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COMPUTER SYSTEM FOR STRUCTURE RECOGNITION OF POLYATOMIC MOLECULES BY I.R., N.M.R., U.V. AND M.S. METHODS

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SUMMARY

A system of algorithms and programs for the recognition of the structures of polyatomic molecules by means of i.r., n.m.r., u.v. and mass spectra is described. Examples of structures identified are cited. The results are promising and suggest that the system could be used for the identification of complex organic compounds.

The problem of structural group analysis and identification of organic molecules in samples of different origin is becoming increasingly important in connection with environmental protection, production of high-purity materials, etc.

This analysis can be effected by various physical and chemical methods. Recent years have seen ever-increasing use of the common spectral methods. Spectral analyses have some advantages over purely chemical methods, as they provide extensive and rapidly obtainable information on the structural groups present in molecules and enable data to be handled by computers. This gives more reliable solutions and increases the ability to identify complex molecules and to analyse a wide variety of solutions which do not contradict the initial experimental data.

Many recent analytical procedures are based on different algorithms designed to identify chemical compounds from their molecular spectra [1—30]. Three successfully developing routes may be mentioned [31]. The first is based on the use of computer catalogues including spectra of different nature and on the development of information retrieval systems [1—7]; the second utilizes algorithms for pattern recognition and computer learning [8—12]; the third is based on artificial intelligence units [13—30]. The above routes all have advantages and disadvantages which are discussed below.

The identification of molecules by comparison of their spectra with those in atlases appears to be logically very simple and, at first sight, rather attractive, particularly when it is considered that current atlases give descriptions of more than 10^5 chemical compounds [32]. However, this approach suffers from a significant shortcoming: the number of chemical compounds currently

known exceeds by one order of magnitude the number of substances coded in the atlases. Moreover, synthesis of new compounds continuously yields structures which are not included in the atlases.

The coding of atlas spectra to be inserted into a computer is inevitably accompanied by various simplifications, which result in a partial loss of information. Consequently, a spectrum to be identified and the one taken from an atlas cannot generally be compared with absolute accuracy; it is probable that in some cases the identity of the two spectra will not be established.

In fact, experience in operating information search systems based on the atlas approach, shows that the computer output frequently contains more than one, and even several tens of "similar" compounds, so that further visual comparison of the experimental and atlas spectra becomes necessary. Accordingly, laboratories must have a full set of the atlases, and this is not easily achieved. Nevertheless, even with the variety of "similar" chemical structures selected by the computer, the specialist obtains valuable information for planning further experiments.

The approach based on pattern recognition algorithms often gives good results, but it cannot be considered universal, since in each particular case the computer has to recognize some new classes of compounds. As a rule, pattern recognition algorithms are used to solve only individual problems, without any real versatility being claimed.

Computer learning aimed at the recognition of a new class of compound cannot be carried out successfully unless the training data are adequate and representative. The narrow specialization of the identification systems incorporating pattern recognition algorithms is a major disadvantage of the approaches mentioned above.

The third route, based on artificial intelligence elements, may be characterized as having the most complex logical structure. However, this route holds much promise for the development of algorithms and systems which identify polyatomic molecules according to their spectra. An algorithm of this kind can be formulated by employing information of different origin and includes general and well-established logical connections of a chemical nature. Specific experience gained in operating this algorithm shows that the algorithm requires a relatively small set of data on the spectral parameters of individual fragments. The resulting systems of programs can be easily reproduced and complemented; they also show various other advantages.

Another important aspect should be noted. The structural-group analysis of molecules, based on the totality of their molecular spectra, represents one of the variants of inverse spectral problems formulated in a manner characteristic of incompletely defined problems, i.e. problems lacking some initial data. These can be solved only by introducing a number of limitations and by imposing additional conditions on the solutions and using these during the solution searches. Consequently, none of the approaches

can, in principle, neglect the above-mentioned aspect which must be taken into account in solving molecule identification problems, whichever method is used.

In the present paper, a specific algorithm is described and examples of structural-group analysis and identification of molecules by a method based on artificial intelligence are given. This method is characterized by a feedback which consists of constructing a theoretical vibrational spectrum and comparing it with one obtained experimentally in order to rank the expected structures according to their degree of reliability. This algorithm has exhibited sufficiently high reliability and high-speed operation in tests. However, its use is restricted to applications where individual compounds are identified. This grave practical disadvantage is encountered, as far as is known, in all present systems for the identification of compounds.

THE STRUCTURE RECOGNITION SYSTEM STREC

The algorithm and the system of structure recognition programs designated at STREC consists of the components presented in Fig. 1. A brief description of the component applications is given below.

The block for structural-group analysis (1) automatically constructs and solves logical equations reflecting relationships between a spectrum and the structure (see below; also [19, 22]). The block operation is effected by use of an empirical formula, and a library of standard fragments (LSF) containing spectrum-structure correlations for i.r. spectroscopy and for a vibrational spectrum (presented in the form of a logical sum of i.r. and Raman spectra). The operation of Block 1 terminates with the output of all fragment sets which fit the spectrum and are not at variance with the empirical formula. It should be noted that this block may use, instead of i.r., a n.m.r. spectrum together with corresponding correlations.

Within the STREC system, the task of Block 1 consists in reducing the dimension of the problem through the choice of such fragments as would "absorb" a maximum number of the skeleton atoms.

The combinatorial block (2, Fig. 1) then provides for a further reduction in the problem dimension by constructing, from a given set of fragments, combinations which may include several identical fragments on condition that the empirical formula requirements are met. This allows recognition problems for molecules with a large number (more than 20) of skeleton atoms to be solved, if several bulky identical fragments (benzene rings, etc.) are present.

Block 3 carries out mathematical synthesis of the structural formulae of all isomers on the basis of fragment sets chosen by means of the MASS system [33, 34]. In this case any chemical information other than empirical formulae may be taken into account; this allows restrictions to be imposed on the structures synthesized.

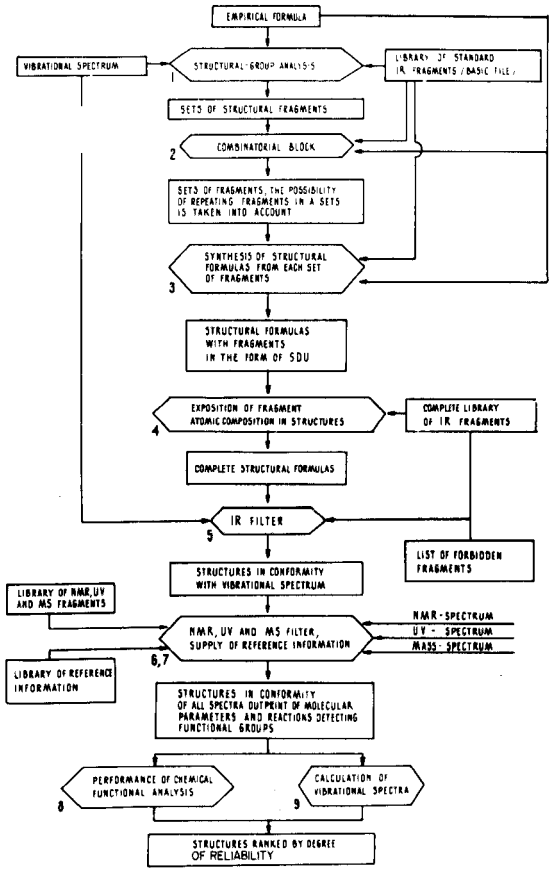


Fig. 1. Block diagram of STREC system.

Block 4 (Fig. 1) exposes the structure of fragments contained in the incidence matrices in the form of "macro-atoms" (structural discrete units, SDU). At this stage, a complete atomic structure of the molecule is obtained, with the non-equivalence of peripheral atoms of some fragments being taken into consideration (e.g., for —COO—, the following structures are formed: R_1—$COOR_2$ and R_2—$COOR_1$).

Block 5 checks the structures being synthesized for the presence or absence of each fragment from the library of standard fragments. When a fragment is found, its characteristic frequency intervals will be compared with the experimental spectrum. If the spectrum confirms the possibility of the presence of all fragments detected in the structure, the structure is tape-recorded. Otherwise (should the presence of any fragment not be confirmed by the spectrum), further analysis of the given structure is terminated, and the program passes over to checking a subsequent incidence matrix. Block 5 makes it possible to solve problems of small dimension (up to 7 or 8 skeleton

atoms) without the use of blocks 1, 2 and 4; the screening of all isomers synthesized by the MASS system, which conform with a given empirical formula, is done by Block 5.

If, in addition to a vibrational spectrum, n.m.r., u.v., and mass spectra are known for the unidentified specimen, the structures selected by Block 5 are checked for the presence or absence of fragments included in the additional library of standard fragments (ALSF). This library is composed of n.m.r., u.v., and mass fragments with appropriate characteristic spectral features (see the section on the Library of Standard Fragments). Structures confirmed by n.m.r., u.v. and mass spectra will be printed. Structural formulae which are not confirmed by the mass spectrum are also printed along with the causes of contradiction (6, Fig. 1).

If Block 6 does not supply a single solution, then at the operator's demand the informational library of standard fragments (ILSF) is accessed. This library comprises information on qualitative reactions for common functional groups as well as geometric, force, and electro-optical parameters for major fragments in organic chemistry.

The program searches the ILSF for fragments in the structural formulae obtained and prints out data relative to each structure (7, Fig. 1).

In cases where qualitative reactions should lead to proper discrimination of structures (e.g., by bromination of O—O or C=C bonds), chemical analyses may be made by the procedures printed by Block 7 (8, Fig. 1).

The last Block (9) of the STREC system is intended to calculate the vibrational spectra of structures which cannot be distinguished by other methods. The spectra are calculated with the help of parameters supplied by Block 7 and compared, by means of a formal criterion, with the experimental spectra, whereupon the structures are ranked according to the degree of reliability. Obviously, Block 9 cannot be operated unless all data necessary for calculation are available, and calculations can be made only for compounds with not too many atoms.

All the algorithms of the system are in FORTRAN.

In the following sections, the major algorithms of the STREC system are described. These are the algorithm for structural-group analysis, the algorithm for mathematical synthesis of all structural formulae, and the algorithm which analyses structural formulae for the presence or absence of given fragments.

ALGORITHM FOR STRUCTURAL-GROUP ANALYSIS OF MOLECULES BY MEANS OF THEIR MOLECULAR SPECTRA

The mathematical establishment of an algorithm which decodes the composition of polyatomic molecules from their spectra is based on the assumption that there is a certain relationship between individual structural units and the molecular spectra. Here, structural units mean not only groups containing a few atoms, but even complete molecules. A collection of

structural discrete units (SDU) makes up one set. Another set is formed by the parameters inherent in the first set, e.g., band frequencies, chemical shifts, etc. The two sets are so interrelated that at least one object from the first set conforms with one of the objects from the second set, and vice versa. Spectrum—structure correlation tables provide an example of such interrelations [35, 36]. After consideration of these aspects, one can formulate rigorously a problem of structural-group analysis in terms of symbolic logic as follows. Let us assume that the spectrum or set of spectra for a certain specimen is known, and that data are available on the relationships between frequencies (or chemical shifts or other parameters) and structural units of the molecules. Let us also assume that among the spectral parameters (for conciseness, only i.r. frequencies are referred to in the subsequent discussion) there are characteristic frequencies, i.e. parameters related only to small structural units of the molecule. If this condition were not fulfilled, analysis with the correlation tables would be impossible, and the problem would have to be solved by identification from an atlas of molecular spectra. However, the atlases may also be included formally in the combination of spectrum—structure correlations. The approach described below could then be extended to cover spectral atlases, and would become quite versatile.

Thus, given a collection of spectrum—structure correlations, the requirement is to establish all possible combinations of functional groups whose presence is consistent with the experimental spectra.

Let $\tilde{\Omega} = \{\omega_j\}$, where $j = 1, 2, \ldots m$, be the set of spectral features (frequencies) observed for the specimen, and $\mathscr{A} = \{A_i\}$, where $i = 1, 2, \ldots N$, be the set of structural units (functional groups) having at least one characteristic frequency among the members of set $\tilde{\Omega}$; the set $\mathscr{A}$ may also include functional groups whose presence or absence in the specimen must be established (i.e. analysis for predetermined groups).

Let $\bar{\Omega} = \{\omega_j\}$, where $j = m + 1, \ldots M$, is the set of frequencies which correspond to some $A_i \epsilon \mathscr{A}$ but are not observed in the spectrum.

Let $\Omega = \bar{\Omega} \cup \tilde{\Omega} = \{\omega_j\}$, where $j = 1, 2, \ldots M$, is the set of all frequencies considered during solution of the problem.

Let $\Omega \cup \mathscr{A} = \{\mathscr{E}_\nu\}$, where $\nu = 1, 2, \ldots M + N$, is the universal set which provides boundaries for all the functional groups and frequencies related to the given problem.

The members of the above sets, as logical variables, can be defined by the following elementary propositions:

A_i: "the molecule contains the group A_i";
$\bar{A}_i$: "the molecule does not contain the group A_i";
ω_j: "there is an ω_j-frequency in the spectrum";
$\bar{\omega}_j$: "there is not a ω_j-frequency in the spectrum".

Then all the logical connections between the members of the sets $\mathscr{A}$ and Ω must be written, and any additional relationships resulting from non-spectral data must be expressed in terms of logical functions. In this case,

when an $A_i \epsilon \mathscr{A}$ group is correlated with a collection of η characteristic frequencies, the latter are connected with the group A_i in the following manner:

$$A_i \rightarrow \omega_1^{(i)} \wedge \omega_2^{(i)} \wedge \ldots \wedge \omega_\alpha^{(i)} \wedge \ldots \wedge \omega_\eta^{(i)}, \omega_\alpha^{(i)} = \omega_j \epsilon \Omega \quad (1)$$

$$(\bar{\omega}_1^{(i)} \vee \bar{\omega}_2^{(i)} \vee \ldots \vee \bar{\omega}_\alpha^{(i)} \vee \ldots \vee \bar{\omega}_\eta^{(i)}) \rightarrow \bar{A}_i \quad (2)$$

where $\omega_\alpha^{(i)}$ are frequencies which occur in characteristic intervals corresponding to group A_i; the signs $\rightarrow$, $\wedge$, $\vee$, and upper dash are logical operation symbols for implication, conjunction, disjunction, and negation, respectively [22].

Expression (1) signifies that, if a molecule contains an A_i group, the spectrum comprises a set of frequencies $\omega_1^{(i)}, \omega_2^{(i)}, \ldots \omega_\eta^{(i)}$. Basically, implication (1) symbolizes the experimental fact that vibrations are characterized by specific frequencies.

According to expression (1), a molecule can contain an A_i structural group only if the conjunction of frequencies $\omega_1^{(i)}, \omega_2^{(i)}, \omega_3^{(i)}, \ldots, \omega_\eta^{(i)}$ is observed in the spectrum (essential condition). Expression (2), analogous to (1), shows that if at least one of the A_i group characteristic frequencies is absent, the sample does not contain an A_i structural group (sufficient condition).

If an ω_j frequency falls in the sets of characteristic frequencies for groups $A_1^{(j)}, A_2^{(j)}, \ldots A_\beta^{(j)}, \ldots A_\gamma^{(j)}$ contained in an $\mathscr{A}$ set, then the expression

$$\omega_j \rightarrow A_1^{(j)} \vee A_2^{(j)} \vee \ldots \vee A_\beta^{(j)} \vee \ldots \vee A_\gamma^{(j)}, A_\beta^{(j)} = A_i \epsilon \mathscr{A} \quad (3)$$

can be written. This expression states that the molecule does include at least one of the functional groups $A_1^{(j)}, A_2^{(j)}, \ldots A_\gamma^{(j)}$, if an ω_j frequency is detected in the spectrum. Expressions (1) and (3) represent basic types of logical relations between groups and spectral features in structural-group analysis.

It is worth noting that an implication by its nature shows that one proposition follows from another, though in principle the same proposition may be derived from other premises. Thus, the conjunction of frequencies $\omega_1^{(i)}, \omega_2^{(i)}, \ldots \omega_\eta^{(i)}$ may result not only from the presence in the sample of an A_i group, but also from other groups or their combinations. Also, the disjunction $A_1^{(j)} \vee A_2^{(j)} \vee \ldots \vee A_\gamma^{(j)}$ may be implied by spectral features other than the ω_j frequency. This suggests that the basic relationships (1) and (3) rigorously reflect the nature of the relations between functional groups and corresponding characteristic frequencies of vibrational spectra.

If a Boolean function $T(\mathscr{A}, \Omega)$ is introduced to describe all the connections between frequencies and structural units in a given problem, then the vibrational spectrum as a combination of frequencies may be represented by the Boolean function $R(\Omega)$.

Logical analysis of this situation, consisting of a simultaneous treatment of the $T(\mathscr{A}, \Omega)$ and $R(\Omega)$ functions must result in a function $f(\mathscr{A})$ which describes all the structural unit combinations whose presence in the sample is possible. This may be symbolized as:

$$T(\mathscr{A}, \Omega) \rightarrow \{R(\Omega) \rightarrow f(\mathscr{A})\} \quad (4)$$

Relation (4) is the most general formulation of the problem of qualitative spectral analysis in terms of Boolean algebra. It represents a logical equation with respect to the $f(\mathscr{A})$ function. In structural-group analysis, this logical problem is solved by calculating the $f(\mathscr{A})$ function, by means of the algorithm described earlier [22]. It should be emphasized that relation (4) is generally applicable and may be used to describe qualitative analyses based on many principles. It is significant that relations of some other types (known from other experiments or essential from the synthetic conditions) may also be included in the scheme described without any change in the algorithm.

In general, the $f(\mathscr{A})$ function will contain K items ($1 \leqslant K \leqslant 2^N$), each of which describes one of the combinations of structural units which are equally probable in the sample. The probability of each combination is $1/K$. This value depends only on the initial data and is not subject to the arbitrary opinion of the specialist.

The probability of the presence of each group is calculated from the formula $P(A_i) = q_i/K$, where q_i is the number of structural unit combinations in the $f(\mathscr{A})$ function containing the A_i group in the affirmative form.

Indefinite analytical results may be characterized by entropy. If the sample is known to be a single compound, the combinations present in the $f(\mathscr{A})$ function will be mutually exclusive, and entropy will be equal to $H = \log_2 K$. When a complete structural-group composition of the sample has been found, the information obtained is numerically equal to the initial entropy. Thus, calculation of the $f(\mathscr{A})$ function provides a measure of the additional information necessary to single out a unique complex of structural groups present in the specimen molecules and allows optimum planning of further experiments.

Clearly, the logic of structural-group analysis amounts to searching for a logical consequence on the basis of all the available experimental and theoretical data, the latter being expressed as Boolean functions and considered to be a set of initial propositions. In this respect, the formulation of initial data in solving a specific structural problem is analogous to the construction of an axiomatic "micro theory". Such an approach allows the initial data to be analysed for their consistency, logical independence and completeness. This is achieved by use of algorithms and an apparatus of designation numbers [22]: if the designation number of the $T(\mathscr{A}, \Omega)$ function is zero, the system of initial data contains contradictions. When the designation number, $\#T(\mathscr{A}, \Omega) \neq 0$, but the product of the designation numbers $\#T(\mathscr{A}, \Omega).\ \#R(\Omega) = 0$, there are latent contradictions between some of the propositions and the experimental spectra.

The algorithm described forms the basis of the first block of the STREC system. Experience in solving the structural-group problems has shown that in most cases the answer proved to be ambiguous, and the solution to be unstable. Such results are characteristic of inverse spectral problems. In this

case, to obtain stable and unique solutions, a number of prior independent restrictions must be introduced which are specified by the type of the inverse problem. It is also necessary that each set of structural discrete units (SDU) detected can be used to synthesize mathematically a molecular structure corresponding both to the expected empirical formula and to valence theory.

The appropriate algorithm, placed in the third block of the system, is described in the following section.

MATHEMATICAL SYNTHESIS OF MOLECULAR STRUCTURES

The algorithm for mathematical synthesis of molecular structures on the basis of atoms and fragments forming SDU is constructed on the following principles [33, 34]. To generate and analyze the structures, graph theory is used [37].

The structural formula of a compound is considered as a connected finite multigraph represented by its incidence matrix. Atoms or fragments which can be introduced in the form of a "macro-atom" with a given valence, i.e. SDU, correspond to the vertices of the multigraph, whereas the bonds correspond to the edges; the multiplicity of the edges and the bonds is the same. The program generates all incidence matrices which satisfy both the empirical formula and the predetermined distribution of valences for the structural units. The matrices obtained are then checked for connectivity. From all the connected matrices fitting a given isomer, the program selects one called canonical.

Each structure has a corresponding set of incidence matrices [37]. Any matrix A_r of this set can be altered to any other matrix of the same set by renumbering the vertices of the graph. The A_r matrix is symmetrical, and its diagonal elements are zeros. For each vertex there is a corresponding row and column of the matrix. If a square B_r submatrix which includes only incidence elements corresponding to SDU is selected, and if a column for hydrogen atoms is added on the right, then the values in this column will indicate the number of hydrogen atoms related to each SDU. Below, an incidence matrix is defined as a B_r matrix. Let us introduce the following number corresponding to the B_r matrix:

$$K_r = \sum_i f_i \sum_{ij} b_{ij} f_j \quad (i,j = 1, 2, \ldots n)$$

where i is the number of the row, j is the number of the column, b_{ij} is the value of a B_r matrix element (bond multiplicity), and f_i, f_j are the weighting factors for a row and a column. The essential condition for the weighting factor is:

$$f_e > (f_{e+1} \cdot V)$$

where V is the maximum multiplicity of the bonds observed in the samples. In the MASS system, $f_1 = 5 \times 10^9$; this value is sufficient for solving problems containing not more than 20 SDU.

A larger K_r represents a "larger" matrix. Generation of all possible structures involves constructing a B_m matrix regarded as the "maximal" one for a given empirical formula, and then successively subtracting from B_m the smallest possible numbers, until K_r becomes zero.

To obtain a B_m matrix, the B_r rows are scanned successively downwards and from left to right, beginning with the element $(i, i + 1)$. Each element is filled with the largest possible number (consistent with valence rules), until the sum of all the entries in the row equals the valence of the SDU corresponding to the row, or until the row ends (in the latter case the remaining valence units make up an element of an additional column).

The "subtraction" is effected by working backwards through the B_r until the first non-zero b_{ij} value is found; the b_{ij} value is then reduced by 1, and a "maximal" matrix is constructed beginning from $(b_{i,j+1})$.

The algorithm developed to check the graph for connectivity is called "graph vertex convergence". The concept of the algorithm is realized by successively uniting the adjacent vertices of the graph in one vertex, the edges remaining as they are except for those connecting the above vertices. If this operation results in only one vertex, the graph is connected, otherwise it is not.

Each graph has $n!$ incidence matrices (n is the number of vertices) [37]. To single out one incidence matrix of set A, the set must first be ranked, and then one matrix must be chosen according to the principle of ranking [38]. This matrix is called the canonical matrix. In accordance with the algorithm of generation, canonicity is attributed to the "maximal" matrix among those possible for the graph. Then an algorithm for checking canonicity must analyse whether the B_r rows could be renumbered in such a way as to produce a B_p matrix with $K_p > K_r$. If this proves to be impossible, the matrix obtained may be classified as canonical. There is no need for the inequality to be checked by calculating K_r and K_p; the numbers obtained and the f_1 value needed are too large. It is sufficient to calculate the sequences of K_{ri} and K_{pi} values for the B_r and B_p rows and to compare them; the matrices may also be compared element by element.

The system allows for human control of generation by means of subroutines which, depending on the problem, make it possible to generate structures pertinent to a certain class of organic compounds as well as to a combination of classes. These subroutines also allow isomers containing a certain number of predetermined functional groups or fragments to be included or excluded. For example, any output of isomers which are unlikely to be found in their natural form, can be forbidden. Data on forbidden isomer structures are input beforehand as a computer catalogue of the corresponding fragments (e.g.  ,  , etc.).

It should be noted that rotational, optical and stereo-isomers are not distinguished during the generation, and molecular geometry is not taken into account. Therefore, the same incidence matrix will be valid both for *cis*- and *trans*-isomers.

An important feature of the MASS system in applications to identification problems should be emphasized. When the empirical formula contains chemical elements not incorporated in the STREC system or when the unknown compound includes a priori some fragments absent from the library of standard fragments (LSF), the MASS system may be used as a self-contained system. Under such conditions, the system is entered via fragments whose presence is confirmed by spectral and chemical considerations. At the same time, restrictions are imposed which explicitly forbid some combinations of fragments or of atoms in the empirical formula. Structures synthesized mathematically under these conditions are printed out and reviewed visually in an attempt to estimate their reliability. This variant of the system has been checked experimentally with positive results [30].

ALGORITHM FOR ANALYSIS OF STRUCTURAL FORMULAE

As was mentioned in the introduction, the system is characterized by a feedback which compares the experimental and theoretical spectra. Theoretical spectra are constructed at two levels: (a) approximate "characteristic" spectra which are produced by additive summarizing of the spectra of discrete units forming a "suspected" structure (obviously, the library of standard fragments must contain all the indispensable discrete units); and (b) exact spectra which are constructed, only for i.r. spectroscopy, on the basis of molecular vibration theory, by means of programs described earlier [39, 40].

The STREC algorithm does not allow the construction of "characteristic" spectra as such. This procedure is replaced by analysis of structural formulae, in order to detect the presence or absence of fragments from the i.r. LSF and/or the additional LSF. The structural formulae are also examined for certain atomic groups, in order to use the informational LSF which, in particular, contains data essential for forming vibrational equations and calculating intensities in i.r. spectra.

For this purpose, a general algorithm which detects predetermined fragments in structural formulae was developed. The concepts are as follows.

Let us assume a certain set of structural units $\mathscr{A}$ (chemical graph) represented by its incidence matrix A; the latter includes only those incidence elements corresponding to the skeleton atoms or, in the general case, to SDU with a given valence. Any incidence matrix A_r obtained from A by r-th renumbering of SDU is provided with the number K_r, which is used to check matrices for canonicity. The present problem consists of establishing the presence or absence of predetermined fragments (subgraphs) in the $\mathscr{A}$ graph.

It is assumed that the initial set which defines the incidence matrix A of the $\mathscr{A}$ graph comprises N structural discrete units, and that $N = \sum_{k=1}^{f} n_k$, where f is the number of SDU types (T_1, T_2, . . . T_k, T_f) and n_k is the number of SDU of the K type. For example, the compound $C_5N_2O_3H_{10}$ is characterized

by $C \epsilon T_1$, $N \epsilon T_2$, $O \epsilon T_3$, $f = 3$; $n_1 = 5$, $n_2 = 2$, $n_3 = 3$. The requirement is to establish the presence or absence in the $\mathscr{A}$ graph of a subgraph $\mathscr{B}$, the vertices of which contain P structural discrete units ($P \leqslant N$); $P = \Sigma_{k=1}^{f} P_k$ (P_k-numbers of SDU of the K type in the subgraph $\mathscr{B}$). For the subgraph $\mathscr{B}$, an incidence matrix B, called a subgraph matrix is constructed. Obviously, the order of matrix B is equal to P, and the number $K_{r\mathrm{B}}$ can be calculated for this matrix, by the method described in the section on Mathematical Synthesis of Molecular Structures.

From matrix A, rows and columns corresponding to the rows and columns of the subgraph matrix B are selected by using the following criterion: a row of the incidence matrix A is considered to correspond to a given row of the matrix B, if for each element $B_{T_i T_j}$ in the subgraph matrix B row, an element $A_{T_i T_j}$ can be found in a row of matrix A, such that $B_{T_i T_j} = A_{T_i T_j}$. Here and below, i and j are values of $K (K = 1 - f)$ for which $P_k > 0$.

A submatrix D is then constructed by combining the rows chosen in the incidence matrix A and the columns which coincide by number with these rows. Suppose that the submatrix D comprises d_i vertices of the T_i type, d_j vertices of the T_j type, etc. Obviously, if at least one T_k does not obey the inequality $d_k \geqslant P_k$, then the subgraph $\mathscr{B}$ must be absent from graph $\mathscr{A}$ ($d_k < P_k$ will mean that the number of K-type SDU in graph $\mathscr{A}$ under analysis is insufficient for constructing the graph $\mathscr{B}$).

If the above condition does not indicate the absence of subgraph $\mathscr{B}$, the following procedure must be applied. Since the inequality of $d_k \geqslant P_k$ ($P_k > 0$) is valid for every $T_k \epsilon D$, all combinations of d_i vertices P_i at a time ($C_{d_i}^{P_i} = C_i$), of d_j vertices P_j at a time ($C_{d_j}^{P_j} = C_j$), etc. are listed, and an arbitrary choice is made of one combination from C_i, one from C_j, etc. The chosen combinations are united in one set S_u of vertices; obviously, as $\Sigma_K P_k = P$, the number of vertices in set S_u equals the number of SDU in the subgraph $\mathscr{B}$. In accordance with the number of possible methods for forming set S_u, the symbol u will run through the values of 1 to M, where $M = C_i C_j \ldots = \Pi_k C_k$ ($k = i,j$; the k values taken are such that $P_k > 0$).

Then a recursive procedure R is applied to the family of sets S_u ($u = 1 \ldots M$): $S_u = \mathrm{R}\, S_{u-1}$. The procedure R consists of successive ranked examination of all sets S_u differing at least in one element. At each step, the rows and columns of matrix D are chosen according to the meanings of the elements of set S_u (the numbers designating the vertices). These rows and columns are used to form a matrix D_u. The examination of all the S_u sets may be interpreted by operation of a counter, the j-th place of which has C_j positions. Conditionally, the subsets $C_{d_i}^{P_i}$, $C_{d_j}^{P_j}$, . . . are arranged in some order (e.g. in increasing order of the numbers in the incidence matrix rows; these numbers correspond to a given type of SDU). Thus, successive use of the R procedure initially generates all combinations of the $C_{d_i}^{P_i}$ subset elements, P_i at a time, and $S_u = \mathrm{R}\, S_{u-1}$ is formed; then after C_i steps, a subsequent combination of the $C_{d_j}^{P_j}$ is generated, with another S_u being formed; this is followed by repeated generation of all $C_{d_i}^{P_i}$, and so on, until all the counter places are

filled up, i.e. until the sets S_u differing in at least one element have been examined. It is clear that the number of possible procedures R is equal to M.

The matrix D_u obtained at the u-th step is checked for connectivity by the method described above. If matrix D_u proves to be connected, all the matrices D_{ul} ($l = 1 \ldots P_i! P_j! \ldots$) are then generated, each matrix resulting from the permutation of rows pertinent to one of the SDU types in matrix D_u. For each matrix D_{ul}, K_{rul} values are found. If for a given value l, $K_{rul} = K_{rB}$, then the graph $\mathscr{A}$ under analysis does contain a subgraph $\mathscr{B}$. Otherwise, if $K_{rul} \neq K_{rB}$ for all the l values, a further step of the recursive procedure R is required. The total number of row permutations for all the D_u formed, which is needed to confirm the absence of a $\mathscr{B}$ subgraph in graph $\mathscr{A}$, is equal to $M \cdot L$, where $M = \Pi_k C_k$ and $L = \Pi_k P_k!$ From this,

$$M \cdot L = \prod_k (C_k P_k!) = \prod_k \frac{d_k!}{(d_k - P_k)!} = \prod_k A_{d_k}^{P_k}$$

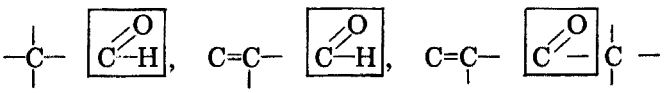
where $A_{d_k}^{P_k}$ is the number of arrangements of d_k vertices, P_k at a time.

The algorithm described above is very general and mathematically rigorous. Though it contains a substantial number of computational operations, the computer time required is negligible compared to the time needed to check the incidence matrices for canonicity during the generation of structures with predetermined topological properties. In the STREC system, the algorithm is realized for not more than 5 SDU (subgraph vertices). The overwhelming majority of fragments contained in the correlation tables [35, 36, 41] meet this requirement. Moreover, to describe dynamic interactions in molecules, it is sufficient, in practically all cases, to consider structural units containing no more than 5 atoms. Therefore, the use of the algorithm described does not offer significant difficulties.

LIBRARY OF STANDARD FRAGMENTS

The libraries of standard fragments for each spectral type have been composed from literature data. The i.r. library of standard fragments is composed of structural units taken from correlation tables [35, 36, 41]. The fragments are distributed between two files. The basic file receives fragments which possess sufficiently stable and highly informative characteristic spectral features, such as —C—CO—C—, —CH_2OH, —C≡CH, H_2C=CH—C≡, etc. These are used to form, automatically, logical equations of structural-group analysis, and as a filter for the examination of hypothetical structures in order to detect contradictory features. The fragments of the auxiliary file participate only in the structure filtering. Two kinds of fragment are incorporated in this file: (a) those having only a small number of relatively uninformative features (—C—O—C—,  , etc); and (b) major functional groups without the indication of near environment, but with broad frequency intervals (C=O, 1640—2300 cm^{-1}; OH, 3100—3650 cm^{-1}, etc.).

To identify accurately a class of chemical compounds which has its own fragment within the library of standard fragments (each fragment belongs to one class), a nucleus which includes the integral part of the given structural unit, is selected from the fragment structure; the neighbouring atoms with a given multiplicity of their valence bonds are also separated, e.g.



Empirical formulae for the nucleus and the environment are indicated separately. Environmental requirements are defined uniquely by specifying a list of forbidden fragments (LFF) and a list of obligatory fragments (LOF) which accompany the given nucleus.

The LOF enumerates the possible permissible variants of the nucleus environment which do not cause a change in any of the characteristic frequency intervals belonging to the nucleus. The LFF imposes necessary bans; e.g. if the fragment is a disubstituted benzene ring, addition of hydrogen atoms to the ring is forbidden.

The structure of fragments is represented by their incidence matrices. Thus, the fragment C—CH—OH (secondary alcohol) is characterized by the
C
nucleus incidence matrix (a) and matrix (b) from the LOF pertaining to the fragment:

01		1			1
03	1				1
		2			

(a)

01		1	1	1	1
01	1				
01	1				
03	1				1

(b)

Here carbon atoms are designated by 01, and oxygen atoms by 03, the last column indicates the number of hydrogen atoms added to the corresponding SDU. SDU can be either skeleton atoms or fragments, provided that their valence is given. The fifth row of the nucleus incidence matrix contains elements the values of which are equal to the number of valences not utilized by the SDU in the nucleus (in this example, the nucleus carbon has two free valences). If a fragment contains non-equivalent atoms, this is fixed by inserting several matrices corresponding to the nucleus. Thus, in the fragment C(O)—CH, the carbon atoms are not equivalent. Therefore, an epoxy group is described by two matrices:

01		1	1			1
01	1		1			
03	1	1				
	1	2				

and

01		1	1			
01	1		1			1
03	1	1				
	2	1				

Because of the LOF and LFF built into the system, certain groups, such as $-CH_3$, $C(CH_3)_2$, and $C(CH_3)_3$ can be confidently distinguished as independent. The system is capable of handling the notion "benzene ring", without the latter being related to any specific type of substitution.

The vibrational spectrum is introduced into the computer as a sequence of experimental frequencies.

In forming the library of standard n.m.r. fragments, data on the chemical shift intervals for protons [17, 36, 41] were used. Along with the chemical shift intervals, the expected multiplicities *M* were indicated on the assumption that they correspond to separations characteristic of first-order spectra. In cases when confident prediction of the multiplicity was impossible, constraints by *M* were not imposed. The experimental n.m.r. spectra were expressed in terms of chemical shift values and corresponding multiplicities. When reliable determination of the value *M* was impossible, only boundaries within which the complex signal occurred were specified. With the algorithm, the presence of a fragment in the structure was regarded as confirmed if the chemical shift in the spectrum fell within the characteristic interval of the fragment, provided that there was no contradiction from the *M* values.

For u.v. spectra the positions of the band maxima were used. Wavelength intervals characteristic of the fragments were taken from the literature [36, 41].

The library of m.s. fragments was formed on the basis of correlation tables listing characteristic mass numbers [42]. Each *m/e* value [42] was provided with a corresponding set of probable fragments. Low-resolution m.s. was introduced as a sequence of the most intensive peaks (not more than 20).

The list of forbidden fragments (LFF) forms a separate file in the library, and is common to the whole STREC system; LFF is analogous to BADLIST [13]. As indicated above, the LFF includes unstable atom groups, and those not found naturally. At the operator's option, all the available chemical information may be used in solving any specific problem. This can be achieved by entering additional atomic groups in the common LFF and indicating a list of obligatory fragments (LOF). Additional constraints may be introduced during data preparation and during the operation of the STREC program, particularly after output of a logical equations solution.

The library of reference information is based on the principle of the library of standard fragments. It contains the functional groups commonly encountered, as well as chemical reactions for their detection [43] and the geometrical, force, and electro-optical parameters characteristic of standard fragments of organic molecules [39, 41, 44].

The STREC system library consists of tape or disk files, each of which is called successively to the computer memory. The library can therefore contain practically any number of fragments together with related data. During tests of the STREC system, the sizes of the library of standard fragments were: i.r. = 80, n.m.r. = 150, m.s. = 270, u.v. = 20 fragments.

RESULTS

To estimate the efficiency of the STREC spectroscopic recognition of molecular structures, more than 150 spectral problems taken from manuals and spectral atlases were solved by operating the system under various conditions. Molecules containing 4—16 skeleton atoms served as samples. During the first tests, the ability of the system to solve problems when only a vibrational spectrum was available was checked. Table 1 presents examples showing the number of solutions obtained in identifying various compounds from their i.r. spectra. Table 2 provides data on the structure of isomers which could not be distinguished by the system from their characteristic i.r. frequencies. In most cases, analysis of i.r. spectra provides a small number of isomers, and these almost always contain the required structure. Incorrect answers were given when the spectrum contained features characteristic of fragments absent from the sample; e.g. a doublet at 1380 cm^{-1} was accepted by STREC as evidence for an isopropyl or t-butyl group. Sometimes the block of logical equation solutions did not yield fragment sets, either because the empirical formula was overfilled or because the spectrum did not contain any characteristic frequencies in the basic i.r. file. In such situations the program took an alternative decision: when the number of skeleton atoms in the empirical formula did not exceed 7 or 8, the computer began automatically to synthesize all the isomers of a given composition, screening them during the filtering. When the number of skeleton atoms in the empirical formula exceeded 8 the program required additional information to avoid excessive losses of computer time.

Several examples showing the effectiveness of individual blocks of STREC are given below.

Example 1

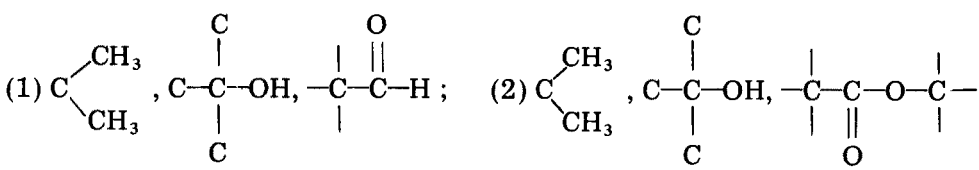
Empirical formula: $C_5H_{10}O_3$

I.r.: 970, 1150, 1200, 1270, 1370, 1380, 1450, 1740, 2980, 3500.

N.m.r.: 1.4 (1)*, 3.7 (1).

M.s.: 27, 28, 29, 30, 31, 32, 33, 38, 39, 40, 41, 42, 43, 44, 45, 58, 59, 61, 118.

Selected sets of fragments:



*The numbers in parentheses designate multiplicities. The symbol (M) shows that the multiplicity was not established.

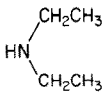
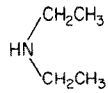
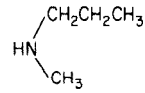
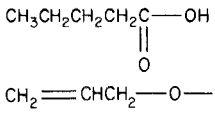
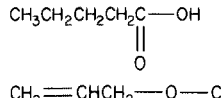
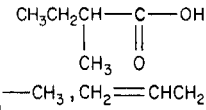
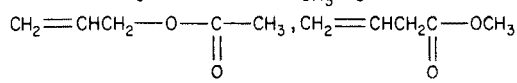
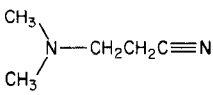
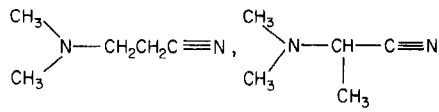
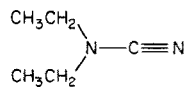
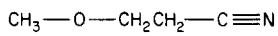
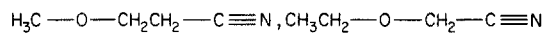
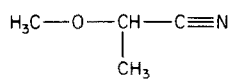
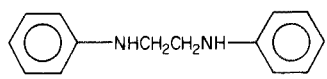
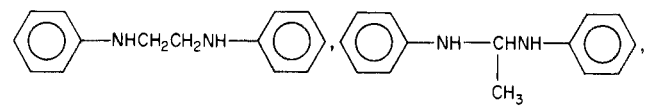
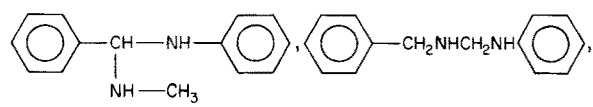
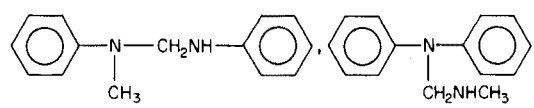
TABLE 1

Results of structure recognition by i.r. spectroscopy

Empirical formula	Compound	Number of solutions	Empirical formula	Compound	Number of solutions
C_2H_7NO	2-Aminoethanol	1	$C_{13}H_{10}$	Fluorene	1
C_6H_{12}	2-Methylpentene-4	1	$C_3H_6O_2$	Glycidol	1
C_7H_8	Toluene	1	$C_8H_4N_2$	Isophthalonitrile	1
$C_4H_{10}O$	Butanol-1	1	$C_8H_4N_2$	Terephthalonitrile	1
$C_4H_{10}O$	t-Butanol	1	$C_2H_5NO_2$	Nitroethane	1
C_3H_6O	Acetone	1	$C_3H_7NO_2$	Nitropropane	1
C_4H_8O	Methyl ethyl ketone	1	$C_4H_{10}O$	Butanol-2	1
C_7H_5NO	Phenyl isocyanate	1	C_7H_5N	Benzonitrile	1
$C_8H_8O_2$	*p*-Methoxybenzaldehyde	1	C_7H_9N	*o*-Methylaniline	1
C_4H_5N	Allyl cyanide	1	$C_3H_6N_2$	Dimethylcyanamide	1
C_3H_6O	Allyl alcohol	1	$C_{12}H_{10}O$	Diphenyl ether	1
$C_9H_{10}O$	Methyl benzyl ketone	1	C_2H_5N	Ethyleneimine	1
C_8H_8O	Acetophenone	1	C_3H_9N	Trimethylamine	1
$C_3H_6O_2$	Propionic acid	1	C_6H_6	Hexadiyne-1,5	1
C_9H_{12}	Isopropylbenzene	1	$C_4H_{11}N$	Diethylamine	2
C_4H_9N	Pyrrolidine	1	$C_4H_8O_2$	Ethyl acetate	2
C_6H_6O	Phenol	1	$C_5H_{10}O_2$	Valerianic acid	2
C_3H_7N	*N*-Methyl-ethyleneimine	1	$C_5H_8O_2$	Allyl acetate	2
C_7H_8O	*m*-Cresol	1	C_6H_{14}	3-Methylpentane	2
C_7H_9N	Benzylamine	1	$C_5H_{10}O_2$	Isovalerianic acid	2
$C_6H_{10}O$	Cyclohexanone	1	C_3H_9NO	3-Aminopropanol-1	2
$C_4H_{11}N$	t-Butylamine	1	$C_5H_{10}O_2$	Acetopropanol	2
$C_3H_6O_3$	1-Methoxyacetic acid	1	$C_4H_{11}N$	n-Butylamine	2
$C_8H_{11}N$	*N,N*-Dimethylaniline	1	C_7H_8O	*p*-Cresol	2
C_8H_{10}	Ethylbenzene	2	C_7H_8O	*o*-Cresol	2
C_8H_{10}	*p*-Xylene	2	$C_3H_4O_2$	Epoxypropionic aldehyde	2
$C_5H_{10}O_2$	Trimethylacetic acid	2	C_6H_{10}	Hexadiene-1,5	2
$C_{11}H_{16}O$	*p*-t-Butyl-anisole	2	C_4H_8O	Tetrahydrofuran	3
$C_6H_6O_2$	Pyrocatechol	2	$C_8H_4N_2$	Phthalonitrile	3
$C_5H_{10}N$	3-Dimethylamino-propionitrile	3	C_8H_7N	*m*-Toluonitrile	3
$C_{12}H_{10}$	1-Phenylhexadiyne-2,4	3	C_6H_{12}	Methylcyclopentane	4
C_4H_9NO	Methoxy-propionitrile	3	$C_4H_8O_2$	1,4-Dioxan	6
$C_4H_4N_2$	Succinonitrile	3	$C_{13}H_{11}NO$	Benzildene-*o*-aminophenol	6
$C_{13}H_{11}N$	Benzilidene aniline	4	$C_{14}H_{16}N_2$	*N,N*-Diphenylethylene-diamine	6
$C_4H_8N_2$	2-(Methyl-amino) propionitrile	4	$C_9H_{12}O_2$	Phenylglycidolic ether	10

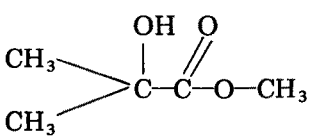
TABLE 2

Examples of isomeric structures found from their i.r. spectra

Structures to be found	Solutions found
	 
	  
	
	
	 
	  

Total structures examined: 116.

After the i.r. filter, 3 structures remained, after the n.m.r. filter 1 structure, and after the m.s. filter 1 structure (which was consistent with the answer):



Example 2

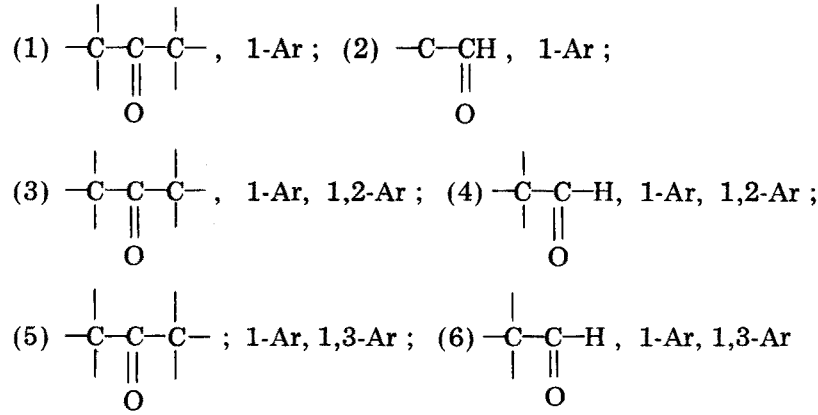
Empirical formula: $C_{15}H_{14}O$

I.r.: 695, 731, 752, 1056, 1120, 1333, 1447, 1500, 1610, 1725, 2970, 3040.

N.m.r.: 3.55 (1), 7.1 (M).

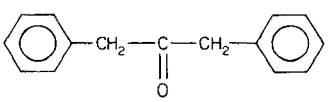
M.s.: 39, 41, 51, 63, 65, 91, 92, 118, 119, 210.

Selected sets of fragments:



Total structures examined: 46.

After the i.r. filter 5 structures remained, after the n.m.r. filter 1 structure, and after the m.s. filter 1 structure (which was consistent with the answer):



Example 3

Empirical formula: C_8H_7N

I.r.: 703, 815, 950, 1022, 1041, 1120, 1180, 1292, 1385, 1456, 1506, 1608, 2230, 2940.

N.m.r.: 2.35 (1), 7.3 (M).

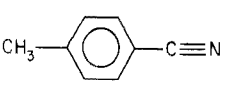
M.s.: 27, 37, 38, 39, 41, 43, 44, 50, 51, 61, 62, 63, 64, 65, 75, 76, 89, 90, 91, 117.

Selected sets of fragments:

(1) —C≡C—, 1,4-Ar; (2) —C≡N, 1,4-Ar

Total structures examined: 5.

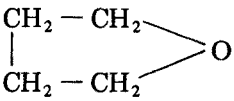
After the i.r. filter 2 structures remained, after the n.m.r. filter 2 structures, and after the m.s. filter 1 structure (which was consistent with the answer):



Example 4

Empirical formula: C_4H_8O
I.r.: 900, 1060, 1170, 1220, 1285, 1365, 1460, 2900.
N.m.r.: 1.79 (3), 3.63 (3).
No fragments were selected.
Total structures examined: 26.

After the i.r. filter 6 structures remained, and after the n.m.r. filter 1 structure (which was consistent with the answer):



Example 5

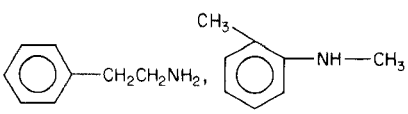
Empirical formula: $C_8H_{11}N$
I.r.: 700, 740, 830, 1030, 1070, 1370, 1435, 1500, 1605, 2960, 3020, 3390.
N.m.r.: 0,9 (1), 2.7 (M), 7.1 (M).
M.s.: 28, 30, 32, 39, 41, 42, 50, 51, 52, 58, 63, 65, 77, 78, 84, 90, 91, 92, 103, 121.
Selected sets of fragments:

(1) >NH, 1-Ar; (2) $-NH_2$, 1-Ar; (3) >NH, 1,2-Ar;

(4) $-NH_2$, 1,2-Ar.

Total structures examined: 16.

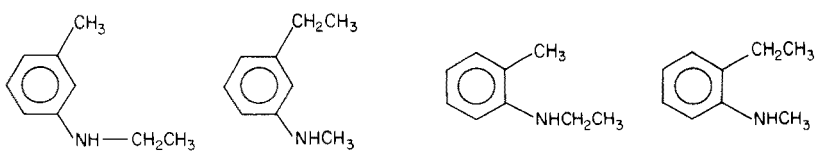
After the i.r. filter 7 structures remained, after the n.m.r. filter 2 structures, and after the m.s. 2 structures (the first structure consistent with the answer):



Example 6

Empirical formula: $C_9H_{13}N$
I.r.: 688, 770, 990, 1170, 1245, 1310, 1370, 1435, 1500, 1600, 2970, 3030, 3390.
N.m.r.: 1.1 (3), 2.2 (1), 2.9—3.1 (M), 6.2—7.1 (M).
M.s.: 39, 51, 52, 53, 65, 70, 77, 91, 120, 135.
Total structures examined: 56.

After the i.r. filter 14 structures remained, after the n.m.r. filter 8 structures, and after the m.s. 4 structures (the first structure consistent with the answer):



It can be seen that application of the recommended methods during the i.r., n.m.r. and m.s. filtering, significantly increases the degree of selectivity of the answer.

As indicated above, when a problem cannot be solved unambiguously, the system provides for ranking the structures according to probability by calculating their vibrational spectra and checking each spectrum calculated for closeness to the experimental one. This approach appears promising, at least for non-bulky molecules [45].

Overall, the results of the STREC system tests are promising. The system should be particularly effective when orientated to solving problems of several special classes. It would then be expedient to use special-purpose libraries of standard fragments, consideration being given to the specific problems of a particular class. The library of standard fragments of the STREC system has been constructed on such principles so that it can easily be adapted to solve new types of problems.

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A MICROCOMPUTER-CONTROLLED TITRATOR FOR AUTOMATED INDIVIDUAL ANALYSIS

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SUMMARY

A titration unit for automatic analysis systems is described. The titrator performs different titrations specified by strings of digital parameters. It has 4 independent titration stations with individual electrode systems. A multiburette with 20 cylinders provides all stations with the necessary reagents. End-point titrations, and incremental and equilibrium titrations are controlled by a microcomputer. In combination with a sample transport and a desk calculator, the titrator can be used to process automatically samples of different natures which require different treatment.

Advances in automating analytical procedures during recent decades have produced a wide variety of automatic analyzers [1—3]. Nevertheless, much of the routine work done in analytical laboratories resists automation because often only a few samples can be treated in exactly the same way. It is often impossible to collect large series of samples which can be processed by automatic or semiautomatic instruments. It is, therefore, of interest, to have automatic instruments capable of treating samples individually, each according to a particular analytical method.

Recently, a new concept of automation in which these features are considered, has been discussed [4]. It is based on the evidence that most analytical methods make use of only a small number of fundamental laboratory operations. Automatic processing units, each performing one or several laboratory operations (such as dilution, extraction or titration), can be grouped along a sample transport. A central control system individually guides samples to the processing units required by the appropriate analytical method. From the central control, each processing unit receives the necessary parameters specifying how the operation has to be performed in the case of the actual sample. An analytical system of this design realizes the concept of automated individual analysis, i.e. the automatic processing of samples requiring different treatment.

The automatic processing units must meet a number of special requirements. The unit must operate under an autonomous and highly flexible control, for at least 8 h without manual intervention. All the reagents needed for the different methods have to be available at the unit. The units must be self-cleaning, so that no cross-contamination can be detected. Finally, every possible irregularity in the process must be recognized in order to prevent the generation of misleading results.

The first processing unit following the concept outlined is presented here: the SR10 System Titrator, which can be used in combination with a sample transport and a desk calculator.

TECHNICAL ASPECTS

The technical aspects of the titrator will be discussed in two parts: (1) aspects which are not specific to the titrator, but valid for any automatic processing unit (APU) of the general system; and (2) specific aspects relevant to an automatic performance of different titration methods.

General features of analytical processing units

Every APU must be able to perform one or several laboratory operations in the various manners specified by the parameters of different analytical methods. Conventional analytical equipment is programmed by adjusting switch positions and turning knobs. In contrast, the APU receives a digital string of parameters from the central system control for each sample to be treated. The APU interprets these parameters and performs the specified operations under autonomous control. Finally, the results generated are sent to the central system control for final calculation and printing of the analysis report.

The APU is controlled by a microcomputer (Fig. 1). Part of the microcomputer firmware, the monitor, manages all the resources (except the hardware specific to the APU), among which is the main interface to the central system control. Through this data communication line, the APU receives the parameter string which is checked by the monitor for character parity and checksum. The monitor responds by sending an acknowledge character.

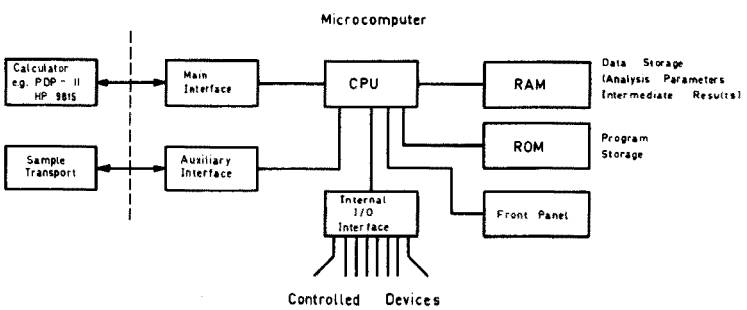


Fig. 1. Block diagram of microcomputer control unit.

With the aid of the auxiliary interface, the monitor coordinates the internal sample carrier of the APU with the system sample transport. A start command signals that the central control has directed a sample to the exchange position of the APU, and the monitor causes the internal sample carrier to transfer the covered sample into the APU, where the cover is lifted from the sample cup. At this moment, control of the APU internal operations is transferred to the APU-specific firmware described in the next section. After completion of the analytical operations, the sample is covered and replaced on the sample transport. This action must be coordinated by the monitor with the central system control, because the sample transport can in the meantime be used for other transport tasks in the system.

The front panel of the APU displays the actual status of the unit, which is generally either BUSY or STANDBY. If an error occurs, a red ERROR is displayed. In addition to this, POWER FAIL signals a previous loss of mains power.

For maintenance purposes, it is necessary to carry out certain operations under local control. A lid in the front of the control unit of the APU gives access to a control panel. There, the mode of operation can be switched from "automatic" to "maintenance", which allows various individual operations to be started manually, the type of operation being defined by the function select switch. The parameters specifying the particular operation are entered by two multiswitches. A blinking light at the front panel, displaying MAINTENANCE, reminds the user of the changed mode of operation.

During any operation, if the microcomputer control of the APU perceives an irregular condition, which necessitates stopping the operation, the error condition is displayed on the front panel, the type of error being specified in an error diagnostics section of the control panel. Depressing the CANCEL key will interrupt any operation or remove an error condition and restore the regular STANDBY condition. Of course, this operation will be successful only in the case of a hardware defect after the source of error has been eliminated. The central system control can also order an APU to cancel an error condition if the APU is in the automatic mode. This enables a system to restart the sample treatment automatically after a power failure. Only the sample being treated in the APU at the moment of the break has to be discarded.

Specific features of the titrator

The titrator is expected to process different samples automatically. It should therefore be capable of adding various reagents to the sample, doing various types of titrations, and cleaning all parts that have come into contact with the sample. For theses purposes, the titrator is equipped with a set of specially developed devices. All these specific hardware components interfaced to the microcomputer (Fig. 2) are controlled by the APU-specific firmware of the microcomputer.

Different titrations may require several measurement systems. By incorporating four independent titration stations, four different electrode systems

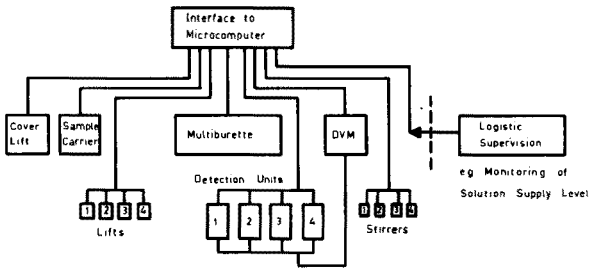


Fig. 2. Block diagram of titrator control.

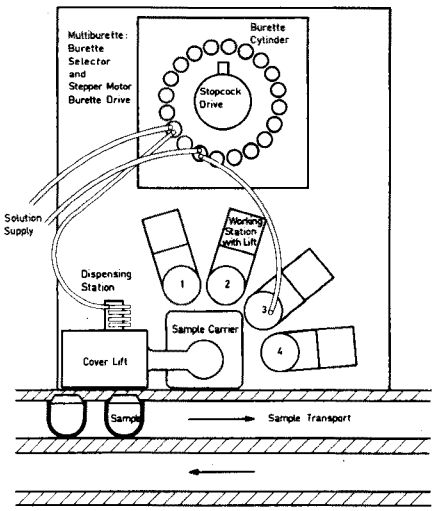
can be made available. This concept also makes it possible to work in different solvent media, so that non-aqueous titrations can be performed. Figure 3 shows the arrangement of the titrator components. A possible configuration would dedicate working stations 1—4 to aqueous acid/base titrations, aqueous redox titrations, aqueous argentimetric titrations and acid/base titrations in methanol (or acetic acid), respectively.

Different solutions are dispensed by a microcomputer-controlled multiburette. The individual burette cylinders can be connected either to a titration station or to a dispensing station, where all samples pass before being transported to the selected titration station. This additional station allows the 20 multiburette channels to be well exploited.

The electrical system for data acquisition. Depending on the analytical method, one of the four electrode systems is selected for a particular titration. This electrode system must be connected to the measuring amplifier which in turn is connected through an A/D converter to the microcomputer.

The resistance of common electrodes can be as high as 10^9 ohm. If the accuracy is to be 0.1% and the maximum error ±1 mV, the input impedance of a measuring amplifier should be above 10^{12} ohm and the input bias current should be less than 10^{-12} A. For many applications, the input should also be floating, to minimize influences from the mains or static charges. The input of an amplifier meeting these demands cannot simply be switched reliably from one electrode to the other. However, four complete measurement systems would be uneconomic. A modular measurement system has therefore been developed which allows the amplifier module to be matched with the specification of the particular electrodes.

Figure 4 shows how, for each of the four electrode systems, a module is inserted between the electrodes and the isolation amplifier. Depending on the impedance of the electrode, a pre-amplifier module reducing the impedance, or a simple switch module, is selected. The switches of the modules are controlled by the microcomputer. With both modules, different grounding systems can be selected which allow any disturbing effects of electrode geometry, solvent medium, etc., to be minimized. For two cases, Fig. 5 shows recommended grounding schemes. In non-aqueous media, static charges are often present; as is usual, the best results are obtained if shielded



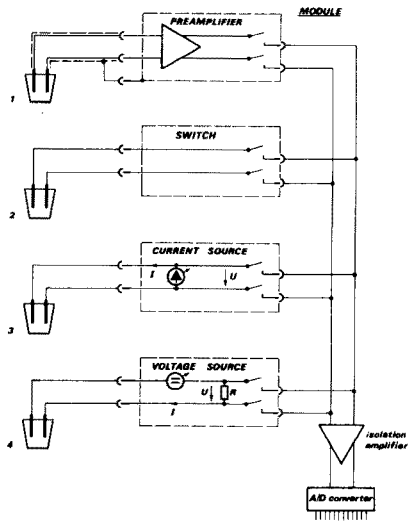


Fig. 3. Arrangement of mechanical units.

Fig. 4. Modular measurement system. Modules are: 1, for high-impedance electrode systems; 2, for low-impedance electrode systems; 3, for potentiometry at constant current; 4, for amperometry.

TABLE 1

Measurement amplifier modules

Module	Specification	Application
Preamplifier	$Z_i = 10^{13}$ ohm	glass electrodes, liquid membrane electrodes, non-aqueous media
Switch	$Z_i = 10^{10}$ ohm	metal electrodes, some solid-state electrodes
Current source	0.1—20 μA	potentiometric titrations at constant current
Voltage source	1—2000 mV	amperometric titrations

cables are used for the electrodes, and if the amplifier is highly symmetrical.

Because of the modular concept, current- and voltage-source modules can replace the amplifier module, and working stations can be used for potentiometric titrations at constant current or amperometric titrations, respectively. For both sources, two independently adjustable levels can be selected under microcomputer control.

Table 1 shows all the measurement amplifier modules developed so far. Obviously, the modular concept can be extended further.

For some analyses, it may be important to record the titration curve for control purposes; this may also help in optimizing parameters when a method

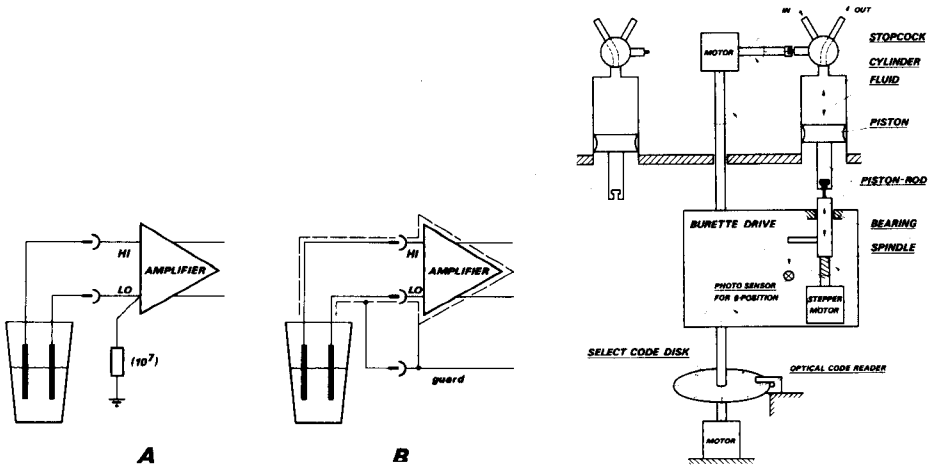


Fig. 5. Grounding schemes for (A) aqueous, (B) non-aqueous media.

Fig. 6. Schematic representation of multiburette functions.

has to be adapted to the system. Therefore a recorder interfaced to the microcomputer can be activated by a parameter in the analytical method. The recorded sample number on the chart allows easy identification. In addition, a large dynamic recording range is available because of the bucking voltage source which is automatically switched in with a hysteresis.

The multiburette. The accuracy of a titrimetric analysis depends — inter alia — on the accuracy with which the titrant can be dispensed. The construction of the burette is therefore of primary importance. For a single burette, the conventional principle of a precision motor burette has been adopted (Fig. 6). A stepping motor directly controlled by the microcomputer drives the burette piston with a resolution of 2000 steps per total volume of the burette. This corresponds to a resolution of 5 or 10 μl for a burette cylinder of 10 or 20 ml, respectively. The burette drive is strong enough to dispense at a maximum rate of one burette volume in 4 s, which is used for rinsing purposes. The burette stopcock is also directly controlled by the microcomputer. The exact positioning is accomplished by a code disk mounted on the axis of the motor and an optical code reader interfaced to the microcomputer.

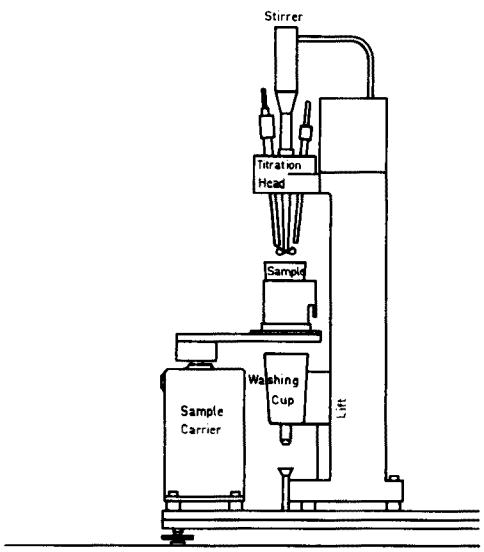
In contrast to an ordinary motor burette, the multiburette can operate up to 20 such burettes with a single piston drive and a single stopcock drive. Depending on the parameters, a particular burette can be selected by the microcomputer control. After the drives of burette and stopcock have been decoupled, they can be rotated by a third motor to any other burette. The positioning is accomplished in the same way as described above for the stopcock (Fig. 6).

The titration station. The sample cup is transported by the sample carrier to one of the four working stations (Fig. 7), where a mechanism lifts the

sample cup to the titration head. The titration head holds a stirrer, one or two electrodes of standard size and a maximum of 7 burette tips. All these items, except an overfill detector, can be rinsed through tiny surrounding channels. This rinsing operation usually eliminates the sample residue on the surfaces to the extent that no cross-contamination is detected*.

Samples requiring different treatment may be analyzed in random sequence. Therefore, perfect cleaning becomes critical. The washing cup protecting the titration head when it is not in use, is useful for this. Solvent or a special washing solution can be dispensed into the cup, and the solution can be vigorously stirred during a selected period, the washing procedure being automatically matched to the last sample processed. The washing cup also serves to store the electrodes in a conditioning solution during times of inactivity. It may be equipped with either a stopper or a valve, so that the cup can be emptied whenever it is lowered to give access for a new sample; it also has an overflow outlet near the top leading to a drain.

The functions available in maintenance mode. Three functions can be carried out in the maintenance mode under local control. First, a burette can be emptied; a changed refill cycle allows air trapped in the burette to be expelled. Secondly, one of the washing cups can be lowered to give access



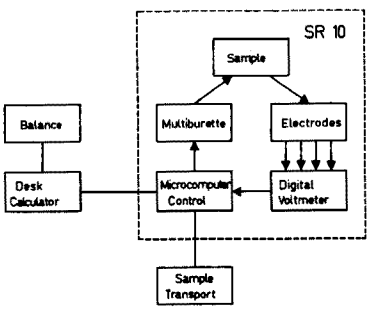


Fig. 7. Working station with lift.

Fig. 8. Block diagram of automatic titration system.

*An aqueous dye solution was used to determine colorimetrically the total residue: 15 μl of the original solution were found after rinsing with 20 ml of water and less than 1 μl after rinsing with 20 ml of methanol or other organic solvents, compared to 400 μl without rinsing.

to the elements of the titration head. Thirdly, one of the measurement systems can be activated, e.g. to check the electrode response on a standard solution.

SEQUENCE OF OPERATIONS FOR SAMPLE TREATMENT

The sample treatment in the titrator is defined by a string of digital parameters which constitutes the analytical method. For every sample, the central system control transmits the appropriate parameters to the titrator. A set of available functions is listed in Table 2, all of which can be called repeatedly. The only limitation to be considered is the maximum volume of 80 ml of liquid in the sample cup when it leaves the unit.

After addition of reagents at the dispensing station, the burettes used at the selected working station (equipped with the necessary electrodes) can be initiated. This means that 2% of the burette volume of the reagent named is expelled into the washing cup and the burette is refilled. This operation ensures that genuine reagent is present in the tube up to the burette tip, and eliminates any adulterations by diffusion, which might occur while the burette tip is immersed in washing, conditioning or another sample solution.

The electrodes, stirrer and burette tips of the selected working station are rinsed into the washing cup before the sample cup is transferred to the titration head. There, the rate of dispensing can be specified for all reagent additions within the range 1—40 ml min^{-1}. A homogenizing period allows dissolution of solid samples, flushing with an inert gas, or awaiting the end of a chemical reaction.

Three different types of titration control are available. If the potential at the equivalence point is known, a fast method is end-point titration, where the rate of dispensing depends on the deviation of the signal from the end-point within a specified band. If the incremental titration is selected, fixed volume increments of titrant are added to the sample at regular intervals. Before addition of a new increment, the potential is read and transmitted to the central system control which will do the approximation calculation for the evaluation of the equivalence volume. An additional parameter can be

TABLE 2

Sequence of sample treatment functions

1. Dispensing at dispensing station
2. Initiation of burettes used at working station
3. Dispensing at working station at a specified rate
4. Homogenizing period with normal or elevated stirring intensity
5. Pre-dosing of titrant at a specified rate
6. End-point titration or incremental titration with or without waiting for a specified equilibrium state between increment addition
7. Dispensing into washing cup
8. Stirring period

used to specify a threshold value which describes a minimum potential change between two increment additions. Once the threshold value has been exceeded, the titration proceeds only until the potential change again decreases. This procedure helps to save time and reagent.

The only difference between the incremental titration and the equilibrium titration is that an equilibrium condition must be attained before the next increment is added. The equilibrium condition is specified by a tolerated potential drift. This titration control gives the most accurate results, for it takes into consideration slow reaction rates and homogenizing problems. For both incremental titrations, it is important to be able to add swiftly a specified initial amount of titrant, in order to reduce the overall time for the analysis.

The titration step can be repeated like the other functions. Back-titrations are therefore possible as well as multiple titrations for multicomponent analyses.

SYSTEM CONFIGURATIONS

The automatic titration unit can be used in systems of various designs. One possible configuration is shown in Fig. 8. The titrator is combined with an automatic sample transport which is used as sample storage. A desk calculator with printer and magnetic tape unit acts as the central control system. A balance for weighing the samples is directly interfaced to the calculator.

The operating system is easy to use by means of the 15 key functions of the calculator. Up to 150 analytical methods can be stored on the magnetic tape. New methods can be entered through a dialogue procedure designed for direct adaptation of established manual titration methods. A method editor allows quick changes of parameters during optimization of a method. For automatic performance of a number of analyses, some information has to be entered for each sample when it is weighed. The method number which specifies the treatment of the particular sample has to be given. A numerical identification will correlate sample, origin of sample and the analytical result. Again, a dialogue procedure has been selected. Weighing limits can be specified in the method, so that the sample weight can be checked and an error message produced if the weight lies outside the limits.

The system can be loaded with up to 96 different samples at a time. These can be processed without attendance, so that the system can be run overnight. The analytical performance is illustrated in Table 3.

For automatic analysis systems having other processing units in addition to the titrator, a more powerful central system control is needed. A minicomputer-controlled system will be the subject of a future paper.

TABLE 3

Some results obtained with the SR10 system titrator

Substance	Titrating solution	Type	Number	$\bar{x}$	s_r [%]	t [n
Sodium carbonate	1 M HCl	Equilibrium	10	99.2	0.2	5
Sodium hydrogencarbonate	1 M HCl	Equilibrium	4	100.35	<0.1	5
Potassium hydrogenphthalate	0.1 M $HClO_4$ in acetic acid	Equilibrium	18	99.98	0.11	3
Sodium chloride	0.1 M $AgNO_3$	Equilibrium	20	100.05	<0.1	5
Benzoic acid	0.1 M TBAH in isopropanol/methanol	Incremental	7	100.20	0.18	5
4-Aminobenzenesulfonic acid	0.1 M $NaNO_2$	End-point with potentiometric indication at constant current	6	100.4	0.2	8

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AUTOMATED DEVELOPMENT OF ANALYTICAL CHEMICAL METHODS
The Determination of Serum Calcium by the Cresolphthalein Complexone Method

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SUMMARY

The cresolphthalein complexone method for serum calcium determination was investigated by means of a modified Technicon Autoanalyzer II under computer control. Simplex optimization of reagent concentrations, followed by response-surface mapping in the region of the optimum produced a method yielding 8.5% greater calcium sensitivity and 15% lower baseline absorbance than the standard method, with comparable insensitivity to interferences, and only a very slight sacrifice in linearity; a comprehensive operational understanding of the chemical system was also obtained.

In the future, many new analytical chemical methods will need to be developed and many old methods must be improved in order to satisfy the requirements of established disciplines as well as to meet the growing demands of newer areas of science and technology. If the experiments associated with the development of analytical methods can be carried out more efficiently, then for a given expenditure of time, manpower, and other resources, these methods can be developed more thoroughly.

The following three stages appear to be fundamental for the complete development of any analytical method: (1) to obtain a response that is related in some way to the substance being determined; (2) to improve the response; and (3) to understand the response.

Obtaining a response

The first stage of development requires that a measurement technique (colorimetry, for example) and a sequence of procedures that constitute the framework of the analytical method, be specified [1]. The efficiency of this stage is highly dependent on the analyst's general chemical background and experience, and can be improved often by increasing the number of persons involved. Once an effective framework has been specified, the desired substance can be determined.

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Many initiating laboratories carry the development of an analytical method through this first stage only [2]. Questions of optimal operating conditions, interferences factor tolerances, and general "ruggedness" are often not fully investigated. Instead, these questions may be answered later by expensive and time-consuming interlaboratory studies: if a method is found to be adequate, it is retained; otherwise it is discarded or improved. The abundance of papers reporting "improved" methods results, in part, from inadequate original development of the methods. It is for these reasons that the second and third stages of methods development assume such great importance in producing good analytical methods.

Improving the response

The improvement of response is often taken to mean increasing the sensitivity of the response with respect to the amount of substance being determined (the determinand [3]). But improvement may also mean decreasing the sensitivity to some interfering substance, decreasing a background contribution, improving the reproducibility, etc. Consideration of these and other possible improvements [4—9] leads to the specification of performance characteristics for "ideal" analytical methods: high accuracy, high reproducibility, low noise, low background, no interferences, good sensitivity to the determinand, low cost, high throughput, etc. Unfortunately, it is usually impossible to obtain "perfect" values for all characteristics simultaneously. For example, improvement of the sensitivity of a method to the determinand is often accompanied by an increase in sensitivity to interferences, and suitable compromises must be reached [10, 11]. An "optimal" analytical method is thus one for which the best compromise of the important characteristics has been achieved.

Understanding the response

Finally, it is important to understand how the responses are affected by the variables associated with the method. This need not be a fundamental understanding of the theoretical chemical and physical processes. An operational understanding of how the responses change when the variables are changed often suffices.

First, such an understanding allows the specification of factor tolerances [8] within which an independent variable (factor) must be controlled if a dependent variable (response) is to change by less than a specified amount. It is important for the widespread success of an analytical method that these tolerances be specified [2]. This factor tolerance, e.g. to pH, should be established by the initiating laboratory and must be reported if large interlaboratory and/or intralaboratory variations in the response are to be avoided.

The second advantage of understanding how the response is affected by variables is realized when the method must be modified. If an operational understanding of the method exists, then knowledgeable decisions can be

made about what changes are possible. In the work reported here, for example, the sensitivity of the method with respect to the determinand could be improved by increasing the concentration of one of the reagents, but the absorbance of the reagent blank also increased. Thus the sensitivity to the determinand cannot be increased and the absorbance of the reagent blank decreased, simply by changing the concentration of this one reagent only.

Given these apparent advantages for the complete, three-stage development of analytical chemical methods, why are not more methods fully developed? One reason is that of economics. The complete development of analytical methods by manual execution of the necessary non-routine experiments and traditional experimental designs has required large amounts of time, manpower, and other resources. The resulting costs are often unjustifiable [10, 11].

Automation of the necessary developmental experiments and the use of more recent experimental designs are two means of making the complete development of analytical methods more efficient. In this paper, the combined use of these two techniques is illustrated for the further development of a determination of calcium in blood serum.

Automation

Over the past decade, papers describing the automated development of analytical methods have appeared at an increasing rate [12—20]. It is now clear that automated instruments capable of performing non-routine, investigative experiments can greatly assist methods development. To be cost effective, such instrumentation must be capable of being used for the development of many methods. Because of the widespread success of continuous-flow analyzers [21, 22] and the potential need for future development of methods for these systems, a Technicon Autoanalyzer II (AA-II) has been modified so that it can be used for the automated development of many continuous-flow methods. The instrument is similar in concept to that previously described by King [23, 24].

Experimental designs

The classical approach to improving and understanding response has been to answer the questions [25]: (1) Does an experimentally measured response depend on certain factors? (2) What equation does the dependence best fit? (3) What are the optimal levels of the important factors? However, if optimization is the desired goal, questions of significant factors and functional relationships are usually of interest only in the region of the optimum [26—28]; excessive experimentation involving conditions outside this region is obviously inefficient.

A more efficient approach to improving response is to answer, in reverse order, essentially the same questions: (1) What are the optimal levels of all factors? (2) In the region of the optimum, what is an equation relating the

response to all the factors? (3) Which factors have the most significant effects on the response in the region of the optimum?

The success of this alternative approach requires efficient optimization to answer the first question. Sequential simplex methods [29, 30] have been shown to be effective multifactor experimental optimization techniques that can rapidly and efficiently locate the region of an optimum [25, 26, 28, 31]. The sequential simplex method [26, 29, 30, 32] was used in this work to find the optimal levels of all factors simultaneously.

The success of this approach also requires an efficient experimental design to investigate the effects of several variables on a response. Such designs are well developed [33]. In general, a full second-order model fit to a three-level experimental design is sufficient to describe adequately the functional relationship between a response and the factors in the region of an optimum [34]; a Box—Behnken design [35] was used in this work to map the region of the optimum.

ANALYTICAL METHOD

The determination of calcium in serum is important in clinical chemistry. Calcium levels significantly less than "normal" are often indicators of hypoparathryoidism, nephritis, or pancreatitis; calcium levels significantly greater than "normal" indicate the possibility of hyperparathyroidism, hypervitaminosis, or multiple myeloma [36, 37]. Because the so-called "normal" range of calcium levels is narrow (8.7—10.7 mg Ca dl^{-1} [36]), the determination of calcium in serum must be both accurate and precise.

The standard AA-II method for determining calcium in blood serum [38] (Fig. 1) was used as the framework for this study. The method involves a 1:9 dilution of the serum with aqueous 0.3 M HCl, dialysis of the calcium into an acidic (0.2 M HCl) aqueous solution of cresolphthalein complexone (CPC, 0.007%), and subsequent adjustment to an alkaline pH by an aqueous solution of diethylamine (DEA, 3.75%) to develop the color of the Ca—CPC complex. Small amounts of 8-hydroxyquinoline (8-HQ, 0.25%) are added both before and after dialysis to minimize interferences from magnesium(II) [39]. Potassium cyanide (KCN, 0.05%) is added to the diethylamine solution to mask heavy metals which might otherwise form a colored complex with CPC, and to prevent deterioration of the DEA solution [38].

Magnesium(II) and protein are the two most serious known interferences in the determination of calcium in serum. The effect of magnesium is presumably caused by co-dialysis with calcium and the subsequent formation of a colored Mg—CPC complex [39]. The effect of protein has been ascribed to binding of calcium to the protein [40], and to a Donnan-type effect [41]. Because the amounts of magnesium and protein vary considerably from sample to sample, their interference also varies and appears as noise or inaccuracy in the determination of calcium [42]. Clearly these interferences must be minimized.

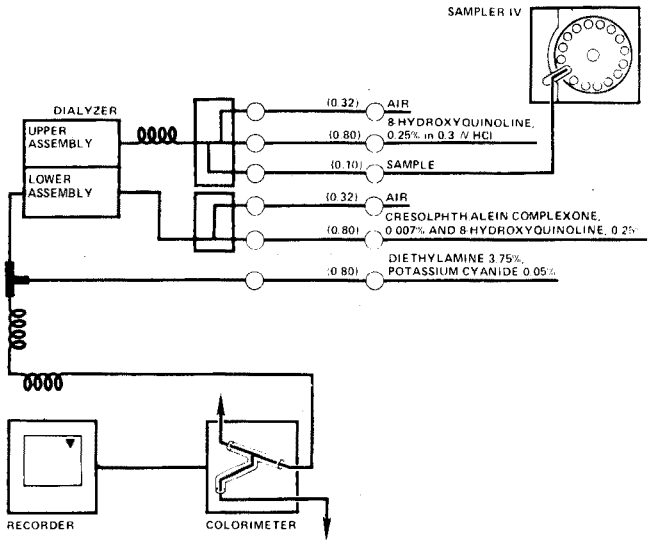

Fig. 1. Flow system for standard AA-II calcium Method No. SE4-0003FJ4. (Courtesy Technicon Instruments Corp.). Figures in parentheses signify flow rates in ml min^{-1}.

Figure 2 is a systems diagram [43] showing many of the important inputs (factors, or independent variables) and outputs (responses, or dependent variables) associated with the determination of calcium in serum.

Inputs to the system are of two general types: controlled and uncontrolled. A controlled factor is one set at a specified level, e.g. in the AA-II calcium method, the reagent levels specified by the standard method. An uncontrolled factor is one that is not set at a specified level, e.g. in serum samples drawn from a human population, the levels of calcium, magnesium, and protein vary more or less randomly. A controlled factor may be set at different specified levels, e.g. in evaluation of the effect of different levels of HCl.

It is also important to realize that factors which may ultimately be uncontrolled in a field method may be controlled in the laboratory. For the method shown in Fig. 2, calcium, magnesium, and protein are necessarily uncontrolled factors in clinical work, but can be controlled in the developmental laboratory.

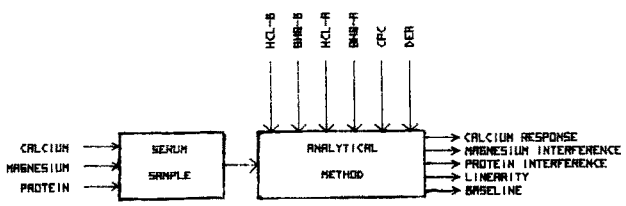

Fig. 2. Systems theory representation of calcium method. HCl—B = HCl before dialysis. 8-HQ—B = 8-HQ before dialysis. HCl—A and 8-HQ—A are the concentrations after dialysis.

The primary output from the system shown in Fig. 2 is the sensitivity of response with respect to calcium. The magnesium and protein interferences have been discussed above. In this colorimetric method for calcium, uncomplexed CPC and other reagents contribute to the absorbance of the reagent blank; to maintain good photometric precision, this baseline absorbance must be kept reasonably low. Finally, a precise, first-order (linear) relationship between absorbance and calcium concentration is desirable.

Objectives

Given the framework for the determination of calcium shown in Fig. 1, it was desired to develop the analytical method further by varying the factors HCl—B, 8-HQ—B, HCl—A, 8-HQ—A, CPC, and DEA so as to maximize the calcium response, minimize the magnesium and protein interferences, maintain the baseline absorbance at an acceptably low level, and maintain good linearity. It was also desired to understand (in an operational sense) how each of the six controlled factors, singly or in combination, affects each of the five responses.

EXPERIMENTAL

Computer

A Hewlett-Packard 9830A minicomputer (Hewlett-Packard, Palo Alto, CA) with approximately 16K bytes of read/write memory was used for experimental control, data acquisition, and data treatment. Read-only-memory (ROM) options included extended input/output (I/O), advanced programming, plotter control, matrix operations, string variables, and mass memory. Peripherals included a 9866A thermal printer, 9862A X-Y plotter, 9867B disc drive, and a 9868A I/O expander. I/O operations were carried out through three modified eight-bit parallel, byte serial 11202A I/O cards. All programs were written in Hewlett-Packard BASIC.

Interface. Signals between the computer and the AA-II system were transmitted via a bidirectional, synchronous serial interface operated at a rate of 250,000 baud. Information was transmitted in the form of time-multiplexed 32-bit words. Sixteen of the bits constituted data, while the other 16 bits were used for device and subdevice addresses and for various synchronization and control functions.

AA-II system

Components comprising the analytical system for serum calcium determination ([38] Fig. 1) include a sampler, proportioning pump, calcium manifold, single-channel colorimeter, voltage stabilizer, and strip-chart recorder. The sampler was operated with a 40 sample/h cam having a 1:1 sample/wash time ratio. A 1.5-mm i.d. flow cell with 15-mm path length was used for the sample stream. No fluid was pumped through the reference cell. Interference filters (570 nm) were employed in both sample and reference beams.

Several major features were added to the basic system to make it as completely automated as possible. To maintain synchronization of the data acquisition routine with the appearance of sample peaks from run to run, a computer-addressable, solid-state relay was used to control power to the sampler. A microswitch (actuated by the sampling cam) was installed to ensure reproducible positioning of the cam whenever the sampler power was switched off. The absorbance signal from the colorimeter was sampled by an instrumentation amplifier (model AD521J, Analog Devices, Cambridge, MA) and sent to a 12-bit analog-to-digital converter (ADC) (model ADC-12QZ, Analog Devices). Conversions were made at 1-s intervals.

With the standard AA-II system, reagent amounts are determined by specifying pump tubing diameters and stock solution concentrations. Each stock solution often contains several reagents and all pump tubes go through a single, fixed-speed peristaltic pump (Fig. 1): variation of individual reagent amounts is difficult with this system. An alternative approach is to use several stock solutions, each of which contains only one reagent, and pump each solution with its own peristaltic pump.

Nine stepper motors (model K82815-P1, North American Phillips, Cheshire, CT), each fitted with a pump head from a peristaltic pump (part number 190-B406-01, Technicon Instruments Corporation) were used to provide for essentially continuous variation of the flow rates of each of the six reagents being investigated. Each of these motors was individually addressable by the computer via the interface. Each motor had its own control circuitry which provided a range of speeds of 0—200 steps/s (0—1 rev/s), adjustable to one part in 4096.

Components of the pre-dialysis and post-dialysis solutions were brought together by means of glass fittings before reaching the calcium manifold (Fig. 3). For each group of reagent pumps, a diluent pump was provided so that concentrations of individual reagents in the mixtures could be varied truly independently of one another, while maintaining the same total flow of each group of reagents at all times.

Flow-rated pump tubing (Technicon) was used in all pumps. For a given size tube, the rated flow(rated for the AA-II proportioning pump) was achieved at approximately one-sixth of full speed on the individual pumps. Relatively small tubes (flow-rated for the proportioning pump at 0.23 ml min^{-1}) were selected for use in this study so that the motors could run at higher speeds to provide both smoother pumping action and a higher degree of resolution for reagent flow rates.

Serum samples (0.10 ml min^{-1}) and air bubbles (0.32 ml min^{-1}) were delivered via the proportioning pump.

Reagents

Concentrations of stock reagents were chosen to be several times greater than those in the standard AA-II reagent mixtures, both to compensate for dilution effects when the stock reagents were combined in the flow streams

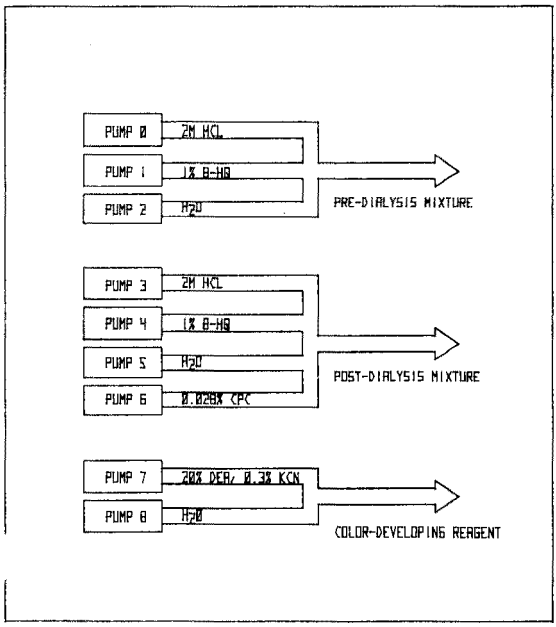

Fig. 3. Reagent addition scheme.

(Fig. 3) and to allow the method to be investigated at reagent concentrations higher than those normally used.

It was originally intended to investigate seven factors, including the effect of KCN in the developing reagent. However, a major function of the KCN appears to be preservation of the DEA solution; a stock DEA reagent prepared without KCN rapidly discolored. Accordingly, the relative concentration of KCN and DEA was fixed by combining them in a single reagent solution.

The following stock solutions were prepared in 9-l quantities and stored in polyethylene bottles: (1) hydrochloric acid (Baker, AR grade), 2 M, to provide additional HCl both before and after dialysis; (2) 8-hydroxyquinoline (Fisher, Certified ACS), 1% in 0.1 M HCl (needed to dissolve the 8-HQ), to provide 8-HQ both before and after dialysis; (3) cresolphthalein complexone (phthalein purple, Fisher, Certified), 0.028% in 0.1 M HCl (needed to dissolve the CPC); (4) diethylamine (Fisher, Reagent Grade), 20% by volume with 0.3% KCN (Fisher, Certified ACS).

Samples

The 20 solutions used as samples were obtained by first preparing 20 intermediate solutions containing specified amounts of calcium, magnesium, and protein, and then mixing these intermediate solutions 1:1 with Scale II serum (Technicon). Table 1 shows the final concentrations of calcium, magnesium, and protein in each of the samples. Each intermediate solution

TABLE 1

Sample composition and representative responses

Order of evaluation[a]	[Ca], mg dl^{-1} [b]	[Mg], mg dl^{-1} [b]	[BSA], g dl^{-1} [b]	Absorbance[c]
1[d]	15.75	3.00	5.80	0.943
2[h]	11.75	7.00	5.80	0.726
3[j]	9.75	1.00	6.80	0.607
4[j]	13.75	1.00	4.80	0.825
5[i]	11.75	3.00	7.80	0.740
6[j]	9.75	5.00	6.80	0.607
7[j]	9.75	1.00	4.80	0.593
8[j]	13.75	1.00	6.80	0.839
9[e]	11.75	3.00	5.80	0.718
10[f]	7.75	3.00	5.80	0.489
11[j]	13.75	5.00	4.80	0.825
12[d]	15.75	3.00	5.80	0.959
13[i]	11.75	3.00	3.80	0.720
14[j]	9.75	5.00	4.80	0.645
15[f]	7.75	3.00	5.80	0.493
16[h]	11.75	9.00	5.80	0.708
17[g]	5.75	3.00	5.80	0.366
18[g]	5.75	3.00	5.80	0.364
19[j]	13.75	5.00	6.80	0.829
20[e]	11.75	3.00	5.80	0.726

[a]In all experiments. [b]Calculated on the basis of average reported values for Scale II serum (11.5 mg Ca dl^{-1}, 2 mg Mg dl^{-1}, 7.6 g protein dl^{-1}); added amounts of Ca, Mg, and protein; and 1:1 mixing. [c]For point 16 of mapping study. [d,e,f,g]Replicate star points in Ca. [h]Star points in Mg. [i]Star points in protein. [j]Factorial points.

(50 ml) was prepared by combining an appropriate number of 5.00-ml aliquots of each of the following stock solutions.

Calcium solution. (40.0 mg dl^{-1} in 0.9% saline). This was prepared from 3.4962 g of dried chelometric standard grade $CaCO_3$ (Fisher, Certified ACS), suspended in ca. 100 ml of water, and dissolved with 12 M HCl. After dilution to the mark in a 1-l volumetric flask with distilled water, the resultant solution was mixed with 2.500 l of water and 31.50 g of NaCl (Fisher, Reagent Grade) and stored in an amber bottle.

Magnesium solution (40.0 mg dl^{-1} in 0.9% saline). This was prepared by dissolving 1.4002 g of dried magnesium turnings (Fisher) with 11 M HCl, and diluting to the mark with distilled water in a 1-l volumetric flask. The solution was poured into an amber bottle containing 2.500 l of water and 31.50 g of NaCl.

Protein solution (20 g dl^{-1} in 0.9% saline). This was prepared by placing 2.25 g of NaCl and 49.1 g of bovine serum albumin (BSA)(Technicon; or, for the comparison study, fraction V, grade B, Calbiochem, La Jolla, CA) in a 250-ml volumetric flask. Water was added slowly as the material dissolved to yield a total volume of 250 ml.

To minimize evaporation effects, 4-ml sample cups were used, each covered with Parafilm with a small hole melted in the top to allow entry of the sampler probe. The entire tray was protected from drafts and light by a plastic cover. A total of three sample trays was used, one during the optimization phase of the study, and one during each of the two blocks of the mapping phase. Each tray was prepared from the same sample solutions. To promote a smoother transition from the end of one tray's use to the beginning of the next, the sample solutions were kept unrefrigerated, even though this allowed some deterioration of the serum components in the samples.

Experimental designs

An "experiment" consisted of running the set of 20 samples (described previously) through the AA-II system under conditions of reagent composition specified by the optimization, mapping, or comparison studies. The designs may be described in terms of both structure within an experiment and structure between experiments. Before discussing these designs, it is useful to consider the requirements necessary for measuring each of the responses.

Responses. Each experiment was designed to allow the evaluation of all five responses shown in Fig. 2. The "baseline" response was simply the absorbance of the reagent blank (compared to the absorbance of water set equal to zero); its evaluation was based on a least-squares fit of reagent blank data acquired 1 min before and 1 min after the series of sample peaks.

The "linearity" response was related to calcium, at fixed magnesium and protein levels, and was expressed as the standard deviation of residual absorbances (a measure of imprecision) about the best least-squares straight line (see Sample design).

The three other responses (Ca, Mg, and protein) are expressed in terms of sensitivity, and are taken to be the slopes of the absorbance vs. [substance] curves: absorbance/(mg Ca dl^{-1}), absorbance/(mg Mg dl^{-1}) and absorbance/(g protein dl^{-1}), respectively. (The variation of protein levels is, on an absolute basis, about 1000 times greater than the variation in Ca and Mg levels so that the sensitivities are, in fact, expressed on a roughly "per normal variation" basis.) Because the specification of a slope and intercept requires two parameters, at least two different levels of calcium, two different levels of magnesium, and two different levels of protein are necessary.

Sample design (Structure within an experiment). The three factors that were varied in this design ([Ca], [Mg], and [protein]) to determine several of the response outputs (Fig. 2) are those which can be controlled in the development but not in the ultimate implementation of the method. Figure 4 illustrates the position of each serum sample listed in Table 1, plotted in the three-factor space of calcium, magnesium, and protein. The points constitute a

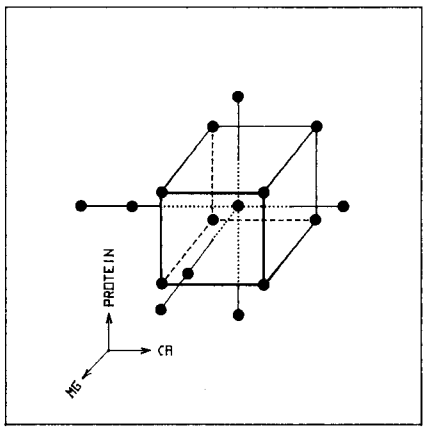

Fig. 4. Composite design for concentrations of calcium (horizontal axis), magnesium (proximal-distal axis), and protein (vertical axis), in the 20 sample solutions.

type of central composite design (a three-factor, two-level factorial design plus additional "star" points) [34] with the factorial design centered on values of 11.75 mg Ca dl^{-1}, 3.00 mg Mg dl^{-1}, and 5.80 g protein dl^{-1}. The design contains six levels of calcium, five levels of magnesium, and five levels of protein.

The eight points for which the levels of magnesium and protein are fixed (at 3.00 mg dl^{-1} and 5.80 g dl^{-1}, respectively) constitute a subset for which the total number of points is 8, the number of treatment combinations is 4, and, if a linear first-order model is applicable,

$$\text{absorbance} = \beta_0 + \beta_1 [\text{Ca}] \quad (1)$$

the number of parameters (β_0 and β_1) is 2. Thus, the standard deviation of residuals (the "linearity" response) is based on 8—2 = 6 degrees of freedom.

If all 20 points are taken as a set, the first-order linear model,

$$\text{absorbance} = \beta_0 + \beta_1 [\text{Ca}] + \beta_2 [\text{Mg}] + \beta_3 [\text{protein}] \quad (2)$$

may be fitted by least-squares methods [44]. Then β_1 is the partial derivative of absorbance with respect to calcium (i.e., the calcium sensitivity), β_2 is the magnesium sensitivity, and β_3 is the protein sensitivity.

Other models containing additional curvature terms, e.g., $\beta_{11} [\text{Ca}]^2$, and/or interaction terms, e.g., $\beta_{12} [\text{Ca}] [\text{Mg}]$, may also be fitted to the data from this design.

Sequential simplex design. The use of sequential simplex designs to obtain improved response has been discussed previously [26, 32]. The six factors that were varied in this design (HCl—B, 8-HQ—B, HCl—A, 8-HQ—A, CPC, and DEA) to optimize the five response outputs (Fig. 2) are those which are controlled, in both the development and the implementation of the method.

Table 2 contains the starting vertex coordinates and the step size in each factor used to generate the initial simplex [29]; large step sizes were chosen to

TABLE 2

Simplex parameters[a]

	HCl—B	8-HQ—B	HCl—A	8-HQ—A	CPC	DEA
Start	500	500	250	250	250	1000
Step	1000	1000	500	500	500	500
Lower boundary	110	110	110	110	110	110
Upper boundary	2320	2320	2290	2290	2290	2360
Summation boundary		2320		2290		2360

[a]Values output from computer to pump drive circuitry. Pump speed = value × 200/4096 steps s^{-1}.

encompass a large volume of the factor space [45]. Upper and lower single-factor boundaries are also shown. Flow rate summation violations were assessed (see Table 2) during the simplex study so that the later mapping study could be centered on the best vertex and have none of the points in the mapping design exceeding the total flow capacity of any reagent supply lines.

The choice of an appropriate objective function for driving any simplex optimization is usually difficult. In this study, it would have been inadequate to improve calcium response only; unacceptable magnesium interference, protein interference, baseline, and linearity might have resulted. To avoid this unacceptable behavior, either (a) permissible limits can be placed on these other responses (response boundary violations can then be used to keep the simplex design within acceptable regions of factor space), or (b) the responses can be incorporated into an objective function. Both possibilities were examined.

The objective function (OF) of responses that was optimized is

$$OF = \beta_1 - |\beta_2| - |\beta_3| \tag{3}$$

where β_1 is the calcium sensitivity (see eqn. 2), $|\beta_2|$ is the absolute value of the magnesium sensitivity, and $|\beta_3|$ is the absolute value of the protein sensitivity. This objective function will be maximum when the calcium sensitivity is greatest and both the magnesium and protein interferences are zero.

Boundary violations in responses were set as follows: if the absolute numerical value of the magnesium interference was greater than 10% of the calcium sensitivity, or if the absolute numerical value of the protein interference was greater than 10% of the calcium sensitivity, or if the absorbance of the baseline was greater than the average absorbance above baseline of the two samples highest in calcium level (15.75 mg dl^{-1}), then a response boundary violation was considered to have occurred.

It was decided in advance to terminate the simplex after 25 vertices.

Mapping study design. The mapping study utilized a Box—Behnken design [35] consisting of 54 treatment combinations (sets of individual reagent pump speeds) shown as coded levels (−1, 0, or +1) in Table 3. The Box—Behnken designs are selectively fractional, three-level factorial designs that efficiently allow the fitting of full second-order linear models. Of the 54 treatment combinations, six points (8, 16, 24, 32, 40, and 48) were replicates of the center point (coded 0, 0, 0, 0, 0, 0). The design was centered at the best simplex vertex and was divided into two orthogonal blocks of 27 experiments each. Sample trays were changed between blocks; thus, the block effect was, among other things, a measure of differences in the sample trays. The replicate center points were run at equal time intervals to assess long-term trends in the system. The time order of all other treatment combinations was randomized within each block.

Comparison study. Interpretation of the results of the mapping study suggested a set of reagent conditions that would produce the best compromise among responses within the various limitations of the chemical and instrumental systems. These conditions corresponded to the coded point HCl—B = 0, 8-HQ—B = +1, HCl—A = +1, 8-HQ—A = 0, CPC = 0, DEA = 0 in the previous mapping study.

A direct comparison of this modified method with the standard AA-II calcium method was made. Three reagent mixtures with the above characteristics were prepared as substitutes for the AA-II reagent mixtures. The composition of these reagent mixtures is shown in Table 4.

Serum samples for the comparison study were prepared with BSA from a source different from that used for the samples in the other studies; the protein response had to be corrected for additional calcium (10.37 mg Ca dl^{-1} determined by atomic absorption) introduced as an impurity in this BSA.

TABLE 3

Coded levels of factors used in mapping study[a]

	−1	0	+1
HCl—B, M	0.370	0.450	0.530
8-HQ—B, %	0.456	0.496	0.536
(HCl, M)[b]	0.046	0.050	0.054
HCl—A, M	0.266	0.346	0.426
8-HQ—A, %	0.133	0.173	0.212
(HCl, M)[b]	0.013	0.017	0.021
CPC, %	0.0018	0.0030	0.0041
(HCl, M)[b]	0.006	0.011	0.015
DEA, %[c]	10.45	11.28	12.10

[a] At point of entry to calcium manifold in flow system.
[b] Add to appropriate level of HCl to obtain actual HCl concentration.
[c] By volume; all other percents by weight.

TABLE 4

Reagent concentrations for comparison study

	Technicon method	Modified method
I. Pre-dialysis reagent:		
(1) 8-HQ, %	0.25	0.536
(2) HCl, M	0.3	0.504
II. Post-dialysis reagent:		
(1) 8-HQ, %	0.25	0.173
(2) HCl, M	0.2	0.454
(3) CPC, %	0.007	0.0030
III. Developing reagent:		
(1) DEA, %[a]	3.75	11.276
(2) KCN, %	0.05	0.169

[a]By volume; all other percents by weight.

The AA-II was set up as shown in Fig. 1 (i.e., all pumping done by the Technicon Proportioning Pump III). The experiments (as defined under Sample Design) were carried out twice with standard Technicon reagent mixtures and twice with the modified reagents.

Software

All computer programs used were on a magnetic disc and could be quickly loaded into the 9830A's memory. A master program determined the proper course of action: (1) begin the simplex study; (2) call the simplex algorithm at the point at which a new vertex was to be calculated; (3) terminate the simplex and call the mapping program; or (4) conclude the study.

The simplex and mapping programs calculated the next set of pump speeds and stored these in a data file. A general data-collection routine was then called by the simplex and mapping programs. This routine set the pump speeds, turned the sampler on and off, and collected and plotted the data. The computer then halted and allowed the experimenter to check for system malfunctions. If a malfunction was noted, the data-collection routine (remaining in memory) could be re-started after the problem was corrected.

The data were then stored. The data analysis program calculated and subtracted the reagent-blank baseline from the data, extracted the peak heights, stored these in a file, and performed regression analysis on the peak heights to determine the calcium, magnesium, and protein sensitivities.

A program was called which computed the linearity of the data and combined the sensitivities and protein effect into an objective function, yielding a response for the set of experimental conditions. This response was stored and the master program again called.

Procedural note. The experimental system was constructed to be as completely automated as possible. Theoretically, the system could have been

started up, and then left entirely alone until the study was completed. However, the possibility of minor flow system problems (leaks around fittings, protein precipitation in the sample inlet, etc.), the need to change recorder paper and sample trays at intervals, and the desire to plot out the data continuously at the computer as it was received, made it convenient to place a STOP in the programming after the completion of each "experiment" (each set of 20 samples).

To minimize long-term trends and large-scale changes in the system, sample, and reagent conditions, the system was run continuously (24 h/day) over the four-day period required to complete the study.

RESULTS

Within an experiment

Table 1 contains absorbance values above baseline for each of the 20 serum samples in a representative experiment (point 16 in the mapping study, one of the center point replicates). The parameters of the model expressed

TABLE 5

Regression results of first-order linear model for mapping study point 16

	Parameter estimates		
Parameter[a]	Value	Calculated t[b]	Confidence level, %[b]
β_0	0.0313	1.367	80.9
β_1	0.0576	55.553	100.0
β_2	0.000374	0.252	19.6
β_3	0.001847	0.562	41.8
	Analysis of variance		
Source	Sum of squares	Degrees of freedom	Variance
From regression (corrected for mean)	0.536	3	0.1780
Residuals	0.002762	16	0.0001725
Lack of fit	0.002598	12	0.0002163
Pure experimental uncertainty	0.0001635	4	0.0000411
Total (corrected for mean)	0.536	19	—

Significance of regression:
$F_{3,16}$ = 1032 (100.0%) based on variance of residuals.
$F_{3,4}$ = 4357 (100.0%) based on pure experimental variance.

Significance of lack of fit:
$F_{12,4}$ = 5.29 (94.0%) based on pure experimental variance.

[a]See eqn. (2).
[b]Based on sum of squares of residuals.

by eqn. (2) were fitted to the factor levels shown in Table 1 and the experimentally obtained absorbance values by means of a matrix linear least-squares regression program. Typical results are shown in Table 5. Residual analysis showed no time trends. Parameter values from this analysis of the data were used to form the objective function (eqn. 3).

A measure of linearity with respect to calcium level was obtained by fitting the model expressed by eqn. (1) to serum samples 17, 18, 10, 15, 9, 20, 1, and 12 (calcium replicates, Mg and protein levels constant; see Table 1) and calculating the standard deviation of points about the regression line. Figure 5 shows the regression line fit to the four pairs of replicates from mapping study point 16.

Table 6 lists the results of a regression analysis for mapping study point 16 fit to the full second-order linear model:

$$\text{absorbance} = \beta_0 + \beta_1[\text{Ca}] + \beta_{11}[\text{Ca}]^2 + \beta_2[\text{Mg}] + \beta_{22}[\text{Mg}]^2 + \beta_3[\text{protein}] + \beta_{33}[\text{protein}]^2 + \beta_{12}[\text{Ca}][\text{Mg}] + \beta_{13}[\text{Ca}][\text{protein}] + \beta_{23}[\text{Mg}][\text{protein}] \quad (4)$$

Simplex progress

The progress of the simplex is detailed in Table 7. Points 8, 10, 12, 14, and 17 (not shown in the Table) represented conditions which would have violated the boundaries of one or more of the factors; no experiments were carried out at these points. Points 3 and 7 were re-evaluated in accordance with the $k + 1$ rule of Spendley et al. [29]; the position of these points in the Table indicates the relative time at which they were re-evaluated.

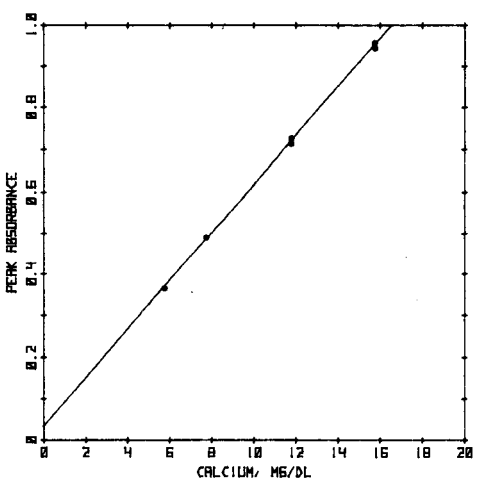

Fig. 5. Linearity plot (regression line) for mapping study point 16 fit to eqn. (1). Standard deviation of residuals = 0.0131 absorbance.

TABLE 6

Regression results of full second-order model for mapping study point 16

Parameter estimates

Parameter[a]	Value	Calculated t[b]	Confidence level, %[b]
β_0	0.0549	0.370	28.1
β_1	0.576	5.048	99.1
β_{11}	−0.000445	1.622	86.4
β_2	0.0533	3.852	99.7
β_{22}	−0.000871	2.179	94.6
β_3	−0.0315	1.008	66.3
β_{33}	0.00121	0.582	42.7
β_{12}	−0.00190	2.347	95.9
β_{13}	0.00265	1.640	86.8
β_{23}	−0.00397	2.458	96.6

Analysis of variance

Source	Sum of squares	Degrees of freedom	Variance
From regression (corrected for mean)	0.536	9	0.0596
Residuals	0.000836	10	0.0000836
Lack of fit	0.000673	6	0.000112
Pure experimental uncertainty	0.000163	4	0.0000409
Total (corrected for mean)	0.536	19	—

Significance of regression:
$F_{9,10}$ = 712 (100.0%) based on variance of residuals.
$F_{9,4}$ = 1457 (100.0%) based on pure experimental variance.

Significance of lack of fit:
$F_{6,4}$ = 2.74 (82.6%) based on pure experimental variance.

[a]See eqn. (4). [b]Based on variance of residuals.

Mapping study

The mapping study was centered about the best simplex vertex, vertex 23, with levels of each factor as shown in Table 3. Table 8 contains the results, sorted by CPC levels, with replicate center points listed at the bottom.

A cell mean is the average response of all data points for which one or more factors is at a specified level. Cell mean plots of all six responses for each of the six factors are shown in Fig. 6. These plots represent the average main effect of each of the factors on each of the responses.

Because of the possibility of interaction among factors, a full second-order linear model was fitted to the mapping study data for each of the responses. The form of the model is

$$\mathrm{RESP} = \alpha_0 + \sum_{i=1}^{6} \alpha_i f_i + \sum_{i=1}^{6} \alpha_{ii} f_i^2 + \sum_{i=1}^{6} \sum_{j=i+1}^{6} \alpha_{ij} f_i f_j \qquad (5)$$

TABLE 7

Simplex progress

Vertex	Factors						Responses					
	HCl—B	8-HQ—B	HCl—A	8-HQ—A	CPC x 100	DEA	OF[a]	Ca[a]	Mg[a]	Protein[a]	Linearity[a,b]	Baseline
1	0.419	0.202	0.221	0.098	0.288	8.26	49.73	60.68	0.67	10.29	14.14	0.455
2	1.144	0.280	0.306	0.137	0.399	9.06	42.88	49.55	1.96	4.69	9.55	0.435
3	0.610	0.566	0.306	0.137	0.399	9.06	53.98	56.23	1.27	−0.99	11.23	0.496
4	0.581	0.280	0.590	0.137	0.399	9.06	48.54	54.75	1.98	4.23	4.89	0.386
5	0.581	0.280	0.320	0.276	0.399	9.06	49.09	50.20	0.53	0.55	5.54	0.514
6	0.581	0.280	0.321	0.137	0.806	9.06	47.91	55.16	3.56	3.72	8.86	0.864
7	0.581	0.280	0.306	0.137	0.399	11.98	54.75	55.90	0.53	0.61	15.57	0.589
9	0.851	0.298	0.325	0.145	0.423	9.23	50.26	54.14	2.45	−1.42	7.40	0.512
11	0.592	0.299	0.333	0.146	0.595	9.25	52.16	56.51	1.40	2.95	6.49	0.692
13	0.593	0.300	0.446	0.147	0.408	9.27	51.74	59.24	1.84	5.66	11.33	0.475
15	0.594	0.302	0.322	0.205	0.409	9.28	50.54	54.35	0.85	2.99	10.58	0.540
16	0.278	0.353	0.320	0.145	0.409	9.79	58.05	68.52	4.57	5.87	7.08	0.603
18	0.664	0.498	0.456	0.207	0.585	11.29	54.45	56.43	0.38	1.60	7.67	0.667
19	0.512	0.463	0.401	0.100	0.523	10.93	54.65	64.28	3.13	6.51	8.31	0.665
20	0.574	0.342	0.342	0.179	0.437	9.70	54.77	57.46	1.19	1.50	10.03	0.576
3[c]	0.610	0.566	0.306	0.137	0.399	9.06	53.52	58.55	1.23	3.78	11.85	0.524
21	0.506	0.479	0.241	0.170	0.534	11.09	50.56	55.46	2.47	2.43	8.86	0.783
22	0.572	0.345	0.395	0.152	0.439	9.72	64.52	74.03	6.33	−3.18	22.71	0.576
7[c]	0.581	0.280	0.306	0.137	0.399	11.98	69.37	73.32	3.76	0.22	14.18	0.582
23	0.501	0.496	0.375	0.173	0.294	11.26	72.12	77.79	2.89	2.77	27.77	0.433
24	0.455	0.594	0.397	0.187	0.145	12.27	30.62	37.88	3.50	3.78	37.42	0.303
25	0.447	0.206	0.425	0.194	0.457	12.19	56.65	63.63	1.09	5.89	5.56	0.617

[a](x 1000). [b]Standard deviation about regression line of eqn. (1). [c]Re-evaluated vertexes.

where RESP is the response being fitted (OF, Ca, Mg, protein, linearity, or baseline), f_i and f_j are the factors exerting an effect (HCl—B, 8-HQ—B, HCl—A, 8-HQ—A, CPC, or DEA) and the α's are parameters of the model. The parameters for each of the responses are listed in Table 9.

Comparison study

Results from the comparison study are given in Table 10.

DISCUSSION

Within an experiment

The use of a composite design to specify levels for the three sample factors, calcium, magnesium, and protein, allowed the calculation of three of the responses which were to be investigated — calcium sensitivity, magnesium interference, and protein interference (β_1, β_2, and β_3 in eqn. 2). The non-zero offset term observed in each experiment (β_0 in eqn. (2) and Table 5, not to be confused with the absorbance baseline) is probably attributable to unknown additional interferences in the serum samples, and/or to the lack of a precise assay of the absolute amount of calcium in the Scale II serum. This offset does not invalidate the estimate of the other parameters.

If mapping study point 16 is taken as a representative example (Tables 1 and 5), replication of four of the treatment combinations allows the significance of the lack of fit of the four-parameter model (eqn. 2) to the data to be assessed; the calculated value of $F_{12,4} = 5.29$ is significant at the 94.0% confidence level. A model containing more parameters might more closely describe absorbance as a function of calcium, magnesium, and protein. It was felt, however, that this simple model (eqn. 2) was adequate to characterize the calcium sensitivity, magnesium interference, and protein interference, and thus could serve as the basis for the objective function that was used to drive the simplex algorithm.

The magnesium and protein interferences (β_2 and β_3 in Table 5) are not highly significant in these 20-sample experiments. This is probably because (a) the effects are small; (b) the ranges of magnesium and protein levels in the sample design were relatively small; (c) there is a finite pure experimental uncertainty in the sample absorbances; and, (d) any curvature in the effects of these factors would show up as an additional contribution to the variance of residuals in the first-order model.

Table 6 presents the results from mapping study point 16 fit to the full second-order model of eqn. (3). The lack of fit of this model to the data is significant only at the 82.6% level of confidence. The calcium sensitivity (β_1) remains highly significant. In this model, the first-order effect of magnesium (β_2) is statistically significant, but the second-order (curvature) effect (β_{22}) is also significant, suggesting that for mapping study point 16, possibility (d) above could be a major reason for the lack of statistical significance of the magnesium effect in the simple first-order model. (It is

TABLE 8

Mapping study results

Evaluation	Factors							Responses				
	CPC	HCl—B	8-HQ—B	HCl—A	8-HQ—A	DEA	OF[a]	Ca[a]	Mg[a]	Protein[a]	Linearity[a,b]	Baseline
3	−1	−1	0	0	−1	0	39.97	48.91	2.02	6.94	15.11	0.36
30	−1	−1	0	0	1	0	35.98	43.47	2.61	4.87	13.53	0.36
10	−1	0	−1	−1	0	0	42.27	45.59	2.20	1.13	14.52	0.38
25	−1	0	−1	0	0	−1	40.53	45.37	2.10	2.73	11.29	0.34
34	−1	0	−1	0	0	1	42.92	45.24	1.56	−0.75	11.12	0.36
37	−1	0	−1	1	0	0	42.92	45.04	0.44	1.68	8.96	0.30
50	−1	0	1	−1	0	0	44.52	46.05	0.89	0.63	6.88	0.34
35	−1	0	1	0	0	−1	43.77	45.37	0.44	1.17	8.74	0.34
26	−1	0	1	0	0	1	42.45	46.05	1.80	1.82	8.52	0.36
21	−1	0	1	1	0	0	42.25	44.74	1.84	0.65	7.32	0.34
39	−1	1	0	0	−1	0	44.15	46.96	2.51	0.30	10.96	0.32
23	−1	1	0	0	1	0	40.01	41.60	0.30	−1.31	7.58	0.36
2	0	−1	−1	0	−1	0	57.06	61.32	0.51	3.74	14.50	0.47
44	0	−1	−1	0	1	0	48.40	57.91	1.58	7.93	8.60	0.45
15	0	−1	0	−1	0	−1	51.13	58.51	2.39	5.00	6.92	0.49
52	0	−1	0	−1	0	1	55.76	57.66	0.97	−0.93	4.75	0.49
42	0	−1	0	1	0	−1	51.45	59.28	1.78	6.03	8.62	0.40
13	0	−1	0	1	0	1	53.94	60.37	3.09	3.34	9.30	0.47
53	0	−1	1	0	−1	0	59.58	61.55	0.51	1.46	7.32	0.45
11	0	−1	1	0	1	0	51.64	55.92	2.59	1.68	9.51	0.49
6	0	0	0	−1	−1	−1	52.24	57.97	1.86	3.88	7.18	0.47
45	0	0	0	−1	−1	1	50.79	57.87	1.01	6.09	8.09	0.49
54	0	0	0	−1	1	−1	52.49	54.04	−0.06	1.48	8.84	0.45
14	0	0	0	−1	1	1	51.70	53.33	0.77	−0.83	8.58	0.51
43	0	0	0	1	−1	−1	58.03	59.48	0.97	−0.45	11.06	0.40
18	0	0	0	1	−1	1	57.28	60.54	1.78	1.46	6.94	0.47
5	0	0	0	1	1	−1	48.04	53.25	1.36	3.84	6.11	0.42
31	0	0	0	1	1	1	51.31	53.60	2.29	0.00	6.98	0.45

36	0	1	−1	0	−1	0	51.76	57.04	3.24	−2.04	8.52	0.47
20	0	1	−1	0	1	0	50.91	53.54	−1.03	1.58	11.06	0.47
49	0	1	0	−1	0	−1	51.05	53.76	1.03	1.66	8.66	0.45
22	0	1	0	−1	0	1	51.56	52.30	0.18	0.53	11.33	0.51
4	0	1	0	1	0	−1	51.09	53.92	1.21	1.62	11.06	0.42
46	0	1	0	1	0	1	51.39	55.48	1.64	2.47	4.89	0.44
1	0	1	1	0	−1	0	54.85	56.98	1.64	−0.49	5.28	0.44
47	0	1	1	0	1	0	50.50	52.04	−0.55	0.99	7.28	0.44
29	1	−1	0	0	−1	0	58.19	63.83	2.51	3.13	5.70	0.61
9	1	−1	0	0	1	0	50.99	60.84	1.46	8.39	8.88	0.59
33	1	0	−1	−1	0	0	53.96	58.55	1.76	2.81	6.01	0.63
38	1	0	−1	0	0	−1	57.64	59.56	0.42	1.52	4.93	0.55
7	1	0	−1	0	0	1	52.51	60.60	2.63	5.44	10.17	0.59
12	1	0	−1	1	0	0	59.20	61.77	1.70	−0.87	10.13	0.55
17	1	0	1	−1	0	0	56.47	58.90	1.34	1.07	10.60	0.63
27	1	0	1	0	0	−1	54.97	59.26	2.89	1.40	6.51	0.57
51	1	0	1	0	0	1	54.73	59.69	1.90	3.05	6.63	0.57
28	1	0	1	1	0	0	57.85	59.95	0.38	−1.72	8.37	0.53
19	1	1	0	0	−1	0	57.30	60.76	1.90	1.58	6.88	0.59
41	1	1	0	0	1	0	52.18	55.50	0.00	3.30	5.24	0.55
8	0	0	0	0	0	0	52.10	56.94	0.67	4.17	7.18	0.45
16	0	0	0	0	0	0	55.40	57.64	0.38	1.84	6.11	0.47
24	0	0	0	0	0	0	54.47	57.04	1.68	0.87	9.08	0.47
32	0	0	0	0	0	0	56.37	56.71	−0.24	0.12	5.83	0.47
40	0	0	0	0	0	0	52.63	57.64	1.92	3.09	4.57	0.44
48	0	0	0	0	0	0	52.30	56.79	0.49	4.00	7.36	0.44

[a](× 1000). [b]Standard deviation about regression line of eqn. (1).

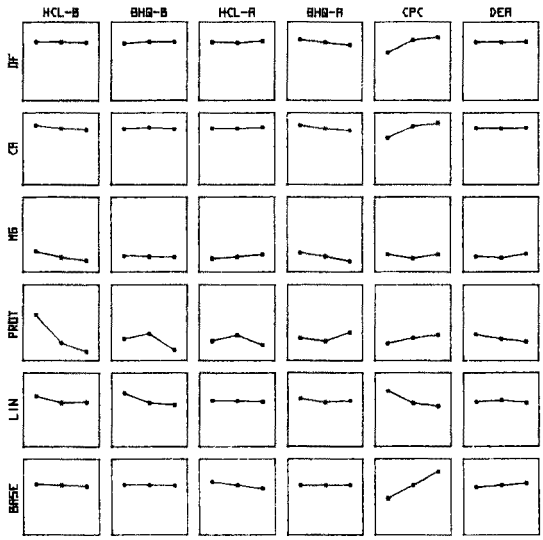

Fig. 6. Cell mean plots of all six responses (rows) for each of the six factors (columns). Points represent responses at the three coded levels (horizontal axis; see Table 3) of each factor. Vertical scaling: OF, 0.000—0.069; Ca, 0.000—0.069 absorbance/(mg dl^{-1}); Mg, 0.0000—0.0069 absorbance/(mg dl^{-1}); protein, 0.0000—0.0069 absorbance/g dl^{-1}); linearity, 0.000—0.014 absorbance; baseline, 0.00—0.69 absorbance.

to be noted that an effect might be statistically significant, yet be so small that it makes a negligible contribution to the overall response.)

The linearity of absorbance with respect to calcium replicates was good in all experiments; Fig. 5 shows the precise reproducibility and lack of serious curvature for a representative experiment.

Simplex study

The simplex progress is presented in Table 7, where an overall trend toward increasing objective function values is seen. During the evaluation of point 7, the manifold sample inlet became blocked by an apparent precipitation of protein. After this and all subsequent experiments, the sample inlet was cleaned to remove any built-up obstructions. Thus, while the responses for the re-evaluation of vertex three are reasonably consistent with the original vertex three responses, the re-evaluation of vertex seven gives much higher responses than the original vertex seven.

Calcium sensitivity is seen to increase throughout the study. There is no clear trend in either the magnesium or protein interferences, but they remain generally low; it appears that the objective function of eqn. (2) and the use of boundary violations in these responses are successful in minimizing the interferences while increasing the calcium sensitivity. The standard deviation of residuals shows some tendency to increase throughout the optimization; this might be due to an increased curvature of absorbance

TABLE 9

Parameters of eqn. (5) relating responses to coded factor levels

Parameter	Response × 1000					
	OF	Ca	Mg	Protein	Linearity	Baseline
α_0	53.879[a]	57.125[a]	0.817[a]	2.350[a]	6.683[a]	462.179[a]
α_1	−0.305	−2.071[a]	−0.415[a]	−1.723[a]	−0.582	−9.379[a]
α_{11}	−1.052	−0.008	0.291	0.595	0.878	−1.784
α_2	0.564	−0.207	−0.061	−0.550	−1.118[a]	−3.200
α_{22}	0.750	0.071	−0.055	−1.147	0.785	−2.255
α_3	0.451	0.540[a]	0.172	−0.186	−0.111	−29.271[a]
α_{33}	0.291	−0.392	0.014	−0.924	0.619	−0.427
α_4	−2.375[a]	−2.427[a]	−0.380[a]	0.263	−0.222	1.019
α_{44}	−0.489	−0.152	0.0008	0.061	0.661	0.091
α_5	6.844[a]	7.283[a]	0.010	0.386	−1.436[a]	119.623[a]
α_{55}	−4.990[a]	−4.229[a]	0.546[a]	0.396	1.009	3.770
α_6	0.162	0.125	0.134	0.340	0.109	18.488[a]
α_{66}	−0.947	−0.320	0.409	0.447	0.008	−0.473
α_{12}	−0.386	0.024	−0.269	1.183	−0.095	−10.232
α_{13}	0.166	−0.014	0.012	−0.425	−1.292	−0.388
α_{14}	0.833	−0.099	−0.831[a]	−0.146	0.099	0.305
α_{15}	−0.993	−0.574[a]	−0.030	0.773	0.957	−0.930
α_{16}	−0.789	−0.016	−0.038	1.042	−0.247	1.121
α_{23}	−0.852	−0.366	0.227	0.045	−0.047	7.979
α_{24}	−0.346	−0.461	0.386	−0.765	0.945	7.024
α_{25}	−0.225	−0.229	0.083	−0.289	0.955	0.247
α_{26}	0.144	0.026	−0.162	0.233	−0.641	−5.839
α_{34}	−2.146[a]	−0.583[a]	0.380	1.521[a]	−0.882	2.453
α_{35}	1.034	0.767[a]	−0.030	−0.884	0.880	−15.311[a]
α_{36}	0.148	0.451[a]	0.360	0.214	−0.617	3.471
α_{45}	−0.520	0.320	−0.164	1.329[a]	0.811	−11.535
α_{46}	0.582	−0.164	0.229	−1.286	0.479	4.270
α_{56}	−0.801	0.115	0.053	1.052	0.720	1.604

[a]Significant at the 95% confidence level.

TABLE 10

Results of comparison study

Method	Response × 1000					
	OF	Ca	Mg	Protein	Linearity	Baseline
Technicon	44.44	50.85	2.08	4.33	4.00	0.475
Technicon	45.83	51.47	1.64	4.00	2.95	0.475
Average	45.14	51.16	1.86	4.17	3.48	0.475
Modified	49.39	55.36	1.27	4.71	7.81	0.398
Modified	47.69	55.66	2.65	5.34	6.84	0.407
Average	48.54	55.51	1.96	5.03	7.33	0.403

with respect to calcium concentration, or it might be due to increased pure experimental uncertainty.

The best value for the objective function was obtained at vertex 23; this was used as the center point of the subsequent mapping study.

Mapping study

Although the objectives of this work did not include obtaining fundamental information about the chemical system, many of the observed main effects (see Fig. 6) are consistent with known chemical behavior.

Calcium response. For the conditions used, increasing the amount of HCl before dialysis tended to cause the calcium sensitivity to decrease. Increasing the amount of HCl after dialysis caused the calcium sensitivity to increase. These effects can be attributed to a dialysis membrane equilibrium involving calcium and hydrogen ions: increasing the acid concentration on the recipient side and decreasing the acid concentration on the donor side favor this transfer of protons and thus increase the rate of calcium dialysis [41].

For the reasons noted in earlier work by Amador and Neely [46], the calcium sensitivity is relatively unaffected by the 8-HQ before dialysis, but is decreased with increasing 8-HQ after dialysis, as the alkaline 8-HQ also complexes the calcium.

The increase in calcium sensitivity with increasing CPC is to be expected, because of the relatively low equilibrium constant for the reaction of calcium with cresolphthalein complexone [47].

The effect of DEA on calcium sensitivity is negligible in the region of the design, presumably because a large enough excess of DEA exists at all levels to make the solution sufficiently basic [47].

Magnesium response. The magnesium interference, shown in Fig. 6 on a scale magnified by a factor of ten compared to calcium sensitivity, shows effects similar to those of calcium for HCl—B, 8-HQ—B, HCl—A, and 8-HQ—A. The effects of CPC and DEA on magnesium response are not well understood; however, from an operational point of view, the 0-level of CPC and DEA appear to give minimal interference from magnesium.

Protein response. The positive protein interference (indicating that as more protein is present, a greater fraction of calcium is dialyzed) has been explained previously by Lott and Herman [41] as being caused by a Donnan equilibrium: protonated, positively-charged, non-dialyzable protein molecules favor the transfer of calcium through the membrane. The observed effect of HCl—B is consistent with this phenomenon.

Baseline response. The two reagents before dialysis (HCl—B and 8-HQ—B) and the 8-HQ after dialysis have little effect on the baseline response.

A major contribution to the baseline absorbance arises from the color of the uncomplexed CPC in alkaline solution [48]; thus, lowering the pH would be expected to lower the baseline. The observations that increased HCl—A and decreased DEA (each contributing to a less basic solution) lower the baseline response are consistent with this explanation.

Increasing the amount of CPC also causes the baseline to increase approximately linearly, as would be expected.

Comparison study

The coded mapping study levels 0, 1, 1, 0, 0, 0 (see Table 3) were chosen as the conditions for the comparison study for the following reasons.

A low (−1) level of HCl—B produces good calcium sensitivity, but the protein interference is relatively large; a high (+1) level produces the opposite effects. A compromise may be achieved if the middle (0) level of HCl—B is used. (The objective function actually shows a slight maximum at this level.)

The 8-HQ—B was arbitrarily set at the +1 level; there are no strong arguments to be made for setting it at any other level.

HCl—A was set at the high (+1) level primarily to reduce the baseline, even though an increase in magnesium interference was to be expected.

The choice of level for 8-HQ—A (0) was a compromise between increased calcium sensitivity and decreased magnesium interference.

CPC trade-offs were more readily apparent: too little CPC greatly decreased the Ca sensitivity; too much CPC increased the baseline absorbance. A middle level (0) of CPC was chosen as an adequate compromise.

Finally, a DEA level of 0 was arbitrarily chosen; the OF is flat with respect to DEA in this region. Some decrease in baseline could have been achieved if less DEA had been used.

From the results presented in Table 10, it can be seen that the modified method gives a higher calcium sensitivity and lower baseline than the Technicon method. The magnesium and protein interferences are approximately the same for the two methods. Although the linearity with respect to calcium is better for the Technicon method than it is for the modified method, the worst-case linearity of 0.00781 absorbance standard deviation corresponds to a relative standard deviation of only 1.4% at a calcium level of 10 mg dl^{-1}.

Conclusions

Automation of methods development and the use of recent experimental designs are two means of making the complete development of analytical chemical methods more efficient. In this work, efficient experimental designs required a total of only 80 experiments: 22 in the optimization study, 54 in the mapping study, and four in the comparison study. The operation of the automated system required only one analyst to prepare samples, reagents, and flow stream before the studies began, and to detect and correct minor malfunctions (e.g., blockage of sample inlet) and carry out a few non-automated procedures (e.g., change sample trays) after the studies had begun.

The modified method is improved in two important aspects (calcium sensitivity and baseline absorbance) and is adequate in others (magnesium interference, protein interference, and linearity). Of even greater importance,

however, is the comprehensive operational understanding of the system that has been easily achieved by automated methods development and efficient experimental designs.

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COMPUTER OPTIMIZATION OF MULTI-COMPONENT SORBENTS IN CHROMATOGRAPHY

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SUMMARY

Development of multi-component sorbent chromatography has been hampered by the difficulty of choice of optimum composition for a given analysis. The authors have previously presented a computer-based strategy for binary mixtures and now describe an extension which allows a choice of optimum mixture composition to be made from up to five sorbent components comprising the stationary phase. The computer program, DIACHOR, is described and shown, in a comparative study, to be superior to the best alternative previously reported in the literature.

Although multi-component solvents have been used to improve a number of separations in gas—liquid chromatography (g.l.c.), the requirement that the choice of solvent compositions has until recently had to be made entirely on an empirical basis, has significantly limited applications. In 1975, a quantitative relation between the infinite dilution solute liquid/gas partition coefficient, K_R, and binary stationary phase mixtures (composed of additive, A, of volume fraction, ϕ_A, in solvent, S, of volume fraction, ϕ_S) was proposed by Purnell and Vargas de Andrade [1, 2]

$$K_R = \phi_A K^0_{R(A)} + \phi_S K^0_{R(S)} \quad (1)$$

where $K^0_{R(A)}$ and $K^0_{R(S)}$ are the infinite dilution solute partition coefficients with pure A and pure S, respectively. Equation (1) was later shown by Laub and Purnell [3, 4] to be obeyed by the several hundred solute—mixed solvent g.l.c. systems described in the literature in sufficient detail for its application and was, consequently, called the law of diachoric solutions [3]. The equation appears to describe the behaviour of a remarkable range of solvent and solute types, including systems where weak complexing appears to occur [4].

The relative retention, α, for a pair of solutes, 1 and 2, with binary stationary phase, A + S, is, following eqn. (1),

$$\alpha = K_{R_2}/K_{R_1} = (\phi_A K^0_{R(A)_2} + \phi_S K^0_{R(S)_2})/(\phi_A K^0_{R(A)_1} + \phi_S K^0_{R(S)_1}) \quad (2)$$

This equation allows the prediction of the separation of solutes as a function of ϕ_A and ϕ_S. The optimum solvent composition of any particular solvent

pair for any solute mixture can therefore be calculated unambiguously, and this has proved highly successful [5, 6]. In practice [5], it has been found simpler to employ the rearranged form (recognizing that $\phi_S = 1 - \phi_A$),

$$\phi_A = (K^0_{R(S)_2} - \alpha K^0_{R(S)_1})/(\alpha \Delta K^0_{R_1} - \Delta K^0_{R_2}) \quad (3)$$

where $\Delta K^0_R = K^0_{R(A)} - K^0_{R(S)}$. Values of ϕ_A are calculated for discrete values of α (1.00, impossible separation; 1.10, trivial separation) and plots of α vs. ϕ_A then take the form of a set of inverted partially-overlapped triangles. Regions in which no overlaps occur are called "windows", the highest of which gives on inspection the largest α value obtainable at the optimum A + S composition. The procedure has been applied to the separation of a wide range of solute and solvent types [5, 7] and has more recently been further developed to deal with mixtures of initially-unknown composition and complexity [8].

Since application of the window diagram method becomes tedious, even with binary solvents, with ten or more solutes, it has been computerized [6, 9]. The logic is not straightforward, however, since one or more of several situations may arise for which the computer calculation of the best α is no longer unambiguous; for example, a window may have a horizontal top such that α is constant over a range of ϕ_A rather than unique at discrete mixtures of A + S.

Equation (1) has recently been shown to apply to three [7] and then to five [10] solvents and can therefore be written more generally as

$$K_R = \Sigma \phi_i K^0_{R(i)} \quad (4)$$

Hand calculation is essentially impossible when three or more solvent components are taken into account. Hence, the computerized optimization of g.l.c. solvent mixtures is now expanded to include eqn. (4), i.e., *n*-component stationary phases.

It is worth emphasizing that the general validity of eqn. (4) is of no real consequence in the current context since, in practice, column components need not be intimately mixed but can be, and preferably would be present as mechanical mixtures. Clearly, then, the method is not limited to g.l.c. but covers g.s.c. and combinations of g.l.c. and g.s.c. as well. Further, it has every prospect of being applicable in all forms of liquid—solid chromatography.

PROGRAM DESCRIPTION

Volume compositions are represented in an optimization program, shown in Fig. 1, as lattice points on a multi-dimensional surface which is bounded by the condition, $\Sigma \phi_i = 1$. Conceivably, the "highest" *n*-dimensional point of intersection of α vs. ϕ_i tie-lines (or surfaces) could be used to determine the most favourable α and the corresponding optimum column composition. For example, the ϕ-surface for a three-component solvent system is an

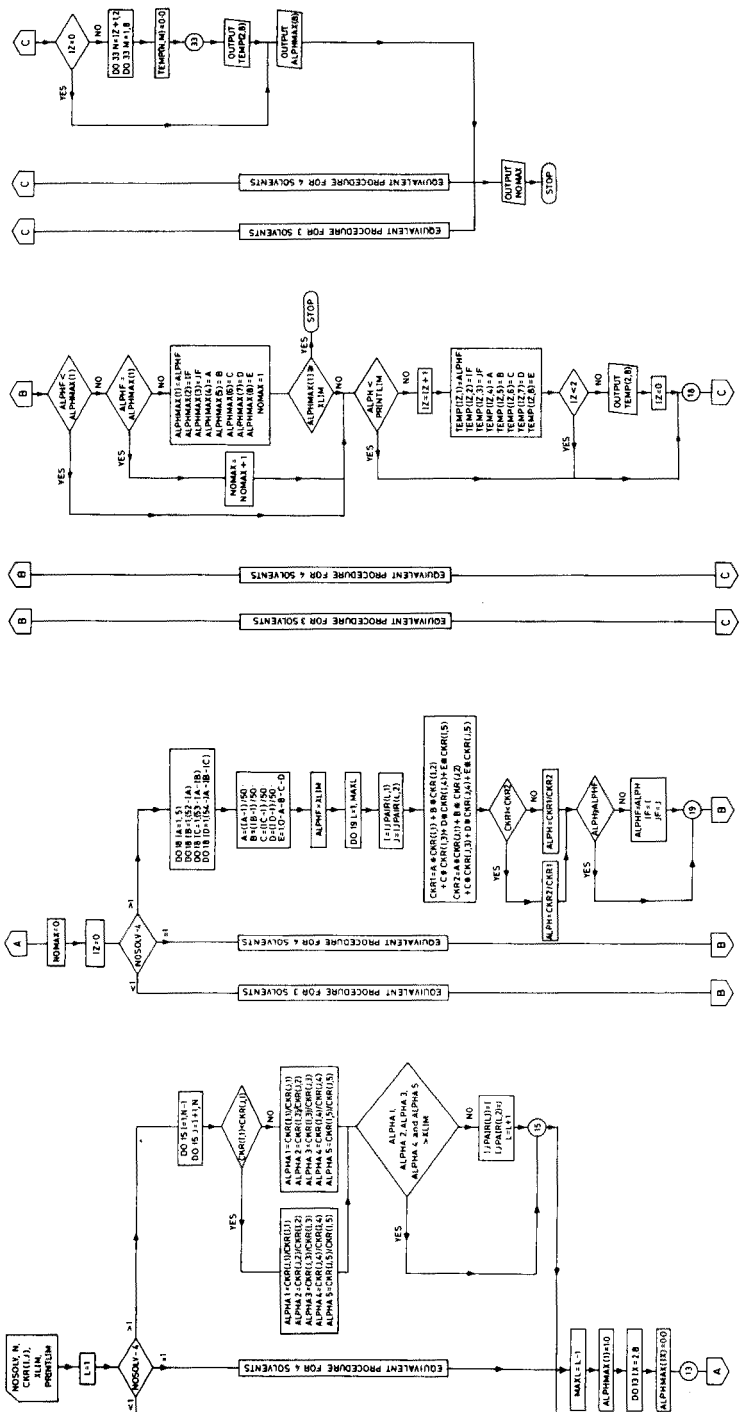

Fig. 1. Flow diagram for program DIACHOR.

equilateral triangle, above which tie-planes of α produce pyramidal windows formed from the intersection of three or more planes and/or the boundaries of the base. In practice, considerable difficulties are encountered in determining which intersections produce precisely the highest window. Moreover, the several complications (such as plateaux) mentioned above for two-component solvents are exacerbated to a considerable degree when three or more liquid phases are considered.

Since both the preparation of a multi-component packing and evaluation of the corresponding $K^0_{R(i)}$ data are unlikely between them to provide data accurate to better than ±2%, an alternative approach to the foregoing has been adopted. Briefly, points of α on the n-dimensional lattice are calculated via eqn. (4) at defined intervals, $\delta\phi_i$, each newly-calculated α value being compared to the largest value previously obtained, the highest being retained. The best α found thus applies to, and is accurate within, an interval of ϕ_i.

Arrays and variables

NOSOLV: the number of solvents to be considered.

N: the number of solutes.

CKR(I,1), ... , CKR(I,5) correspond to $K^0_{R(1)}$, ... , $K^0_{R(5)}$, respectively, where I refers to a particular solute and 1, ... , 5 to a solvent.

XLIM: the maximum value of α which is considered, e.g., 1.10 (solute pairs with $\alpha >$ XLIM for all solvent compositions are ignored). XLIM must, of necessity, be greater than α at the optimum solvent composition.

PRINTLIM: since it is impractical to output all data for every solvent combination considered, PRINTLIM is used to provide that only data corresponding to the pre-defined condition, $\alpha \geqslant$ PRINTLIM, are printed. This aids retrieval of data for solvent mixtures which give α values only marginally less than the finalized optimum (for which the number of solvents may also be less or more).

IJPAIR (L,1), IJPAIR (L,2): the pair(s) of solutes for which $\alpha \leqslant$ XLIM.

ALPHAMAX (8): used to store data corresponding to the optimum solvent mixture.

Main routine

NOSOLV, N, CKR(I,J), XLIM, and PRINTLIM are read. The K^0_R data are searched for all pairs of solutes which have a relevant value of α for a particular combination of solvents and these are stored in IJPAIR (L,1), IJPAIR (L,2). A systematic calculation of α values of the pairs most difficult to separate, using eqns. (2) and (4), is next made (at defined $\delta\phi_i$ intervals) of all possible combinations of solvents. All such combinations which give an α value such that PRINTLIM $\leqslant \alpha <$ XLIM are printed, together with the α value and identification of the corresponding solute pair. Throughout the search, the largest of these α values is stored in ALPHAMAX(8) which is continually updated. At the optimum solvent composition there may, of course, be several pairs of solutes which give the same α value, e.g., if the

optimum is a combination of three solvents, there must necessarily be at least three solute pairs which give the same α (each of which, however, need not be mutually exclusive).

The program has been named DIACHOR.

RESULTS AND DISCUSSION

Choice of $\delta\phi$ interval

The magnitude of the $\delta\phi_i$ interval employed is defined solely on the basis of the computer time available to the user. The number of mixtures, p, available from n solvents divided into m intervals is:

$$p = (m + n - 1)!/m!(n - 1)! \quad (5)$$

Table 1 gives the number of possibilities of solvent mixtures as a function of $\delta\phi_i$. Since K_R (thence α) values must be calculated for each possible solvent combination for all solutes stored in IJPAIR the required number of computations is a multiple of the number of possibilities.

Obviously, use of an interval of 0.01 for five solvents requires a greater amount of computer time than will use of 0.02. Since a difference of as much as 0.01 α units for $\alpha > 1.06$ represents a small difference in the number of theoretical plates required to effect a given separation, and for reasons of accuracy as noted above, there appears to be little to be gained by adopting, as a general case, a $\delta\phi_i$ interval of less than 0.02.

Program test

Hypothetical K_R^0 values used to test the program are listed in Table 2. These have their origin in experiment and so are not atypical of those encountered in chromatography.

Pure solvent A would, for columns of moderate efficiency, show two peaks, the first being solute 2 and the second composed of solutes 1 + 3 + 4 + 5 + 6. Stationary phase B would require a column efficiency of

TABLE 1

Number of combinations of m volume fraction intervals, $\delta\phi_i$, of n solvents

m	$\delta\phi$	n			
		2	3	4	5
1	1.00	2	3	4	5
2	0.50	3	6	10	15
3	0.33	4	10	20	35
4	0.25	5	15	35	70
5	0.20	6	21	56	126
25	0.04	26	351	3276	23,751
50	0.02	51	1378	24,804	341,055
100	0.01	101	5151	176,851	4,598,126

TABLE 2

K_R^0 values for program tests (subscript letters are designated solvents)

Solute No.	$K^0_{R(A)}$	$K^0_{R(B)}$	$K^0_{R(C)}$	$K^0_{R(D)}$	$K^0_{R(E)}$
1	162.1	219.4	126.6	201.9	91.93
2	102.1	138.0	78.37	127.7	93.12
3	158.3	143.9	79.93	124.9	94.22
4	159.7	228.1	125.4	198.8	150.1
5	159.2	221.6	82.96	122.2	90.25
6	164.0	229.2	130.2	120.9	91.04

10,000 plates for partial (4σ) separation of solutes 2 and 3, but would, in this situation resolve solutes 4 and 5 to an unacceptably lesser extent while the pairs, 1 + 5 and 4 + 6, would each appear as single peaks. Stationary phase C would produce similar results: the first peak would comprise solutes 2 + 3 + 5, the second, solutes 1 + 4, followed by a partially resolved third peak, solute 6. Solvent D would be even less useful, giving only two peaks composed of solutes 2 + 3 + 5 + 6 and solutes 1 + 4. Finally, stationary phase E also would yield only two peaks, the first comprising solutes 1 + 2 + 3 + 5 + 6 and the second representing solute 4. Thus, none of the pure solvents will resolve all six solutes satisfactorily except, of course, with effectively infinitely-long columns.

TABLE 3

Calculated best solvent combinations for solutes of Table 2 (order of elution in parenthesis; asterisks designate the most difficult pair to separate in terms of α)

Solvent mixture	$\delta\phi$	α	Solvent composition				
			ϕ_A	ϕ_B	ϕ_C	ϕ_D	ϕ_E
(i)	0.02	1.0949	0.16	0.16	0.22	0.20	0.26
(ii)	0.01	1.0380	0.01	0.65	0.19	—	0.15
(iii)	0.01	1.0365	—	0.67	0.18	0.00	0.15
(iv)	0.01	1.0301	0.00	0.81	0.01	0.18	—
(v)	0.01	1.0294	0.00	0.82	—	0.18	0.00
(vi)	0.01	1.0287	0.09	—	0.10	0.04	0.77

	K_R for solute number					
	1	2	3	4	5	6
(i)	153.2(5)	105.4(1)*	115.4(2)*	168.4(6)	127.1(3)	139.4(4)
(ii)	182.1(4)*	119.6(1)	124.4(2)	196.2(6)	174.9(3)	189.0(5)*
(iii)	183.6(4)	120.5(1)*	124.9(2)*	197.9(6)	176.9(3)	190.7(5)
(iv)	215.3(5)*	135.5(1)	139.8(2)	221.8(6)*	202.3(3)	208.7(4)
(v)	216.3(5)	136.1(1)	140.5(2)	222.8(6)	203.7(3)*	209.7(4)*
(vi)	106.1(5)	93.84(1)	99.79(3)*	150.4(6)	97.00 (2)*	102.7(4)

Table 3 gives the computer-optimized best ($\delta\phi_i = 0.02$) mixed-solvent composition, the respective solute K_R values (with the order of elution in parenthesis), and also the α value of the most difficult pair to be separated. In this instance, a five-component solvent proves best, although this is not generally the case. Table 3 also shows the corresponding data (listed in order of largest α) for the best solvent mixtures of the five possible combinations of four solvents, i.e., A, B, C, E; B, C, D, E; A, B, C, D; A, B, D, E; and A, C, D, E, which, for greater accuracy because of the low α and small difference were computed with $\delta\phi_i = 0.01$. Mixture (ii) requires only a very small amount of stationary phase A which, however, noticeably improves the best value over that obtained with mixture (iii) which does not include A. Similarly, a trace amount of C in mixture (iv) is superior to mixture (v) which omits C. Stationary phase mixture (vi), while containing four components each of non-trivial ϕ, is nevertheless not as useful as (v). It is self-evident that this kind of quantitative reasoning demands computerization.

The program DIACHOR, in conjunction with eqns. (2) and (4), thus allows the computation of vitally useful information from data for pure stationary phases. For the test data, for example, the program indicates that mixture (i) offers, overall, the highest α value obtainable for any combination of these solvents with this particular set of solutes. Further, it can be seen that the use of all five solvents is dramatically superior to any combination of fewer than five. An α value of 1.0949 requires (for k' (the capacity factor) $\geqslant 10$) 4790 theoretical plates for baseline resolution of the solute mixture, while the penultimate value of 1.0380 would need 26,860 plates. Assuming a (not unreasonable) value of 500 plates per foot for packed columns, the α values demand, respectively, columns of 10 ft. and 54 ft. in length.

Alternative programs

Only one alternative approach to the proposed program has been described in the literature [11], a flow diagram of which is given in Fig. 2; this program may be named SORT. Rather than invoke a value of XLIM during a search for significant pairs of solutes, SORT, instead, calculates all K_R values at each solvent composition and stores these in array, PKR(1,2), the first column of which contains the index numbers of the solutes and the second the value of K_R. The first N values of the first column are initialized to $1, 2, 3, \ldots, N$, and the second to zero. The partition coefficients are then calculated and the two columns sorted, where the K_R-column is placed in descending order (a Nottingham Algorithms Group subroutine, M01AFF, a combination of "Quicksort" by C. A. R. Hoare and "Shellsort" by D. L. Shell, was used for sorting). Values of α for each consecutive pair (e.g., solutes 1, 2; 2, 3; 3, 4; etc.) are next calculated and the smallest of these printed, as before, if greater than PRINTLIM. Note that, at a point adjacent to the column composition under consideration, the K_R values will, more often than not, already lie in descending order; sorting may,

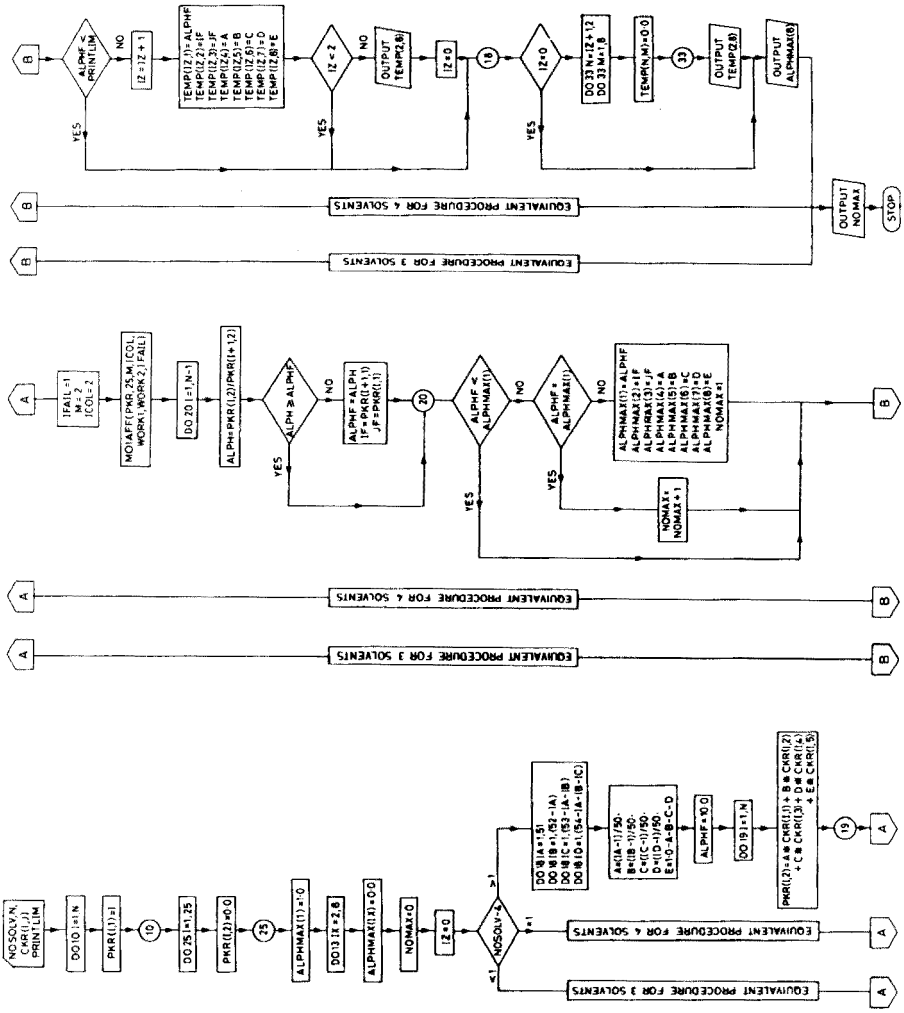

Fig. 2. Flow diagram for program SORT.

TABLE 4

Comparison of computation times for DIACHOR and SORT programs for example of Table 2

Solvent mixture	Computation time (s)		PKR
	DIACHOR	SORT	
(i)	1382	>2000	(15,2)
		1644	(10,2)
		1302	(6,2)
(ii)	661	1379	(15,2)
(iii)	613	1395	(15,2)
(iv)	612	1366	(15,2)
(v)	661	1388	(15,2)
(iv)	661	1395	(15,2)

therefore, be extremely fast since, in such cases, only small alterations to the PKR array will be required.

Table 4 gives the computer times required for DIACHOR and SORT programs to complete the calculations of Table 3. The parameter, PKR, is the limiting factor in the SORT method which may become marginally faster than DIACHOR when PKR is set at the actual number of solutes. However, in practice, the number of solutes in a mixture is frequently not known and often exceeds 15. The computer time needed for SORT for more than 15 solutes with 5 solvents becomes excessive, requiring for example, for fewer than five solvents with PKR set at (15,2), more than double the time of that for DIACHOR.

In terms of computer time and flexibility, therefore, the DIACHOR algorithm described here presents significant advantages for the computer optimization of the use of multi-component stationary phases in gas chromatography. The fact that the method may be used for mixtures of unknown composition and complexity, as shown above, makes it significantly preferable.

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COMPUTER-CONTROLLED PROGRAMMABLE MONOCHROMATOR WITH REPETITIVE OPTICAL SCANNER FOR ACCURATE PEAK DETECTION AND BACKGROUND CORRECTION

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SUMMARY

A 0.5-m grating monochromator is controlled by a minicomputer for multi-element analysis of solutions with a microwave-induced argon plasma as light source. The monochromator slews at a rate of 3.4 nm s^{-1} between wavelengths of analytical interest and remains at a particular wavelength until the measurement is made. A vibrating quartz plate placed behind an entrance slit repetitively scans narrow wavelength regions around the spectral lines of interest. Data acquisition is synchronized with the vibration of the quartz plate so that the profile of each line can be obtained and accurate peak detection and background correction are possible.

With light sources that produce steady signals with time such as flames, microwave plasmas, and inductively coupled plasmas, a multi-element analysis can be performed in a sequential mode of measurement, provided that the amount of sample is not extremely limited. Several authors have described computerized programmable monochromators for multi-element analysis on this principle in atomic fluorescence [1], flame emission [2] and inductively-coupled plasma emission spectrometry [3]. The analytical usefulness of these systems has been discussed with special reference to their high flexibility and low investment.

There has been substantial interest in the use of microwave-induced plasmas for the emission spectrometry of solutions of trace metals [4—10]. Although simultaneous multi-element analysis with these plasmas has been conducted by means of a spectrometer with a vidicon tube detector [11] and a rapid scan spectrometer [12], lack of spectral resolution restricts the application of these systems. Moreover, since they utilize only a small fraction of the signals obtained over a wide range of wavelength, analysis time is used quite inefficiently. These facts prompted the development of a new system.

This paper reports the construction of a programmable monochromator for multi-element analysis of solutions containing up to 20 elements with a low-wattage microwave-induced plasma as light source. The system is based on a computer-controlled, slewed-scan technique and a repetitive

optical scanning over narrow wavelength regions around the spectral lines of interest by means of a vibrating quartz plate. The latter technique permits precise profiles of the lines to be recorded so that the problem of wavelength misalignment is eliminated and an accurate background correction is easily performed. Although this combination of slewing of a grating with wavelength modulation has been suggested by Boumans et al. [3], the details have not been reported previously.

EXPERIMENTAL

Instrumental system

A block diagram of the system is shown in Fig. 1. The wavelength driving motor of a monochromator (Nippon Jarrell-Ash Co., 0.5-m Ebert mounting, reciprocal linear dispersion of 1.6 nm mm^{-1}) was replaced by a stepping motor (Nippon Pulse Motor Co., PF6-36, 72 steps/revolution, maximum rate of 1000 step s^{-1}). The original mechanism of the wavelength drive was used except that the first and second reducing gears were replaced by plastic ones to reduce the inertia and the gearshift mechanism was disengaged. One step of the motor resulted in a wavelength change of 0.0034719 nm. Therefore the maximum slewing rate was 3.4719 nm s^{-1}.

To avoid steps being skipped, a "ramping" procedure in the step rate was employed to start and stop the measure. The time intervals between the steps in the start ramp were calculated sequentially by the following equation:

$$\Delta t_{(n+1)} = [(3n - 2)/3n]\,\Delta t_n, \; (n = 1, 2, \ldots\ldots, 48)$$

which was derived by trial and error so that rapid calculation was possible with a small number of memories. In the stop ramp, the calculation sequence was reversed. With these intervals, the step rate increased almost linearly to, or decreased from, the maximum rate with time within 50 steps and 130 ms. When the wavelength interval was so small that the corresponding step number was less than 130, the motor was driven at a constant step rate of 30 steps/s.

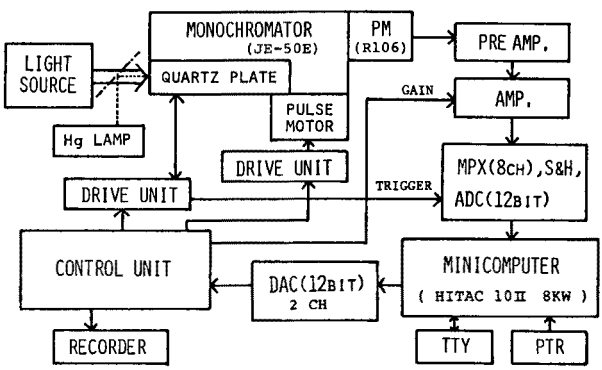

Fig. 1. Block diagram for computer-controlled programmable monochromator.

A quartz vibrating plate described previously [13] was mounted behind the entrance slit of the monochromator to scan a narrow wavelength region. A vibrator and its driving unit were constructed to a design similar to that described by Snellman et al. [14]. With a plate 2 mm thick, a maximum scan width of about 0.3 nm was attained at a vibrating rate of 97 Hz. The defocussing at the exit slit caused by the insertion of the quartz plate in the optical path was compensated by shifting the collimating mirror so that the resolution remained unchanged.

A minicomputer (HITAC 10 II) with 16-bit, 8-kW memories was used to control the stepping motor, via a driving unit (Nippon Pulse Motor Co., PS-3B) and the quartz vibrating plate, and also to process the data from a photomultiplier (HTV, R106). A d.c. amplifier (Sanei Instrument Co., 6L3-1) for the photomultiplier signal was modified to cut off the d.c. component in the signal (i.e., it was used as an a.c. amplifier) and the manual gain control switch of the amplifier was replaced by a relay circuit controlled by the computer. A low-pass filter (1 kHz) was incorporated into the amplifier. A control unit [15] was constructed to interface the output of two D/A converters (Hybrid Systems Co., DAC-372-12) with the driving units of the stepping motor and the quartz plate, strip-chart recorder, etc.

The microwave-induced argon plasma employed has been as described previously [10]. Briefly, a torch-like discharge was induced at the mouth of a quartz tube (1.2-mm i.d., 4.2-mm o.d.) by a 2450-MHz microwave at a power of 100 W, which was coupled to the plasma with a tapered rectangular cavity. The sample solution containing potassium chloride (1 mg KCl ml^{-1}) was introduced into the plasma with an ultrasonic nebulizer (1 MHz) and a desolvation facility.

Software

In trace element analysis, spectral lines to be measured for each element are usually limited to a few lines whose intensities are relatively strong. To simplify the input of the wavelength, a 6-figure wavelength table of 3 spectral lines for each of 71 elements was stored via a punched paper tape. A flow chart of the program for wavelength selection in tape I is given in Fig. 2.

Spectral lines of interest can be selected from the storage simply by typing the alphabetical symbols of the element and the number designated to each of the 3 lines. Lines that are not stored in the file can also be used by typing the elemental symbol, and a number larger than 4, followed by the wavelength (in nm) of interest. The number of the elements to be determined simultaneously is limited to 20 only by the capacity of the memory.

The computer rearranges the retrieved list of the wavelengths in ascending order and calculates the numbers of motor steps necessary to slew the monochromator from a reference line to the selected lines. Retaining the list of the calculated numbers of the steps in storage, the computer loads tape II which contains the programs for wavelength calibration, slewing of the monochromator, data acquisition, and data processing.

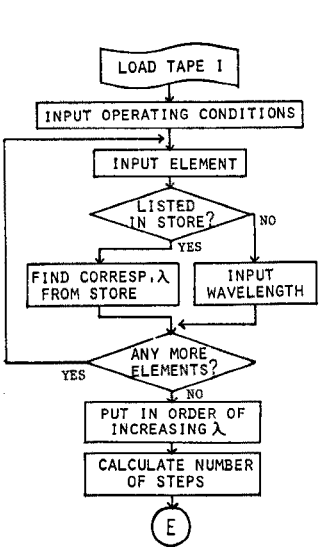

Fig. 2. Flow chart of program for wavelength selection.

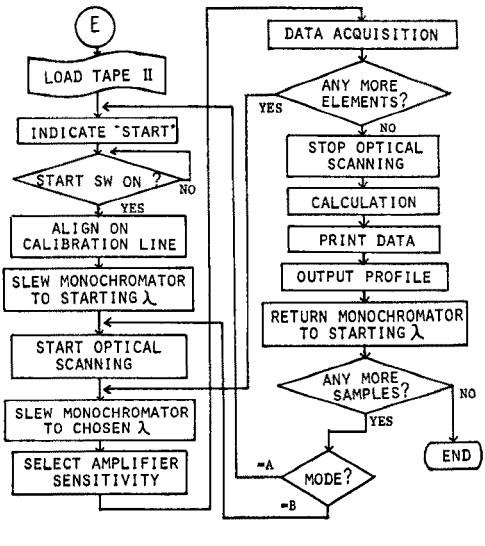

Fig. 3. Flow chart of program for wavelength calibration of monochromator and data acquisition.

A flow chart of the program in tape II is shown in Fig. 3. A mercury line, Hg 253.652 nm, is normally used as the reference line for wavelength calibration of the monochromator, though any line can be chosen depending on available spectral lamps. A mercury lamp is positioned in the optical path with a mirror, the wavelength of the monochromator is set manually at a few tenths of a nm lower than that of the reference line, and the computer is started to find the exact peak position of the reference line, measuring its intensity and slewing the monochromator step by step. Then the monochromator is automatically slewed to a starting wavelength which is lower by a few nm than the lowest wavelength of the lines to be measured. Thus, all the data acquisition is performed with the monochromator slewing in the same direction of ascending order. This procedure overcomes the effect of the backlash of the wavelength drive mechanism.

The computer starts the vibration of the quartz plate and slews the monochromator to the first line. The amplifier is automatically set to a maximum gain at which signals do not exceed the maximum input of an A/D converter (Hybrid Systems Co., ADC-591-12A). Data acquisition is done by synchronizing with the vibration of the quartz plate. In each half cycle of the vibration, 91 data are acquired and stored in independent locations in the storage with double-precision words. The data acquired during the backward scan are also added to each corresponding location of the forward scan. Thus, a spectral region (0.1—0.3 nm) optically scanned by the quartz plate is digitized in 91 channels. This number is determined by both the frequency

(97 Hz) of the vibration and the time required for the single data acquisition (55 μs).

When the predetermined number of scans has been completed, the monochromator is slewed to the next line and the procedure is repeated, with storage of the data in new locations. Two thousand scans are completed in about 10 s. When the final line has been served, the program finds the peak channel in each of the spectral data in the storage. The background intensity in each spectrum is calculated as the mean value of 8 channels of the background, i.e., 4 channels at one end of the spectrum and 4 channels at the other end. The peak intensities corrected for both the background data and amplifier sensitivity, are output with the corresponding elemental symbols on the Teletype.

Then the spectral data are output via a D/A converter on a strip-chart recorder or a monitorscope to visualize the profiles of the spectral lines. These profiles enable one not only to ascertain the normal operation of the instruments but also to find any casual interference line. As the wavelength is modulated in a sine function by the vibrating plate, the time intervals of the data ouput in each spectrum are programmed to be a sine function, so that the time scale of the profiles corresponds linearly to the wavelength.

After all the data have been output, the computer slews the monochromator to the starting wavelength and waits for the next command. When "A" is input from the keyboard, a new scanning cycle is immediately started without wavelength calibration, and when "B" is input, the program begins with the wavelength calibration. If the operator wants to modify parameters including the list of elements, the program tapes have to be reloaded.

RESULTS AND DISCUSSION

A mercury hollow-cathode lamp was used as light source to test the operation and to evaluate the potential value of the system. The spectral profiles obtained simultaneously for 9 mercury and 2 neon lines are shown in Fig. 4. The term "simultaneous" as used here refers to the total measurements in a single run. The gain of the drive unit of the quartz plate was pre-set for each spectral line to be scanned over 0.13 nm. The spectral intensities stored in the memories are multiplied by a scaling factor, computed for each profile, to display the profiles in full scale of the strip chart recorder.

Precise information about the entire line profile and its vicinity is obtained from the chart so that the reliability of automatic background correction and spectral interferences from partly overlapping lines can be easily checked. A mercury doublet, Hg 313.155 and Hg 313.183 nm, shows the resolution of the spectrum in this system when the slit width of the monochromator is 10 μm (spectral band width, 0.016 nm). With this resolution, interferences from adjacent lines can be avoided in many cases of trace element analysis.

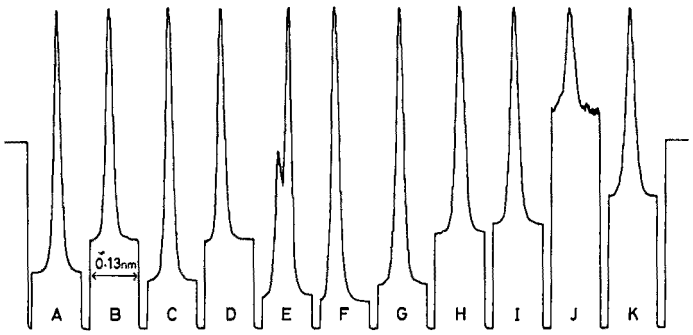

Fig. 4. Spectral profiles for 9 mercury and 2 neon lines obtained by using a hollow-cathode lamp. Wavelength in nm: (A) Hg 253.652, (B) Hg 275.278, (C) Hg 289.360, (D) Hg 302.150, (E) Hg 313.155 and 313.183, (F) Ne 341.790, (G) Hg 365.015, (H) Hg 404.656, (I) Hg 435.835, (J) Ne 471.534 and (K) Hg 546.075.

Although small shifts of the peak position from the center of each band are observed for several lines in Fig. 4, they do not affect the accuracy of measurement of peak intensities, because the computer always finds the peak value in each spectral band. The errors in wavelength setting were measured from profiles for several runs of the operation, with the results shown in Fig. 5. In all runs, the wavelength of the monochromator was calibrated with the Hg 253.6-nm line. The error curves between 250 and 550 nm show a rather complex pattern having a maximum at 302.2 nm. However, none of the curves exceeds ±0.02 nm from the pre-set wavelengths.

The pattern of the error curves seems to be caused by the error of the precision screw of the monochromator. The scatter of the error at each wavelength is considered to be due to the loose contact between the sine bar pivot arm and the nut on the precision screw. These errors cannot be easily eliminated even by rigorous modification of the wavelength drive

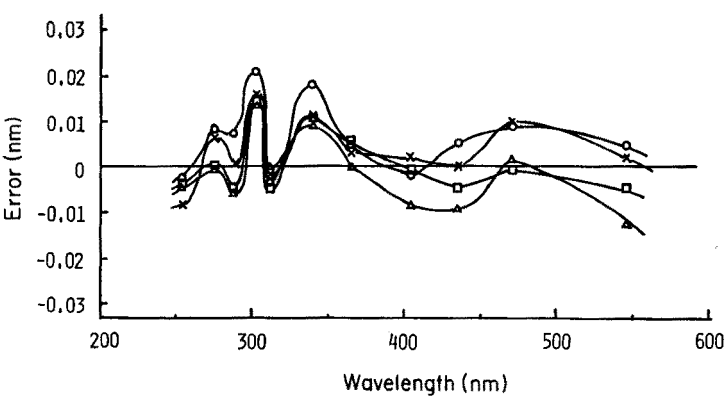

Fig. 5. Error in wavelength setting measured from profile charts. The lines used are the same as those shown in Fig. 4.

mechanism, and would cause severe errors in the measurements of peak intensities if the wavelength modulation system were not adopted.

The reproducibility of intensity measurements was tested with a mercury hollow-cathode lamp and a microwave discharge under the operating conditions listed in Table 1. The results of simultaneous measurements of 6 mercury lines with the hollow-cathode lamp demonstrate that peak intensities can be measured with a relative standard deviation of 0.6--1% (Table 2). Simultaneous measurements of 5 elements (zinc, cadmium, copper, cobalt and chromium) in solutions were conducted with the microwave discharge as light source for the concentrations of 0.1 and 1.0 μg ml^{-1}. The results are shown in Table 3. The single analysis time for these elements was about 2.5 min without wavelength calibration and chart recording of the line profiles. Wavelength calibration was necessary only once for several runs of measurements.

Tables 2 and 3 show that the reproducibility of this system is limited by the stability and the signal-to-noise ratio of the light source used. A more elaborate sample introduction system is necessary to improve the reproducibility. It is a simple matter of software to change the measurement period for each line depending on the signal-to-noise ratio, though it was not attempted here.

TABLE 1

Operating conditions for intensity measurements

Slit width (entrance and exit)	30 μm
Optical scan width	0.2 nm
Number of optical scan (measurement period)	2000 (~10 s)
Hollow-cathode lamp current	4 mA
Microwave discharge	2450 MHz, 100 W
Sample feed rate	0.3 ml min^{-1}
Argon flow rate	0.5 l min^{-1}

TABLE 2

Reproducibility of intensity measurements of mercury lines with hollow-cathode lamp

Wavelength (nm)	Mean relative intensity[a]	R.s.d. (%) (n = 5)
HgI 253.65	34.9	0.68
275.28	1.00	0.64
289.36	2.70	1.00
302.15	14.4	0.95
435.84	34.5	0.95
546.08	13.9	0.80

[a]No correction was made for the wavelength response of the detector and the optical transmission of the monochromator.

TABLE 3

Reproducibility for simultaneous measurements of 5 elements in solutions with microwave discharge

Element	Wavelength (nm)	R.s.d. (%) (n = 7)	
		1.0 μg ml^{-1}	0.1 μg ml^{-1})
Zn	I 213.86	3.7	4.7
Cd	I 228.80	2.8	3.7
Cu	I 324.75	2.5	4.5
Co	I 345.35	6.1	12.8
Cr	I 357.87	7.8	11.6

In conclusion, the system described shows great potential as a technique for rapid multi-element analysis. Owing to the excellent resolution and the capability of accurate background correlation, it will provide an extremely flexible, versatile and inexpensive addition to the multi-element emission spectrometric systems.

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CALCULATION OF RETENTION INDICES IN PROGRAMMED-TEMPERATURE GAS CHROMATOGRAPHY BY IMPROVED LINEAR INTERPOLATION

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SUMMARY

In order to determine the retention indices of compounds in programmed-temperature gas chromatography as precisely as possible, a series of hydrocarbons having successive carbon numbers is mixed with the solution. The addition of reference products to complex mixtures often results in peak interferences; by adding paraffins with even carbon numbers only, the risk of overlapping of peaks diminishes at the expense of precision. This paper describes a method of improved linear interpolation which gives reliable results of the same accuracy as would have been obtained if the whole series of alkanes had been added. Computer programs have been designed for calculations based on linear as well as improved linear interpolations in on-line and off-line applications.

In addition to specific identification techniques such as mass spectrometry and nuclear magnetic resonance, the system of retention indices [1, 2] constitutes an obvious method of carrying out reliable qualitative analysis with less expensive equipment.

As far as isothermal gas chromatography is concerned, the system developed by Kovats is based on the differences in retention volumes of the various compounds, calculated with reference to series of n-hydrocarbons. Multiplication of the carbon number by 100 gives the retention index of the n-paraffins. To calculate the retention index of a compound, a linear interpolation is applied between adjacent hydrocarbons by means of a logarithmic retention-volume scale.

If $V(X)$ is the net retention volume of a compound eluted between two hydrocarbons with net retention volumes $V(1)$ and $V(2)$, the retention index of the solute is defined by

$$I_i(X) = I(1) + [I(2) - I(1)] \cdot [\log V(X) - \log V(1)] / [\log V(2) - \log V(1)]$$

where $I_i(X)$ represents the isothermal retention index. The net retention volumes are obtained by subtracting the dead-space volume.

Complex mixtures of compounds with a wide range of boiling points require programmed-temperature analyses. Van den Dool and Kratz [3], Guiochon [4], and Habgood and Harris [5] have demonstrated that the

equation for isothermal analysis can be applied to programmed-temperature gas chromatography (p.t.g.c.) if the log of the net retention volumes are replaced by the retention temperature T.

For a compound X, eluted between hydrocarbons with retention indices $I(1)$ and $I(2)$ and retention temperatures $T(1)$ and $T(2)$

$$I_p(X) = I(1) + [I(2) - I(1)] \cdot [T(X) - T(1)]/[T(2) - T(1)] \quad (1)$$

where $I_p(X)$ is the p.t.g.c. retention index of the component. If R is the programming rate, the temperature will rise after time $t(X)$, exceeding the pre-programming time $t(0)$, to:

$$T(X) = T(0) + R[t(X) - t(0)] \text{ for } t(X) \geqslant t(0) \quad (2)$$

Replacing temperatures in eqn. (1) by their values according to eqn. (2) gives, after simplification

$$I_p(X) = I(1) + [I(2) - I(1)] \cdot [t(X) - t(1)]/[t(2) - t(1)] \quad (3)$$

where $t(X)$, $t(1)$ and $t(2)$ represent the retention times of the X compound and both hydrocarbons.

In order to obtain more precise results, it is advisable to add n-paraffins with successive carbon numbers. However, with complex mixtures, interferences occur frequently between the compounds and the standards added. The frequency with which overlaps occur can be decreased by adding only n-alkanes with even carbon numbers, although this procedure leads to bigger errors.

As a result of insufficient immobilization of the solutes near the column inlet at injection temperature [5], and because of variations in the carrier gas flow caused by pressure alterations, there is no purely linear relationship between retention indices and retention times. Particularly for the initial values, the calculated errors increase with increase in the difference in index between adjacent standard peaks. A mixture of decane, undecane, dodecane, and tridecane, analysed at a programming rate of 6°C min^{-1} according to condition (C) (see Experimental) gave retention times of 11.60, 14.44, 16.94 and 19.42 min. Undecane, calculated with respect to decane and dodecane, has a retention index of 1106; if tridecane is regarded as the second reference peak, a value of 1109 is obtained.

These systematic errors can be diminished if improved linear interpolation is used. The method involves creating imaginary reference points (Fig. 1) in the middle of the segments formed by hydrocarbons which differ by 200 retention units.

Suppose $t(1)$, $t(2)$, and $t(3)$ are the retention times belonging to hydrocarbons with retention indices $I(1)$, $I(2)$ and $I(3)$. The Kovats index of the supplementary reference point in the first segment is deduced from points A and B (ordinates I_1 and I_2).

I_1 represents the retention index found if a pure linear interpolation is applied: thus $I_1 = [I(2) - I(1)]/2$.

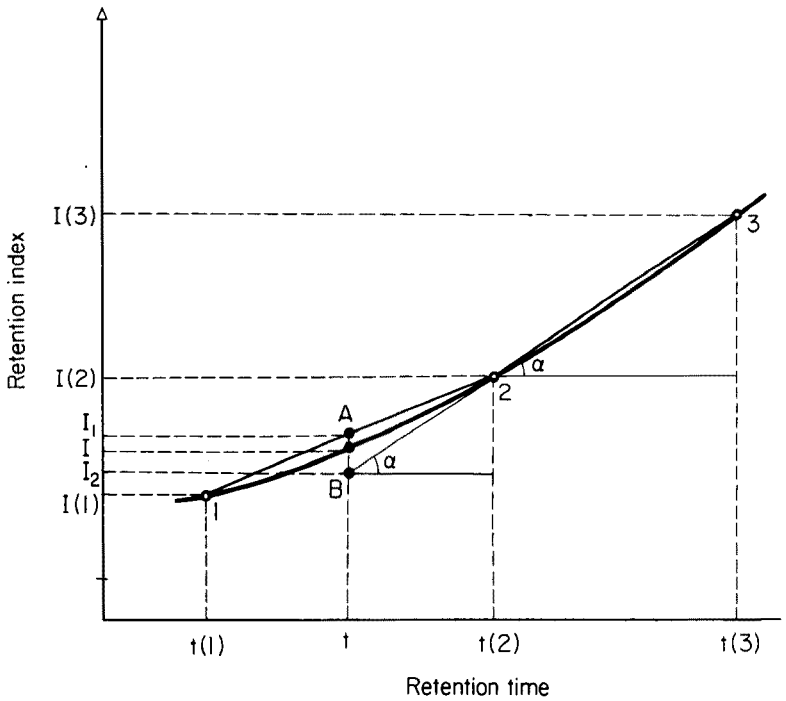

Fig. 1. Construction of an imaginary reference point in the interval formed by reference points 1 and 2, based upon reference points 1, 2 and 3.

I_2 is formed by the intersection of straight lines through reference points 2 and 3, and the perpendicular through A with the time axis, i.e.

$$I_2 = I(2) - [t(2) - t] \cdot \tan\alpha = I(2) - [I(3) - I(2)]\,[t(2) - t]/[t(3) - t(2)]$$

The retention index of the imaginary reference point is calculated from $I = kI_1 + (1 - k)\,I_2$, where $0 \leqslant k \leqslant 1$. Parameter k varies according to the curvature of the line through the reference points. Changes in the slopes of the lines connecting the reference points constitute a measure of this curvature, so that $k = f(\Delta \tan \alpha)$.

Experiments have shown that the best results are obtained by attributing to parameter k the value

$$k = 0.6 - (\tan\alpha_4 - \tan\alpha_3)\,(1 - 0.18\,n)/30$$

where the indices of angle α and parameter n represent the interval number.

As changes between slopes in successive segments are almost constant for the whole range, with the exception of the first few segments where slight variations may occur, $\Delta \tan\alpha$ can be replaced by the slope difference of the third and fourth segment.

EXPERIMENTAL

G.l.c. was carried out with a Varian Aerograph type 2700 gas chromatograph equipped with f.i.d. detector. Detector and injector temperatures were 300°C. The test solution, a mixture of hydrocarbons within the range decane—hexacosane, was injected at an oven temperature of 50°C, by three procedures:

(A) onto a glass capillary column containing SE-52 (55 m × 0.55 mm) at a programming rate of 2°C min^{-1}. The flow rate of the carrier gas (helium) was 4.5 ml min^{-1};

(B) onto a glass capillary column containing SE-52 (35 m × 0.55 mm) at a programming rate of 4°C min^{-1}. The flow rate of the carrier gas was 3.5 ml min^{-1};

(C) as for (B), but at programming rate 6°C min^{-1}.

In each case a pre-programming time of 5 min was chosen. The flow rate of the make-up gas was 30 ml min^{-1}.

The detector signals were processed by a Hewlett-Packard 3352-C data system, consisting of a 21MX-computer with an internal memory size of 32 K, and A/D convertors of the 18562A-type which are mounted in two loops of 15 convertors each. The sampling rate was fixed at 4 Hz.

The computer programs for segmental interpolation are written in the programming language LAB BASIC. As "data transfer commands" [6] are used, the on-line program is only suited for application to the 3352 and 3354 systems of Hewlett-Packard. The off-line program can, however, be applied to every kind of computer provided with a Basic compiler. The Basic program amounts to 2250 words in autocall mode (on-line), and to 1042 words in terminal mode (off-line).

RESULTS AND DISCUSSION

The n-alkanes with even carbon numbers in the test solution were regarded as references.

Ordinary linear interpolations always lead to positive deviations which are most significant in the first part of the analysis, because of the greater curvature of the reference curve. At a programming rate of 2°C min^{-1}, deviations amounting to 5 units are observed for the volatile hydrocarbons. The errors diminish systematically and come to a 2 unit shift for pentacosane. The accuracy is only slightly influenced by the programming rate; a rate of 6°C min^{-1} increases the shifts by 1 unit only.

The results calculated by improved linear interpolation for the hydrocarbons with odd carbon numbers are shown in Table 1 for the three programming rates. Despite the big variations in the range of the retention times at various programming rates, the data indicate that the shifts between real and calculated indices remain restricted to 1 unit. As far as the component tricosane is concerned (eluted at a programming rate of 2°C min^{-1}) an

TABLE 1

P.t.g.c. results for a mixture of hydrocarbons (C_{10}—C_{26})
(The retention indices of alkanes with odd carbon numbers were calculated by means of improved linear interpolation between n-paraffins with even carbon numbers at different programming rates under conditions A, B and C, as in Experimental.)

A (2°C min^{-1})			B (4°C min^{-1})			C (6°C min^{-1})			Compound
RT	*RI*	Shift	*RT*	*RI*	Shift	*RT*	*RI*	Shift	
35.73	1100	—	21.06	1099	−1	14.44	1099	−1	$C_{11}H_{24}$
50.49	1300	—	33.03	1300	—	19.42	1300	—	$C_{13}H_{28}$
63.84	1501	1	39.85	1501	1	23.85	1501	1	$C_{15}H_{32}$
75.80	1701	1	45.94	1701	1	27.80	1701	1	$C_{17}H_{36}$
86.69	1900	—	51.48	1900	—	31.39	1901	1	$C_{19}H_{40}$
96.63	2099	−1	56.52	2099	−1	34.66	2099	−1	$C_{21}H_{44}$
105.88	2299	−1	61.21	2299	−1	37.69	2299	−1	$C_{23}H_{48}$
114.45	2500	—	65.56	2500	—	40.51	2500	—	$C_{25}H_{52}$

TABLE 2

Example of a report obtained on-line by application of the "autocall" program

KOVATS INDICES – TEMP. PROG.
CORRECTED LINEAR INTERPOLATION

CHAN# 2 METHOD: KOVAC SAMPLE: 2G/MIN

RT	RET. INDEX	AREA	AMOUNT	NAME
17.15		141212	64.97	SOLVENT
27.66	1000#	1239	.57	#1000#
35.73	1100	18028	8.29	UNDECANE
43.15	1200#	1377	.63	#1200#
50.49	1300	11175	5.14	TRIDECANE
57.20	1400#	1390	.64	#1400#
63.84	1501	14433	6.64	PENTADECANE
69.83	1600#	1767	.81	#1600#
75.80	1701	8939	4.11	HEPTADECANE
81.27	1800#	1517	.70	#1800#
86.69	1900	5955	2.74	NONADECANE
91.72	2000#	1514	.70	#2000#
96.63	2099	1644	.76	HENEICOSANE
101.33	2200#	1326	.61	#2200#
105.88	2299	1639	.75	TRICOSANE
110.23	2400#	1289	.59	#2400#
114.45	2500	1664	.77	PENTACOSANE
118.49	2600#	1257	.58	#2600#

DONE

error of 1 unit corresponds to a time shift of 2.8 s within the time interval formed by docosane and tetracosane. In terms of temperature, this means an error smaller than 0.09°C. Successive injections with varying concentrations of the compounds always yielded identical results.

Tests carried out with differences of 300 retention units between successive reference peaks did not yield satisfactory results. For the relatively volatile paraffins, deviations from 5 to 6 units were observed. To calculate precise results, it is therefore essential to add reference substances with retention indices which do not differ by more than 200 units.

Table 2 shows a report obtained on-line immediately after completion of

TABLE 3

Results from application of the off-line program in the "terminal mode"

```
                    KOVATS INDICES IN PTGC

GIVE ME AT LEAST 5 REFERENCE-PEAKS (END WITH 0, 0)

      RT    RI
1   ?*11.60, 1000
2   ?*16.94, 1200
3   ?*21.63, 1400
4   ?*25.82, 1600
5   ?*29.59, 1800
6   ?*33.04, 2000
7   ?*36.20, 2200
8   ?*39.12, 2400
9   ?*41.83, 2600
10  ?*0,0

I'LL CALCULATE FOR YOU KOVATS – INDICES
GIVE ME RETENTION TIMES (IN MIN.) IN THE INTERVAL 11.6
41.83 (END WITH 0).

                                  RT          RI
?*14.44                           ----------------
?*19.42                           14.44       1099
?*23.85                           19.42       1300
?*27.80                           23.85       1501
?*31.39                           27.8        1701
?*34.66                           31.39       1901
?*37.69                           34.66       2099
?*40.51                           37.69       2299
?*0                               40.51       2500
```

the g.l.c. analysis. The data from channel 2 were recorded and interpreted by the Kovac method in which the planned Basic program was recalled.

Table 3 shows an example of calculations done in the terminal mode. The underlined data were submitted by the analyst to the computer via the keyboard of a terminal. By means of the off-line program, retention times can be processed even when the equipment is not connected to a computer.

Program listings can be obtained from the author on request.

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ENTWICKLUNG EINES RECHNER-BETRIEBSSYSTEMS FÜR DIE LABORDATENERFASSUNG

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ZUSAMMENFASSUNG

Für einen in der Labordatenerfassung eingesetzten Prozeßrechner IBM-System/7 wurde ein Betriebssystem entwickelt und ausgetestet. Dieses Betriebssystem basiert auf der Methode der ADU-Selbstsynchronisation als Taktgeber für verschiedene Funktionen wie z.B. die Datenübertragung zum Host-Computer. Die Interrupt-Bedienung wird asynchron in die Synchronisationszyklen der Analog—Digital-Umsetzer eingespielt. Die benutzte Strategie eignet sich für mittelhohe bis hohe Rechnerbelastungen durch die angeschlossenen Geräte.

SUMMARY

The development of a computer operational system for data acquisition
An operational controller, run on an IBM System/7, has been developed and tested for analytical data acquisition. This control system is particularly satisfactory with high external interrupt rates. It is based on self-synchronization of the analog—digital converters. The synchronization cycles serve as the trigger for various functions, e.g. for data transfer to the host computer. The interrupt servicing is done intermediately in an asynchronous manner.

Die zentralen analytischen Laboratorien in der chemischen Industrie sind im allgemeinen Servicebetriebe, die, soweit sie für Forschung und Verfahrensentwicklung arbeiten, Routineaufgaben nur in untergeordnetem Umfang zu lösen haben. Ständig wechselnde Aufgabenstellungen erfordern eine Vielzahl von Methoden und Meßverfahren mit einer Vielzahl verschiedener Analysengeräte. Dabei sind einerseits oft mehrere Auswertemethoden für dasselbe Analysengerät erforderlich, andererseits können dieselben Auswertemethoden für mehrere Typen von Geräten sinnvoll verwendet werden.

Die Auswertung über Computer entlastet das Laborpersonal von der mechanischen Tätigkeit des Ausmessens von Registrierkurven und vergrößert damit die Geräteauslastung bzw. den Probendurchsatz pro Mitarbeiter. Gleichzeitig erfordert die eindeutige analytische Bestimmung einer Substanz oft mehrere Analysen nach verschiedenen Verfahren, so daß der durch Korrelation und Zuordnung erforderliche nicht unbeträchtliche Verwaltungsaufwand durch zentrale Auswertung mittels Datenverarbeitung eher noch vergrößert wird.

Die wachsende Zahl von Analysengeräten im Analytischen Labor der BASF zwangen in der Vergangenheit zu einer Trennung der drei Teilaufgaben Datenerfassung, Datenorganisation + Verwaltung sowie Auswertung, so daß eine hierarchische Rechnerorganisation gewählt worden war (Abb. 1) [1]. Erhöhter Durchsatz und weitere Analysengeräte (Verdoppelung der Zahl der Analysen/Tag innerhalb eines Jahres bei leicht reduziertem Personaleinsatz) zwangen im Teilbereich Datenerfassung zum Überdenken des Erfassungssystems und der Methodik der Datenerfassung.

DATENERFASSUNGS-SYSTEM IBM/7

Anzuschließende Labormeßgeräte

Die durch das Computersystem zu bedienenden Labormeßgeräte samt den zu übernehmenden Meßwert-Zählraten läßt sich als Anforderungsmatrix darstellen (Tab. 1). Die insgesamt anzuschließende Zahl der Analysengeräte beträgt 64. Wenn alle Geräte simultan mit maximal möglicher Zählrate betrieben würden, wäre eine Gesamterfassungsrate von 21000 Meßwerten s^{-1} zu erwarten. Da diese Zählrate nicht zu bewältigen ist, muß der simultane Betrieb der beiden schnellsten Geräte ausgeschlossen werden. Unter Wegfall eines der beiden Geräte "Massenspektrometer, hochauflösend" (MSH) und "GC-MS-Kopplung" (MSK) ergibt sich im ungünstigeren Fall eine maximale Zählrate von 13000 Meßwerte s^{-1} und im Normalfall (GC-Geräte mit 2 Meßwerten s^{-1}) eine Zählrate von 12300 Meßwerten s^{-1}. Es ist also ein Betriebssystem zu konzipieren, das erlaubt, aus 11 Gerätetypen mit insgesamt 64 Meßgeräten eine Zählrate von ca. 13000 Meßwerten s^{-1} zu erfassen und an den Verwaltungsrechner IBM 1800 weiterzugeben.

Die Realisierung einer derartigen Aufgabenstellung [2] und Bausteine für die Entwicklung von Betriebssystemen [3—5] sind veröffentlicht.

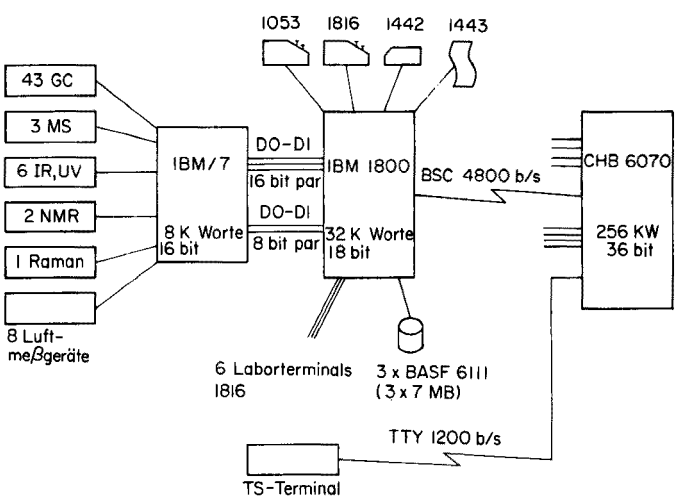

Abb. 1. Rechnerhierarchie für das Analytische Labor der BASF Aktiengesellschaft.

TABELLE 1

Anforderungsmatrix für die Datenerfassungsleistung

Geräteart	Zahl der Geräte	Meßwert Zählrate (s^{-1})	Anschluß Art	Dynamik (bit)	Auflösung (bit)
Gas-Chromatographen Typ 1	36	1—20	analog	23	14+Vorz.
Gas-Chromatographen Typ 2	7	1—20	analog	24	14
Infrarotspektrometer	3 + 1[a]	100—200	analog	14	14
Ultraviolettspektrometer	2	100	analog	14	14
Kernresonanzspektrometer	2	40	digital	12 bit* $10^1/10^2/10^3$	12+Vorz.
Massenspektrometer niedrigauflösend	1	1000	analog	14bit*1 14bit*16	14
Massenspektrometer hochauflösend	1	max. 8000	digital Abszisse + Ordinate	Abszisse: 28 BCD Ordinate: 8	8
GC-MS-Kopplung	1	10000	analog	14bit*1 14bit*16	14
Ramanspektrometer	1	1	digital	ASCII	ASCII
Umweltüberwachung Luftmeßgeräte	8	0,5	analog	14	14

[a]In Vorbereitung.

Betriebssystem-Konzept

Es gibt prinzipiell verschiedene Möglichkeiten, Prozeßperipherie (hier: Labormeßgeräte) zu bedienen, die sich zwischen den Extremen: Bedienung auf Anforderung (Interrupt) und Zyklische Bedienung (Polling) bewegen. Einfache Überlegungen zeigen, daß im Falle der maximalen Auslastung (worst case) die zyklische Bedienung höhere Erfassungsraten erlaubt, als die Bedienung auf Anforderung, wo im allgemeinen pro Meßwert zwei Interrupts zu bedienen sind: 1. Interrupt: Meßgerät fordert Übernahme eines Meßwerts an, Analog—Digital Converter (ADU) wird adressiert. 2. Interrupt: Analog—Digital Umsetzung beendet, Meßwert kann eingelesen werden.

Bei zyklischer Bedienung entfällt dagegen der Geräteinterrupt zur Adressierung des ADU sowie die daran anschließende Wartezeit (Convertzeit), bis der digitalisierte Meßwert übernommen werden kann. Im Falle niedriger Auslastung ist die Bedienung auf Anforderung günstiger, da der durch das Interrupt-Handling notwendige System-Overhead in die ohnedies freien Reserven des Rechners fällt.

In einem Labor mit unvorhersehbarer Anforderungsdichte kann also nur eine Kombination beider Grenzfälle ein praktikables Ergebnis liefern. Im einzelnen muß die Übergangsstelle bzw. die Art der Kombination durch Lastberechnung gefunden werden, d.h. die für die einzelnen Verarbeitungsroutinen notwendigen Designarbeiten müssen durchgeführt und der dazu

erforderliche Code ausgezählt werden. Für die zeitkritischen Routinen sollte wenigstens ein Alternativdesign erstellt und die Optimierungsmöglichkeiten des Codes untersucht werden. Dabei ergibt sich "nahezu von selbst" die Verarbeitungsstrategie und daraus das Gesamtdesign.

Im einzelnen zeigte sich, daß folgende Punkte einer genaueren Untersuchung bedurften: (a) Verwaltung der leeren und vollen Meßwert-Puffer für die verschiedenen Geräte; (b) Strategie der Selbstsynchronisation der ADU's und Generierung einer Convert-Tabelle; (c) Interrupt-Erkennung aufgrund des rechnerspezifischen Hardware-Gruppeninterrupts, Festlegung der Interruptprioritäten; (d) Verteilung der Geräte und Software-Moduln auf die Hardware-Levels des Rechners; (e) Codeoptimierung bezüglich Codelänge (Kernspeicherverbrauch) und Laufzeit. (Je vielfältiger die Anforderungen sind, desto kürzer muß die Laufzeit des Einzelmoduls werden, um überlappte Verarbeitung zu ermöglichen).

Verwaltung der Meßwert-Puffer. Jedem aktiven Labormeßgerät muß ein Meßwertpuffer bereitgestellt werden, in welchem die vom ADU gelieferten digitalisierten Meßwerte eingespeichert werden. Durch Wechselpuffertechnik ist sichergestellt, daß jedem Gerät auch dann ein Puffer zur Verfügung steht, wenn der gerade gefüllte Puffer noch nicht abgearbeitet, d.h. zur IBM 1800 übertragen worden ist. Ein dynamisch verwalteter Pufferpool enthält somit leere und volle Puffer, wobei in einer Verwaltungstabelle jeder Puffer "gekennzeichnet" sein muß.

Für die Pufferverwaltung stehen drei Alternativen zur Auswahl:
(a) Die Verwaltungstabelle enthält die Adressen aller Puffer mit Belegungskennzeichnung. Es wird sequentiell der nächste nicht belegte Puffer gesucht. Das Füllen geschieht "von vorne nach hinten", das Abarbeiten von "hinten nach vorne". Die Puffertabelle entspricht einem "Keller", dessen Füllhöhe ein Maß für die momentane Last darstellt (wichtig für statistische Zwecke). Der Nachteil des Verfahrens besteht darin, daß die Suchzeit nach einem leeren Puffer bei höchster Last am größten ist, weil dann der erste leere Puffer weit hinten steht.
(b) Eine "Einworttabelle" enthält das Belegungsabbild des zugehörigen Pufferpools bzw. Teils des Pufferpools (bit $i = 1$: Puffer i leer, bit $i = 0$: Puffer i voll). Die Suche nach einem leeren Puffer wird nach dem Zweierschrittverfahren (Wägeverfahren) der Einworttabelle durchgeführt, desgleichen die Abarbeitung. Dieses Verfahren hat den Vorteil konstanter Suchzeit unabhängig von der momentanen Rechnerlast. Die Modul-Laufzeit ist also berechenbar.
(c) Es wird in Ringpuffertechnik gearbeitet. Eine Tabelle enthält die Adressen aller Puffer. Zusätzlich existieren drei Pointer; der erste zeigt auf den nächsten freien Puffer (empty pointer), der zweite auf den nächsten vollen Puffer (ready pointer) und der dritte schließlich auf den letzten vollen Puffer (end pointer). Die Suche nach einem leeren Puffer besteht aus der indirekten Adressierung mittels empty pointer, die Abarbeitung geschieht von ready pointer bis end pointer. (Der Unterschied zwischen end pointer und empty

pointer zeigt diejenigen Puffer an, die gerade aktiviert d.h. teilgefüllt sind. Werden anstatt einer Tabelle mit drei Pointern zwei Tabellen mit je 2 Pointern verwendet, erspart man sich einige Prüfungen, was zu kürzeren Laufzeiten führt.

Beim Vergleich der drei Alternativen ergibt sich nach entsprechender Optimierung, daß Verfahren (a) wegen zu langer Laufzeit ausscheidet, Verfahren (b) die kürzeste Laufzeit hat, und Verfahren (c) in mehrere Teilaufgaben auftrennbar ist; die gesamte Laufzeit ist jedoch ca. 15% länger als diejenige nach (b). Bei verschachtelter Abarbeitung der Anforderungen muß das Hauptgewicht auf möglichst kurze Laufzeit der Teilaufgaben gelegt werden, so daß die Entscheidung wegen der Auftrennbarkeit zugunsten Verfahren (c) fiel.

Strategie der ADU-Selbstsynchronisation. Die Forderung nach maximaler Durchsatzleistung verbietet die Realisierung eines reinen Anforderungskonzepts. Es wurde daher davon ausgegangen, daß im Falle eines Meßwert-Übernahme-Interrupts der digitalisierte Analogwert bereits vorliegt und zwar in einem jedem Meßgerät zugeordneten "Einwort-Puffer". Die dem Übernahme-Interrupt zugeordnete Verarbeitungsroutine kopiert nun sofort den aktuellen Meßwert aus dem Einwort-Puffer in den Meßwert-Puffer, ohne die Convertierungszeit des ADU abwarten zu müssen.

Der ADU läuft dabei im "free-running mode", d.h. mit Selbstsynchronisation. Bei IPL (Initial Program Load) wird die erste Adresse des ADU-Multiplexors adressiert und ein Convertbefehl aufgesetzt. Nach Ende der Konvertierungszeit liefert die ADU-hardware einen "Convert-complete-Interrupt". Das zugehörige Modul übernimmt den Digitalwert in den der Multiplexoradresse zugeordneten Einwortpuffer und setzt danach einen neuen Convertbefehl für eine neue Multiplexoradresse auf. Damit verschieden schnelle Geräte verschieden oft vom Converter bedient werden, muß eine "Converttabelle" generiert werden, in welcher die Folge der Multiplexoradressen einzutragen ist. Schnellere Geräte treten also in der Converttabelle häufig auf, mittelschnelle weniger häufig und langsame Geräte nur einmal.

Nachdem die Converttabelle dieses ADU abgearbeitet ist, wird entweder auf einen weiteren ADU und dessen Converttabelle umgeschaltet oder wieder von vorn begonnen. Die zeitaufwendigen Analog—Digital-Umwandlungen werden also in Eigensynchronisation nach einem durch die Converttabelle definierten prioritätsgesteuerten Polling durchgeführt, wodurch auch bei maximaler Erfassungslast die gefürchtete Interruptverklemmung verhindert wird.

Das Erfassungssystem IBM/7 arbeitet mit vier unabhängigen Hardware-Levels (Level 0 bis Level 3).

Auf Level 0 mit der höchsten Priorität wurde ein ADU mit den schnelleren Geräten generiert (ADU 1). Dieser ADU liefert bei einer Convertzeit von 50 μs eine maximale Leistung von 20000 Konvertierungen s^{-1}. Für die Zählrate von 10 kHz für GC-MS-Kopplung enthält die Converttabelle also an jeder zweiten Stelle dessen Multiplexoradresse. Die Adresse des niedrigauflösenden

Massenspektrometers (MSN) tritt an jeder 10. Stelle und diejenige jedes Infrarotgerätes spätestens an jeder 50. Stelle auf.

Auf Level 1 wurden zwei ADU's für die GC-Geräte generiert (ADU 2 + 3). Sie haben wegen der höheren Dynamik eine maximale Wiederholfrequenz von 7000 Konvertierungen s^{-1}. Die Converttabelle enthält jedes angeschlossene Gerät nur einmal.

Die beiden oberen Levels der IBM/7 sind somit jeder für sich "eigensynchron-getaktet". Durch einen externen "Interrupt-Timer" wird, wie in "Codeoptimierung" (s.u.) näher beschrieben, der Betriebszustand und die Arbeitsweise dieser Taktungen überwacht.

In den 20-kHz-Takt von Level 0 wird der Datentransfer von /7 zur IBM 1800 zwangssynchronisiert eingeschleust (1 Wort pro Takt), so daß die maximal mögliche Transferrate mit 20 kHz die Erfassungsleistung an der Prozeßperipherie mit Sicherheit übersteigt. Nach jeweils 220 Worten liefert die 1800 einen Quittierungsinterrupt, der anzeigt, daß ein Makro-Puffer gefüllt und auf den nächsten umgeschaltet worden ist. Da dieser Quittierungsinterrupt mit einer Verzögerung von ca. 500—700 μs abgesetzt wird, muß der Pufferpool der /7 während dieser Zeit die angekommenen Meßwerte aufnehmen und halten können. Die erhöhte Absetzgeschwindigkeit während der eigentlichen Übertragungszeit stellt jedoch sicher, daß ein Überlaufen des /7-Pufferpools ausgeschlossen ist.

In den 7 kHz-Takt von Level 1 werden die Meßwert-Übernahme-Interrupts eines Teils der Meßgeräte von ADU 1 asynchron eingeschleust. Um eine Synchron—Asynchron-Verklemmung auf diesem Level zu vermeiden, werden nur die schnellsten Geräte auf Level 1 bedient. Alle Übernahme-Interrupts der langsameren Geräte von ADU 1 sowie diejenigen sämtlicher Geräte von ADU 2 + 3 werden auf Level 2 bedient; damit wird Level 2 vollständig asynchron, d.h. im Anforderungsmodus betrieben.

Bei dieser Konfiguration muß sichergestellt werden, daß die Summe aller Modul-Laufzeiten die CPU nicht überlasten. Durch Auszählen und Eintragen auf einer Zeitachse kann dies verifiziert werden.

Interrupt-Prioritäten. Da die Hardware der IBM/7 lediglich den Gruppeninterrupt liefert, muß in einer Interrupt-Behandlungsroutine aus den 16 möglichen das richtige Interrupt-bit gesucht, d.h. das unterbrechende Gerät lokalisiert werden.

Die Strategie der minimalen mittleren Erkennungszeit ist einfach; bei Anschluß von Geräten gleicher Priorität wird das Wägeverfahren auf den Interruptvektor angewandt, wodurch sich konstante Suchzeiten ergeben. (In bekannter Weise liegt der "break-even point" der Suchzeit zwischen sequentioneller Suche und Wägeverfahren bei 7 Geräten). Bei Geräten mit verschiedener Zählrate liegt es nahe, die Zählrate als Prioritätsfaktor aufzufassen und sequentielle Suche im Interruptvektor anzuwenden. Das Gerät mit höchster Zählrate wird als Gerät 1, d.h. auf Bit O des Interruptvektors konfiguriert.

Danach folgen die Geräte nach fallenden Zählraten. Ein Interrupt von GC-MS-Kopplung wird somit mit minimaler Suchzeit, d.h. im ersten Schritt erkannt.

Geräte- und Modulaufteilung auf die Hardware-Levels des Rechners. Die IBM/7 besitzt 4 Hardwarelevels (Level 0—Level 3), deren jeder 16 Sublevels (SL 0—SL 15) aufweist. Während sich die Hardwarelevel in ihrer Priorität unterscheiden, sind die Sublevels jedes Levels untereinander gleichrangig. Die Einsprungadressen für die Sublevels werden also nach dem FIFO-Prinzip in die Warteschlange eingetragen. Verarbeitungsmoduln auf tiefer liegenden Levels können durch "Maskierung" vor dem Verlust der Rechnerkontrolle durch Moduln oder Interruptroutinen höherliegender Levels bewahrt werden. Diese Maskierung ist notwendig, um Datenbereiche verschiedenen Levels zugänglich zu machen, deren jedes "Read/Write-Erlaubnis" hat.

Es ist offenbar, daß der maskierte Bereich eines Moduls eine Laufzeit haben muß, die kürzer ist als das jeweilige "Laufzeitloch" zwischen den Moduln der höheren Levels, gegen die die Maskierung wirksam zu sein hat. Darüber hinaus muß bei Maskierungen im einzelnen untersucht werden, ob infolge eines asynchronen Interrupts auf der Synchronebene eine Blockierung eintreten kann, welche z.B. durch einen doppelten Interrupt ausgelöst wird (Interrupt pending). Die Berechnung der maximal zulässigen Maskierungszeit wird naturgemäß umso komplexer, je mehr höherliegende Levels maskiert werden. Dabei kann der zu berechnende Überlapp selbst zeitabhängig sein.

In der vorliegenden Systemstrategie ist es lediglich notwendig, die Pufferverwaltungsroutinen zu maskieren, weil sie von drei verschiedenen Levels angesprochen werden und zwar:

von Level 1 und 2	durch die Meßwertübernahmeroutinen, wenn ein Meßwertpuffer voll ist und ein leerer Puffer bereitgestellt werden muß;
von Level 3	durch die Behandlungsroutinen bei Start bzw. Ende einer Messung, wo jeweils der erste leere Puffer bereitgestellt bzw. der letzte, teilgefüllte Puffer zur Übertragung freigegeben werden muß.

Von den insgesamt 4 Teilroutinen der Pufferverwaltung bedürfen zwei der Maskierung; die Maskierungszeit konnte durch langwieriges Optimieren auf 4 bzw. 6,8 μs beschränkt werden. Im übrigen ist die Systemstrategie so ausgelegt, daß weitere Maskierungen nicht notwendig sind. Die Verteilung der synchron und asynchron betriebenen Levels trug wesentlich zu diesem Ergebnis bei.

Die Verteilung der wichtigsten Verarbeitungs- und Organisationsmoduln auf die vier Hardware-Levels der /7 ist in Tab. 2 zusammengefaßt.

Codeoptimierung bezüglich Codelänge und Laufzeit. Die für die Erfassung von Labordaten eingesetzte IBM/7 ist mit 8K Worte Kernspeicher ausgerüstet. Es konnte vermutet werden, daß dieser Ausbau für die abzuwickelnden Aufgaben ausreicht. Daß dabei optimaler Code vorausgesetzt werden muß, zeigt sich bei überschlägiger Rechnung (Tab. 3).

Um den gerätespezifischen Code mit weniger als 30 Befehlen/Gerät zu

TABELLE 2

Zuordnung von Software-Moduln zu /7-Hardware-Levels

Level 0	ADU 1-Synchr.: 1800-Übertragung: Pufferpoolverwaltung
Level 1	ADU 2 + 3 Synchr.: teilw. Meßwertübernahme für ADU 1 (schnelle Geräte)
Level 2	Meßwertübernahme für ADU 1 (langsame Geräte), ADU 2 + 3
Level 3	Konsole/Messung Start/Ende/Rechnerstatusüberwachung/Error Recovery/ext. Interrupt-Timer

realisieren, müssen die Gerätegruppen zusammengefaßt und durch generalisierte Routinen bedient werden. Da der Compiler keinen reentrant Code liefert, die IBM/7 andererseits maschinenseitig kein BAR (Basisadressregister für im Speicher verschiebliche Programme) aufweist, um das gerade zu bedienende Gerät innerhalb der Gerätegruppe durch einfache Indexierung zu beschreiben, wird eine selbstverwaltete Basisadresse sowie ein Indexwort benutzt, welches erlaubt, ein an einer beliebigen Stelle des Kernspeichers liegendes Programm in einfacher Weise mit einer bestimmten Stelle einer Gerätetabelle zu verknüpfen.

TABELLE 3

Kernspeicherbelegung für ein 8K-Datenerfassungssystem

Verarbeitungsblock	Systemdesign Vorausschätzung	Erreichte optimale Codelänge Worte
Systemroutinen Konfigurationsmakros Pufferpool	3K	2471
Pufferverwaltung, Tabellen Synchronisation, Tabellen	1K	882
Interrupterkennung, Tabellen	1,5K	1502
Verwaltungsroutinen: Messung Start/Ende Rechnerstatus Error-Recovery	0,75K	513
Zwischensumme	6,25K	5368
Rest für gerätespezifischen Code (= Codelänge/Gerät)	1,75K = 1792W (28W)	1457 (23)
Free-core für Erweiterungen	0	1367
Summe	8K	8192

Die konsequente Durchführung dieser Strategie führte zu dem Ergebnis, daß:

die Einzelmoduln extrem kurz werden.
die meisten Moduln mehrfach benutzbar sind.
Tabellen (Gerätetabellen, Pufferpool) von verschiedenen Moduln auf verschiedenen Levels i.a. verschachtelt benutzt werden können; ein Großteil der Maskierungen wird dabei überflüssig;
der Code und die Funktionen leicht durchschaubar und einfach zu dokumentieren sind.
das benutzerorientiertes Fehlerhandling in einfacher Weise gerätespezifisch codiert werden kann, da die Basisadressen der Tabelle sowie die Indexnummern des Gerätes direkt in den Indexregistern stehen.

Allerdings sind nun Kontrollblöcke innerhalb des gerätespezifischen Codes (10—15 Worte je Gruppe) notwendig.

Die Laufzeit eines Einzelmoduls muß dabei umso kürzer sein, je höher seine Priorität ist.

Auf Level 0	sind 7 Moduln implementiert mit Laufzeiten zwischen 2,4 μs und 17,6 μs.
Auf Level 1	beträgt die maximale Laufzeit des umfangreichsten Moduls 20,8 μs.
Auf Level 2	werden die Meßwert-Übernahme-Interrupts bedient mit max. 11 μs Laufzeit.
Auf Level 3	residieren die umfangreicheren Verwaltungsmoduln: Operator-Station mit einigen Sekunden Aktivzeit, Externer-Interrupt-Timer mit 1 s Takzeit, Messung-Start/ Messung-Ende, Lampenschaltung mit ca. 50 μs Laufzeit.

Insgesamt sind nahezu 100 Benutzermoduln implementiert. Das System ist vollständig in Assembler programmiert. Zusätzlich wurden folgende IBM-System-Makros benutzt [6—9] : Konfigurationsmakros, Initialisierungsmakros, Operator-Station, Error-Processing (nicht Error-Handling). Das Verzweigungsmakro SPI ist implementiert, wird jedoch nicht benutzt. Weiterhin wurde der Timer nicht konfiguriert, da sein Zeitverbrauch mit ca. 200 μs [6] für den verlangten Durchsatz zu hoch ist. Es wird daher mit einem Externen Interrupt-Timer gearbeitet.

Externer-Interrupt-Timer. Wegen des hohen Zeitverbrauchs des System-Timers wurde auf einen freien Interrupt-Eingang ein quarzstabilisierter Uhrimpulsgenerator gelegt, der in regelmäßigen Abständen in ein auf Level 3 residierendes Programm-Modul verzweigt. In diesem Modul werden einerseits Betriebszustandsprüfungen für die ADU's durchgeführt, andererseits jene Geräte überwacht, die kein eigenes Start/Stop-Signal liefern. Wenn nötig, werden für diese Geräte die entsprechenden Start-bzw. Messung-Ende-Moduln angestoßen. Wenn eine "Betriebsstörung" auf dem Synchron-Level 1 auftritt, so daß die Selbstsynchronisation der ADU's außer Tritt gerät, wird der betroffene ADU nach Durchlaufen einer Rückstellroutine neu gestartet. Tritt die Störung auf Level 0 auf, wo die schnellen Geräte konfiguriert sind, wird nicht neu gestartet, da Meßwerte verloren gegangen sein können.

ZEITPLAN, PERSONAL-EINSATZ

Das gesamte Entwicklungsprojekt wurde nach zeitlichem Ablauf und personellem Einsatz gegliedert.

Zeitlicher Ablauf. Es lassen sich unschwer 5 Phasen selektieren. Phase 1: Grundsatzdebatte, Vordesign; Phase 2: Suche nach Alternativen, Systemdesign; Phase 3: Programmierung und Vortestläufe; Phase 4: Optimierung und Testläufe; Phase 5: Testweiser On-line-Betrieb, Programmierung des Error-handling und des automatic-restart.

Der zeitliche Ablauf und der Überlapp der einzelnen Phasen ist in Abb. 2 dargestellt. Nach jeder der drei ersten Phasen war eine Aufgabe des Projekts ohne allzu großen verlorenen Personaleinsatz möglich gewesen.

Personeller Einsatz und Verantwortlichkeiten. Es wurden vier Zuständigkeitsbereiche (ZB 1—ZB 4) definiert: ZB 1 Systemdesign (Strategie) und Koordination; ZB 2 Moduldesign, Programmierung und Optimierung; ZB 3 Programmierung und Schnittstelle 1800; ZB 4 Hardware, Leitungsinstallation.

Die Durchführung der Tests ist naturgemäß Aufgabe des Teams. Der personelle Gesamtaufwand bis zur Übernahme des Systems in den Routinebetrieb (März 77) beläuft sich auf ca. 23 Mannmonate; das Aktivitätsprofil ist in Abb. 3 dargestellt.

TESTLÄUFE UND TESTWEISER ON-LINE-BETRIEB

Die Reihe der Testläufe zieht sich nahezu über die Hälfte der Projektzeit. Durch diese permanente Rückkopplung der Ergebnisse auf die Programmierung konnten Fehlentwicklungen vermieden, wertvoller Input für die Optimierungsaufgabe und eine stufenweise fortschreitende Erfolgs-Gewißheit erreicht werden. Der Zeitverlust durch die Notwendigkeit, Hilfsmoduln für Verwaltung und Errorhandling der einzelnen Testphasen mehrmals schreiben zu müssen, wurde hingenommen im Hinblick auf die durch die Tests erreichbare frühzeitige Rückkopplung.

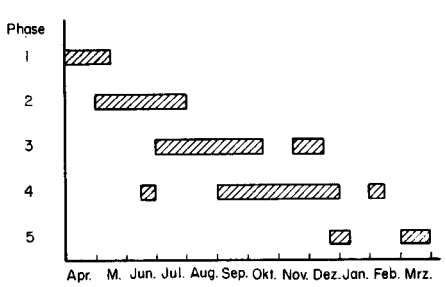

Abb. 2. (links) Zeitlicher Ablauf der Projekt — Realisierung.

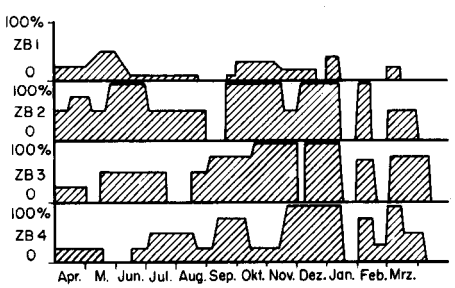

Abb. 3. (rechts) Aktivitätsprofil des Personaleinsatzes nach Zuständigkeitsbereichen.

Einzeltests

Im wesentlichen wurden Tests gemäß Tab. 4, gegebenenfalls mehrmals, durchgeführt, um das entsprechende Planziel (Designziel bzw. Ergebnis von Vorausberechnungen) zu erreichen. Mit den Ergebnissen wurde der Einsatz unter Praxisbedingungen riskiert.

On-line Betrieb

Es war zu erwarten, daß sich im Dauerbetrieb Systemabstürze ergeben würden, die unter Testbedingungen nicht realisiert werden können. Aufgrund der praktischen Erfahrungen muß das Error-recovery und der automatic-restart nach error realisiert werden.

Beim on-line-Einsatz zeigten sich folgende Fehler:
(a) Interrupt-pending auf Level 2, behoben durch Ersetzen des System-Timers durch Extern-Interrupt-Timer.
(b) Asynchroneität des ADU 2 auf Level 1 (intermittierend), behoben durch Extern-Interrupt-Timer.
(c) Überlastung der IBM 1800 bei Vollast auf /7 und gleichzeitig aktivem I/O-intensivem Programm auf 1800, behoben durch organisatorische Maßnahmen in der Jobverteilungstabelle der 1800.
(d) Ausbleiben des Quittierungssignals der 1800 nach 220 übertragenen Meßwerten. Der Fehler trat nur selten auf, Ursache nicht genau bekannt.
(e) Analog-input error, device-busy bei ADU 2, behoben durch error handling-Routine.

Aufgrund dieser Fehler und einiger durch den Vollastbetrieb zutage tretender Hardwareprobleme wurden alle erkannten Hardwarefehler behoben. Während der zwangsweise dabei auftretenden Denkpause wurde eine weitere Codeoptimierung an den weniger wichtigen Moduln durchgeführt und der Systemtimer durch den Externen Interrupt-Timer ersetzt. Dazu mußten nicht nur die entsprechenden Installationsänderungen durchgeführt, sondern auch das dem Timer zugeordnete Verarbeitungsmodul neu geschrieben werden.

Das nunmehr implementierte neue System lief nach kurzer Zeit einwandfrei.

AUSBLICK

Für die Labordatenerfassung im Analytischen Labor der BASF wurde ein Prozeßrechner-Betriebssystem für eine IBM/7 entwickelt, das auf komplexe Arbeitsweise mit hoher Erfassungsrate zugeschnitten ist. Mit Ausnahme der beiden schnellsten Geräte — Massenspektrometer hochauflösend und GC-MS-Kopplung — die nicht gleichzeitig aktiv sein dürfen, können sämtliche 63 Labormeßgeräte simultan in Betrieb sein. Die maximal zu bewältigende Prozeßdatenrate beträgt 12300 bzw. 13000 Meßwerte s^{-1}.

Das Betriebssystem wurde so konfiguriert, daß die reine Datenerfassung im Pollingverfahren mit Prioritätstabelle, d.h. nach einem selbstsynchronisierenden Modus erfolgt, wobei auf zwei verschiedenen Hardware-Levels der IBM/7 zwei getrennte, voneinander unabhängige, gegeneinander asynchron

TABELLE 4

Funktions- und Auslastungs-Tests während der Phase der Systementwicklung

Test	Planziel	Konfiguration	Ergebnis
1	3 ADU synchron auf 2 Hardwareleveln. CPU-last = 65%	Polling mit Priorität, Synchro-Moduln; Auslesen des Digitalwerts aus ADU	CPU-belastung: 64-67%
2	Messung der Lastgrenze für ext. Interrupts	zusätzlich zu 1: Interrupterkennung, (keine Meßwertbehandlung)	Interruptbelastung / lost interrupt ⩽ 17 kHz: 0% 20 kHz: 0,07% 25 kHz: 19,2%
3	Messung der differentiellen Linearität eines ADU-Eingangs bei selbstsynchronisierter Betriebsweise	zusätzlich zu 2: Meßwertübernahme für GC-Geräte	mittl. diff. Nichtlinearität 0,28% max. diff. Nichtlinearität 0,58% bei a. 1,4% Vollaussteuerung
4	Messung der freien CPU-Zeit für Verwaltungsaufgaben in Abhängigkeit v. der Interruptzählrate	wie 3	Zählrate / Idle Zeit 10 kHz: 33,4% 12,5 kHz: 26,3% 17 kHz: 17,6% 18 kHz: 1,7%
5	Messung der Lastgrenze für ext. Interrupts unter Einschluß von Pufferverwaltung und Meßwertübertragung zur 1800	zusätzlich zu 3: Pufferverwaltung Meßwertübertragung zur 1800	Interruptbelastung / lost interrupt ⩽ 13, 5 kHz: 0% 15 kHz: 0,32%
6	Messung der maximalen Belastbarkeit für alle ADU's	zusätzlich zu 5: Meßwertübernahme für alle Geräte	Lastgrenze Level 0-ADU: 11,5 kHz (< 11,7 kHz) Lastgrenze Level-1-ADU's: > 400 kHz (von der Gerätekonfiguration her zu erwartende maximale Belastung: 200 Hz)

7	Entscheidung für Interrupterkennungsroutine: Wägeverfahren oder sequentiell mit Prioritätszuordnung der Geräte	zusätzlich zu 6: sequentielle Interrupt-Erkennung	Level-0-ADU: 10,1 kHz auf Eingang mit höchster Priorität Level-1-ADU: Eingang mittlerer Priorität Zahlräte — lost interrupt 1 kHz — 0% 1,5 kHz — 0% 2 kHz — 0% 2,5 kHz keine Puffer mehr verfügbar
8	Bestimmung der CPU-Reserve für Verwaltungsaufgaben auf niedrigstem Level (Level 3)	zusätzlich zu 7: Hilfsroutine für Level 3	Interruptbelastung: 10,7 kHz CPU-Zeitreserve: ca. 18%
9	On line Test mit 2 schnellen Geräten an Level-0-ADU, zusätzlich 17 Geräte an Level-1-ADU	zusätzlich zu 7: Verwaltungsroutinen ohne error-recovery	Level-0-ADU: 10 kHz + 0,2 kHz Level-1-ADU: 17 + 2 Hz Level-3: 38 Verwaltungsanfragen insgesamt 700 000 Meßwerte in 68 s, lost interrupts: 0

betriebene Synchronisierüngen benutzt werden. Die Datenübernahme, die Pufferverwaltung sowie die allgemeinen Verwaltungsaufgaben werden nach dem Anforderungsverfahren asynchron in die Synchronperioden der Maschine eingestreut bzw. auf einem eigenen Prioritätslevel abgearbeitet. Dabei wurde sichergestellt, daß Geräte, die mit einer dem einen der beiden Synchrontakte ähnlichen Frequenz oder deren Oberwellen laufen, asynchron in den anderen der beiden Synchrontakte eingespielt werden, so daß ein Lastaufschaukeln und daraus resultierende Synchronverklemmung vermieden wird. Für die auf niedrigstem Hardware-Level abzuwickelnden allgemeinen Verwaltungsaufgaben steht bei Maximallast eine mittlere CPU-Zeit-Reserve (idle time) von ca. 18% zur Verfügung. Diese Reserve reicht aus, um das Errorhandling und einen automatic-restart zu realisieren. Dabei muß weitgehend auf das betriebssystemeigene error-handling verzichtet werden, desgleichen reicht die idle-time nicht aus, standardmäßig komfortable Systemmeldungen über die System-Konsole auszugeben.

Bei der Programmierung des error-handling wurde davon ausgegangen, daß ein Fehler im "Zuständigkeitsbereich" des Level-0-ADU (schnelle Meßgeräte) zu so großen Erfassungsverlusten führt, daß ein automatisches Wiederstarten der Anlage nicht sinnvoll ist. Im Bereich des Level-1-ADU (GC-Geräte) ist ein automatic-restart vorteilhaft, wenn sichergestellt werden kann, daß nur wenige, höchstens drei Meßwerte verloren gegangen sind. Dann wird den betroffenen Labors die Tatsache verlorener Meßwerte mitgeteilt, die Datenerfassung geht jedoch weiter; das Labor muß also im Einzelfall selbst entscheiden, ob die mit "Aussetzern" behaftete Messung verwendbar ist oder wiederholt werden muß.

Das Betriebssystem ist vollständig im Assembler programmiert, nur wenige Systemmakros wurden benutzt. Die hohe Durchsatzleistung bei geringem Kernspeicherausbau (8K Werte) erforderte eine ausgeklügelte Programmierung, die erst nach umfangreichen Designarbeiten und Optimierungsläufen durchgeführt wurde. Dabei ergab sich, daß die fünf zeitlichen Phasen (Vorarbeit, Systemdesign, Programmierung, Optimierung und on line-Test) etwa gleich groß waren. Die Programmcodierung selbst erfolgte unter Time-Sharing am Großrechner.

Durch die mit dem neuen Betriebssystem erreichbare hohe Erfassungsleistung ist eine weiter gesteigerte Durchsatzleistung in den Labors zu erwarten. Dadurch steigt der Verwaltungsaufwand in den Labors sowie am Verwaltungsrechner IBM 1800. Es ist deshalb eine Erweiterung dieser IBM 1800-Konfiguration geplant, die am Rechner selbst einen Kernspeicherausbau um 8K, an der Rechnerperipherie eine 50% ige Erhöhung der Zahl der Laborterminals vorsieht. Dadurch soll sichergestellt werden, daß der zu erwartende höhere Analysendurchsatz von den Labormitarbeitern auch bewältigt werden kann, d.h. daß Schlange-Stehen an den Terminals vermieden wird. Eine Erhöhung des Analysendurchsatzes um den Faktor 1,5—2 scheint im Bereich des Möglichen zu liegen.

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SUBJECT INDEX

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