

from contamination by atmospheric carbon dioxide and carbon dioxide produced by microbial metabolic activities. Any moderate losses of carbon dioxide will be inconsequential, since both the radio- and stable-isotopes will be lost in equivalent proportions and so will not influence the answer obtained upon analysis; 5. the method is applicable to waters not suited to analysis by conventional field methods.

**Riassunto.** Si descrive un metodo per determinare il contenuto di anidride carbonica dell'acqua, che prevede il passaggio del  $\text{CO}_2$  dall'acqua attraverso una membrana di lattice ad una soluzione alcalina dove la variazione di

conduttività e radioattività viene misurata e usata per calcolare il livello di  $\text{CO}_2$  del campione d'acqua.

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## On Matrix Rank Analysis of Spectra of Multicomponent Mixtures

Several workers have shown that application of the concept of the rank of a matrix to the spectra of multicomponent mixtures can give the number of components in the mixtures. The practical problem of rank determination has been attacked in various ways including evaluation of determinants<sup>1-3</sup>, annihilation of rank<sup>4,5</sup>, and evaluation of eigenvalues<sup>6</sup>. The rank determined is that consistent with the error of the matrix elements.

Consider a matrix  $\mathbf{D} = [d_{\lambda i}]$  whose columns consist, for example, of absorption spectra of a multicomponent mixture. Here  $d_{\lambda i}$  represents the absorbance at the  $\lambda$ th wavelength in the  $i$ th spectrum. We assume that each  $d_{\lambda i}$  has a variance  $\text{var}(d_{\lambda i})$ , and that the  $d_{\lambda i}$  are observationally independent. To find the rank of  $\mathbf{D}$ , HUGUS and EL-AWADY<sup>6</sup> recently proposed the determination of the number of nonzero eigenvalues of the real symmetric matrix  $\mathbf{DD}^T$ , where  $\mathbf{D}^T$  is the transpose of  $\mathbf{D}$ . These authors determined the eigenvalues of  $\mathbf{DD}^T$  by the Jacobi method. To set up a test for a zero eigenvalue, they considered the propagation of errors in the computation of  $\mathbf{DD}^T$  and the diagonalization of this matrix. The purpose of this communication is to show that when the elements of  $\mathbf{D}$  have the same variance it is easier to proceed as follows by considering the singular values of  $\mathbf{D}$ .

A general  $m \times n$  matrix of rank  $k$  has exactly  $k$  positive nonzero singular values<sup>7</sup>. The singular values are real. Corresponding to each singular value  $\mu_p$  there is a pair of singular vectors  $\mathbf{u}_p$  and  $\mathbf{v}_p$ . These are eigenvectors of  $\mathbf{DD}^T$  and  $\mathbf{D}^T\mathbf{D}$  (for a real matrix  $\mathbf{D}$ ), respectively, corresponding to an eigenvalue  $\lambda_p = (\mu_p^2)$ . We have

$$\mathbf{D}^T \mathbf{u}_p = \mu_p \mathbf{v}_p.$$

Multiplying on the left by  $\mathbf{v}_p^T$  we get

$$\mu_p = \mathbf{v}_p^T \mathbf{D}^T \mathbf{u}_p,$$

since the  $\mathbf{v}_p$  are orthonormal. If  $\mathbf{D} = [d_{\lambda i}]$  we have

$$\mu_p = \sum_{\lambda=1}^m \sum_{i=1}^n u_{\lambda} v_i d_{\lambda i}$$

where  $u_{\lambda}$  and  $v_i$  are the  $\lambda$ th and  $i$ th components of the eigenvectors  $\mathbf{u}_p$  and  $\mathbf{v}_p$ , respectively. By propagation of errors we have

$$\text{var}(\mu_p) = \sum_{\lambda=1}^m \sum_{i=1}^n (u_{\lambda} v_i)^2 \text{var}(d_{\lambda i}).$$

It will be noticed that when the  $d_{\lambda i}$  have the same variance

$$\text{var}(\mu_p) = \text{var}(d_{\lambda i}),$$

since the eigenvectors  $\mathbf{u}_p$  and  $\mathbf{v}_p$  are orthonormal, and the problem of determining the number of nonzero singular values of  $\mathbf{D}$  is greatly simplified. The singular values are computed as the non-negative square roots of the eigenvalues of  $\mathbf{D}^T\mathbf{D}$  or  $\mathbf{DD}^T$  and compared with the standard deviation of the elements of  $\mathbf{D}$ .

We have tested the method of rank determination given here on an  $8 \times 8$  matrix of neutral red spectra consisting of 8 rows at different wavelengths and 8 columns at different pH values of the solution as given by WALLACE and KATZ<sup>4</sup>. These authors found the matrix to be definitely of rank 3 and possibly of rank 4 by considering the propagation of errors after reduction steps consisting of Gaussian elimination with complete pivoting. HUGUS and EL-AWADY<sup>6</sup> declared the matrix to be of rank 3 as a result of computing 3 nonzero eigenvalues for the product matrix  $\mathbf{DD}^T$ . The matrix elements each had an error (standard deviation) of 0.003 absorbance unit.

Diagonalization of the product matrix  $\mathbf{DD}^T$  was carried out by the Jacobi method using an algorithm of RUTIS-

<sup>1</sup> R. M. WALLACE, J. phys. Chem., Ithaca 64, 899 (1960).

<sup>2</sup> G. WEBER, Nature, Lond. 190, 27 (1961).

<sup>3</sup> S. AINSWORTH, J. phys. Chem., Ithaca 65, 1968 (1961).

<sup>4</sup> R. M. WALLACE and S. M. KATZ, J. phys. Chem., Ithaca 68, 3890 (1964).

<sup>5</sup> D. KATAKIS, Analyt. Chem. 37, 876 (1965).

<sup>6</sup> Z. Z. HUGUS and A. A. EL-AWADY, J. phys. Chem., Ithaca 75, 2954 (1971).

<sup>7</sup> D. NOBLE, Applied Linear Algebra (Prentice-Hall Inc., New Jersey 1969), p. 335.

Computations for  $8 \times 8$  matrix  $\mathbf{D}$  of neutral red spectra of WALLACE and KATZ<sup>4</sup>

Eigenvalue of $\mathbf{DD}^T$	Singular value of $\mathbf{D}$
11.570321	3.401518
1.158019	1.076123
0.096982	0.31142
0.008896	0.094317
0.000068	0.008219
0.00001	0.003115
0*	0
0*	0

\* Declared zero after computation as a negative number with small absolute value.

HAUSER<sup>8</sup> in a program<sup>9</sup> written for a Hewlett-Packard 9100B Calculator fitted with a 9101A Extended Memory. The computed eigenvalues and singular values are given in the Table.

Comparison of the singular values of the Table with the standard deviation of the matrix elements indicates 4 singular values greater than twice the standard deviation and, therefore, nonzero. The matrix thus appears to be an approximation to a matrix of rank 4, as originally suggested by WALLACE and KATZ<sup>4</sup>.

**Zusammenfassung.** Neues Verfahren zur Bestimmung des Ranges, wonach die Quadratwurzeln der Eigenwerte mit der Standardabweichung der Extinktionsmessungen

verglichen werden. Die Zahl der Komponenten aus einem Lösungsgemisch kann aus dem Rang der Matrix der Extinktionskoeffizienten bestimmt werden.

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31 January 1974.*

<sup>8</sup> H. RUTISHAUSER, Numer. Math. 9, 1 (1966).

<sup>9</sup> Available on request from the authors.

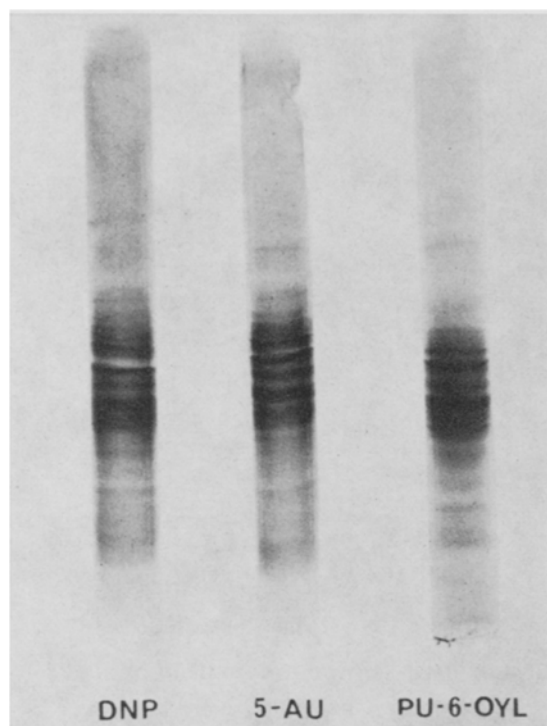
### Multiple Binding Functions of Sheep Anti-DNP Antibodies

An immune serum is generally assumed to exhibit a high degree of specificity against the antigen used to induce the immune response. This general rule has become questionable by the recent findings of several workers<sup>1-6</sup> suggesting that individual antibodies may competitively bind several structurally dissimilar haptens. These observations indicate that hapten binding by the antibody might not be an all-or-none function but rather that the antibody combining site might be a polyfunctional region, capable of binding several structurally dissimilar haptens; however, with different affinities.

The present work was undertaken in order to examine this hypothesis in sheep antibodies towards the 2,4-dinitrophenyl (DNP) determinant and to establish the degree of polyfunctionality of these antibodies.

**Material and methods.** Antibodies were raised by i.m. injection of 5 mg DNP-BGG in complete Freund's adjuvant. In regular 1 month intervals, the sheep were boosted with the same amount of antigen. After 6 boosters the antibody titers reached an average of 0.5–1.0 mg/ml.

Immunoabsorbents consisted of hapten-protein-conjugates mechanically entrapped into polyacrylamide gels according to the method of CARREL<sup>7</sup>. Active protein was desorbed with 0.2 M glycine, pH 2.3. 5-acetyluracil-BSA, purine-6-oyl-BSA, nucleotide- and nucleoside-protein-conjugates were synthesized as described by BEISER et al.<sup>8</sup>, nitroazidophenyl-BSA by the method of FLEET<sup>9</sup>. DNP-BSA and DNP-BGG were prepared according to LITTLE and EISEN<sup>10</sup>. Equilibrium dialysis was performed as described by EISEN<sup>11</sup>, using tritiated  $\epsilon$ -N-DNP-L-lysine (New England Nuclear). The relative association constant with various hapten-protein-conjugates were studied by the inhibition of the binding of radioactively labeled DNP-BSA to an insoluble antibody preparation. This method has the advantage that the reaction with the immunogen itself may be studied. Essentially antibody coupled to bromoacetylcellulose<sup>12</sup> was reacted with <sup>125</sup>I DNP-BSA. After centrifugation and washing, the radioactivity in the sediment was counted, giving 100% of binding. The relative affinities against the cross-reacting hapten-protein-conjugates were calculated



Isoelectrofocusing pattern of the whole anti-DNP antibody population and of the subfractions isolated from the 5-acetyl-uracil-BSA immunoabsorbent and the purine-6-oyl-BSA immunoabsorbent respectively.

<sup>1</sup> D. SCHUBERT, A. JOBE and M. COHN, Nature, Lond. 220, 882 (1968).

<sup>2</sup> B. J. UNDERDOWN and H. N. EISEN, J. Immun. 106, 1431 (1971).

<sup>3</sup> H. N. EISEN, M. C. MICHAELIDES, B. J. UNDERDOWN, E. P. SCHULENBURG and E. S. SIMMS, Fedn. Proc. 29, 78 (1970).

<sup>4</sup> R. W. ROSENSTEIN, R. A. MUSSON, M. Y. K. ARMSTRONG, W. H. KONIGSBERG and F. F. RICHARDS, Proc. natn. Acad. Sci., USA 69, 877 (1972).

<sup>5</sup> W. RIESEN and A. MORELL, Immunochemistry 9, 979 (1972).

<sup>6</sup> W. RIESEN and V. CASTEL, Experientia 29, 608 (1973).

<sup>7</sup> S. CARREL, H. GERBER and S. BARANDUN, Nature, Lond. 221, 385 (1969).

<sup>8</sup> S. M. BEISER, B. F. ERLANGER and S. W. TANENBAUM, Methods in Immunology and Immunochemistry (Ed. C. A. WILLIAMS and M. W. CHASE; Academic Press, New York 1967), vol. 1, p. 180.

<sup>9</sup> G. W. FLEET, R. R. PORTER and J. R. KNOWLES, Nature, Lond. 224, 511 (1969).

<sup>10</sup> J. R. LITTLE and H. N. EISEN, Methods in Immunology and Immunochemistry (Ed. C. A. WILLIAMS and M. W. CHASE; Academic Press, New York 1967), vol. 1, p. 128.

<sup>11</sup> H. N. EISEN, Meth. med. Res. 10, 106 (1964).

<sup>12</sup> J. B. ROBBINS, H. HAIMOVICH and M. SELA, Immunochemistry 4, 11 (1967).