because of possible deficiencies in the CRAIG4 program. In fact the BIOPREP program is based on a completely different approach than that used in CRAIG4, as a careful reading of ref. 8 indicates.

(3) Conclusions derived from CRAIG4

It is worthwhile to examine some of the conclusions [2–5] reached on the basis of CRAIG4 simulations, as a further check on their relative reliability and value. That is, are our findings "reasonable" in terms of what was already known concerning preparative HPLC? And, do these conclusions provide further insight into preparative HPLC? The computer simulations of refs. 2–5 represent an extension of prior work [16–19] dealing with lightly loaded preparative HPLC ("touching band" separations in both isocratic and gradient modes) to the case of larger samples and overlapping bands. The major aim of these studies was to define general conditions for the maximum production rate (g/h) of the desired (purified) product, using the general approach of Knox and Pyper [16] for "touching band" separation.

The main conclusions reached by us in refs. 2–5 are as follows.

First, there is a marked parallelism between lightly and heavily overloaded preparative HPLC in the dependence of the maximum production rate on the sample characteristics (k_0 and separation factor, α) and separation conditions (values of the plate number N_0 and the weight of sample). Relative to the case of an optimized "touching band" separation, production rate can be increased by further increase in sample size and decrease in column plate number —with a corresponding increase in band overlap and decrease in the recovery of pure product.

The optimum choice of the (small-sample) column plate number N_0 can be related to the resolution R_s observed for a small sample. This should be about $R_s = 1.7$[a] for the touching band case (100% recovery of pure product), $R_s = 1.2$ for moderate overlap of the two bands (95% recovery of pure product) and $R_s = 0.9$ for heavy overlap (50% recovery). The production rate increases for these three cases in the ratio of 1 (100% recovery):4 (95% recovery):20 (50% recovery), showing a marked advantage to the use of heavier column loadings.

Finally, the required plate numbers for *e.g.*, 95% recovery of pure product are

[a] Knox and Pyper suggest a value of $R_s = 2$, but this ignores the displacement of one solute by the other.

relatively low: about $N_0 = 200$ for $\alpha = 2$, and $N_0 = 3000$ for $\alpha = 1.25$[a]. In the latter connection, it is probable that most preparative HPLC separations carried out at the present time use columns and flow-rates that provide excessively large N_0 values, in turn yielding below-optimum production rates.

Second, earlier work has shown (for the case of a small sample and closely eluting solute bands) that an isocratic separation can always be duplicated by a gradient run with "corresponding" conditions; *i.e.*, a gradient steepness b which is equivalent to the mobile phase composition (%B) used in the isocratic separation [21] ($1/b \approx k'$). This conclusion was subsequently shown to be true of lightly loaded ("touching band") separations as well [19]. Recent studies based on CRAIG4 simulations [3–5] have now extended this generalization to heavily overloaded preparative HPLC. That is, the various conclusions summarized above for overlapping-band isocratic separation have been shown to apply also to gradient elution, for the case of "corresponding" conditions. This further suggests that the more approximate computer simulations used by us for isocratic elution are in fact adequate in terms of our "practical" objectives.

We leave to the reader the question of whether the above conclusions based on CRAIG4 simulations are (a) reasonable and (b) of practical value.

CONCLUSIONS

In summary, we agree with Czok and Guiochon that our use of an empirical isotherm (in our CRAIG4 program) in place of the exact Langmuir isotherm introduces some error into resulting simulations of preparative HPLC, and there is no compensating advantage in terms of computation time. However this observation must be qualified by several other facts.

First, our use of computer simulations based on CRAIG4 has been aimed at deriving approximate, general guidelines that will be helpful to practical workers. Since the Langmuir isotherm is only a crude approximation for most HPLC separations of practical interest, any generalizations based on an isotherm of slightly different shape are not likely to be much different.

Second, Czok and Guiochon [1] have emphasized certain differences in separations predicted by CRAIG4 *vs.* the exact Langmuir isotherm; *i.e.*, band shape and retention. Our work has instead focused on separation as measured in terms of band overlap and band purity. Such evidence as has so far been reported suggests that band overlap as predicted by our approach (using CRAIG4) agrees within about $\pm 2\%$ with band overlap predicted by the exact Langmuir isotherm.

Finally, our work based on CRAIG4 is an attempt to extend the conclusions of previous workers on lightly loaded ("touching band") separations to the case of heavily loaded (overlapping band) preparative HPLC. The CRAIG4 results suggest that lightly and heavily overloaded separations are remarkably similar, despite striking differences in the shapes of the solute bands in the two cases. Well documented generalizations that apply to the "touching band" case can now be extended (with minor modification) to overlapping band separations.

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[a] Preparative HPLC on a production scale should in most cases involve values of $\alpha > 1.5$, as the result of careful selection of separation conditions; see, *e.g.*, ref. 20.

Finally, in our opinion, the Knox–Pyper model of preparative HPLC [16] continues to provide the best available conceptual picture of these separations. Computer simulations based on more detailed and "exact" models will no doubt continue to add to our understanding of preparative HPLC. However such work (including our own) has not yet significantly modified the elegant (if approximate) guidelines set forth in ref. 16.

APPENDIX

The discontinuity in the isotherms of Fig. 1 (and Fig. 1 of ref. 1) arise from our use of a two-part function to represent the Langmuir isotherm. In an earlier study [10] we showed that the one-solute isotherm can be used as the basis for a reasonable approximation to the two-solute isotherm (thereby simplifying and shortening the calculations needed in computer simulation), if different functions are used for light and heavy loadings of the stationary phase (column). In earlier work (prior to our use of CRAIG4) computer simulation was restricted to rather small samples, allowing use of a single function for the iostherm, with no discontinuity as in Fig. 1. CRAIG4 was intended for application to a wide range of sample sizes, leading to the use of the two-part function illustrated in Fig. 1.

The accuracy of the smaller-sample function that forms part of the CRAIG4 isotherm can be seen in the various comparisons of Fig. 1 and Fig. 1 of ref. 1 (first part of the isotherm). Unfortunately, values of α (which are of major importance in the accurate description of preparative HPLC) begin to deviate from the chosen (correct) values at higher loadings —just beyond the first segment of these isotherms. The second (large-sample) function that comprises the CRAIG4 isotherm is designed to maintain the value of α at a constant (correct) value regardless of sample size.

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