

**WHEN GOING BACKWARDS
MEANS PROGRESS:
ON THE SOLUTION OF
BIOCHEMICAL INVERSE PROBLEMS
USING ARTIFICIAL NEURAL NETWORKS**

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1. Introduction

Many (perhaps most) biochemical problems are actually "inverse" problems, or problems of system identification. In general, if we know the parameters of a system of interest, we can set up a model (or actual) experiment, run the model (or experiment), and observe the behavior or time evolution of the system. However, especially in a complex biological system, the things which are normally easiest to measure are the variables, not the parameters, and it is the variables which depend on the parameters, not *vice versa*.

In the case of metabolism, the usual parameters of interest are the enzymatic rate constants, which are difficult to measure accurately *in vitro*¹ and virtually impossible so to do *in vivo*. Yet to describe, understand, and simulate the system of interest we need knowledge of the parameters. In other words, we need somehow to go backwards from variables such as the steady-state fluxes and metabolite concentrations, which are relatively easy to measure, to the parameters, which are not.

A similar situation exists in biological spectroscopy. If we have chemical standards, whether pure or mixed, it is easy to obtain their spectra. The inverse problem then involves obtaining the concentrations of metabolites, or the overall spectral parameters, from the spectra observed in a complex system.

The purpose of this article is to describe our successful exploitation of artificial neural networks (ANNs) in the solution of such biochemical (and other) inverse problems. We apply the approach specifically to:

- (i) model metabolic pathways studied by computer simulations,
- (ii) parameter identification in biological dielectric spectroscopy, and
- (iii) the extraction of chemical information from pyrolysis mass spectra of intact microbial cells.

2. Artificial Neural Networks

ANNs are collections of very simple "computational units" which can take a numerical input and transform it (usually via summation) into an output (see e.g. Refs 2-12). The inputs and outputs may be to and from the "external world" or to other units within the network. The way in which each unit transforms its input depends on the so-called "connection weight" (or "connection strength") and "bias" of the unit, which are modifiable. The output of each unit to another unit or to the external world then depends on both its strength and bias and on the weighted sum of all its inputs, which are transformed by a (normally) nonlinear weighting function referred to as its activation function.

The great power of neural networks stems from the fact that it is possible to present ("train") them with known inputs (and outputs) and provide some form of learning rule which may be used, iteratively, to modify the strengths and biases until the outputs of the network as a function of the inputs correspond to the desired ("true") outputs. The trained network may then be exposed to "unknown" inputs and will then provide its view of the "true" output(s).

A neural network therefore consists of at least three layers, representing the inputs and outputs and one or more so-called "hidden" layers. It is, in particular, the weights and biases of the interactions between inputs and outputs and the hidden layer(s) which reflect the underlying dynamics of the system of interest, even if its actual (physical) structure is not known. By training up a neural network with known data, then, it is possible to obtain outputs that can accurately predict things such as the (continuing) evolution of a time series, even if it is (deterministically) chaotic¹³.

Other successful uses of neural networks include speech recognition, DNA sequence analysis, the correction of errors in optical astronomy, and the analysis of vapors by arrays of artificial sensors. One may also perhaps mention the successful use of simple neural nets in the analysis of chemical engineering systems¹⁴.

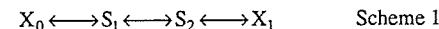
3. Analysis of Metabolic Systems Using Neural Networks

As described in several other contributions in this volume, it is possible by computer simulation to determine steady-state variables such as fluxes and metabolite concentrations as a function of parameters such as the enzymatic rate constants and external metabolite concentrations. It is obviously then possible to change one or more of the parameters and to determine another set of associated variables, and so on. The idea is that having acquired related sets of parameters and variables, we would then be in a position to train neural networks in which the (known) variables were the inputs and the parameters were the outputs. When the nets had successfully learned to reflect the correct parameters when presented with the variables, we would have solved our problem. We could then present the net with "random" (experimental) variables and ask it for the parameters.

The correctness of the network's predictions could obviously be checked by running a simulation with the parameters provided by the network and seeing if they generated the variables used as the input to the net. The result would be that we *could* in fact obtain the (enzymatic) parameters of a metabolic network (and hence the control coefficients and

elasticities) by measuring the variables alone. We have now implemented this strategy, as outlined in what follows.

For computational simplicity we concentrate here on a simple, three-step linear pathway, as shown in Scheme 1, with each enzyme (one substrate / one product) possessing reversible Michaelis-Menten kinetics, and with the steps having equilibrium constants of 567, 13.4 and 2.3 respectively.



The dataset was obtained by varying the V_{\max} values of each enzyme and the first forward K_m value. All other K_m s were held constant. The concentration of S_1 , S_2 and the steady-state flux were recorded for each parameter set. In addition, each parameter set was applied using three different concentrations (1, 10, 100) of the starting metabolite X_0 so that we could obtain three sets of variables for the same set of parameters (in an experimentally realizable fashion), and so aid the training process.

The parameters of the system were varied as follows. Each parameter was first assigned a uniform random number u between 0 and 2.0, which was then used to generate a non-skewed distribution spanning two decades using the formula, 10^u . The reason why the parameters were generated in this manner was so that the distribution of values would not be confined to the upper decades of the range and thus there would be a roughly equal number of random values between 1 and 10 as there would be between 10 and 100. In this way 500 random sets of K_m and V_{\max} values were generated, from which the steady-state concentrations of S_1 , S_2 and the fluxes were obtained. A 12-18-4 net was then trained (with the stochastic backpropagation algorithm) using the 12 values of X_0 , S_1 , S_2 and J as the input and the four varied parameters as the output.

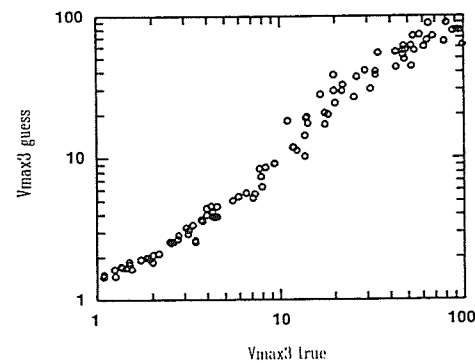


Figure 1. An Artificial Neural Network's estimate of unseen $V_{\max,3}$ values.

After training to an RMS error of ca 0.08 (just under 4000 epochs), the net had successfully generalized, as illustrated in Fig. 1, which shows the network's estimate of the unknown values for $V_{\max,3}$ against the "true" values. Other parameters had been learned to a more or less similar degree.

It is worth pointing out that it is only possible for ANNs to learn something that is actually learnable, so that if a variable is not significantly related to a particular parameter then the network will not learn it; this of course may be used to our advantage, since if the

parameter of interest does not significantly control the variable it may be assumed that one is not interested in studying it anyway. Because updating of the network is based on the overall RMS error, "bad" relationships interfere with the learning of "good" ones; our experience to date suggests that any linear correlation coefficient (of logarithmic parameters) below some 0.4 will inhibit the net from converging.

4. Solution of the Inverse Problem in Biological Dielectric Spectroscopy

In biological dielectric spectroscopy, where dispersions are substantially broader than that expected from a purely Debye-like process, it is not always possible, due to technical limitations, to obtain data over a wide enough range of frequencies to encompass the entire dispersion(s) of interest. Similarly, because of the breadth of the dispersions, it is common to seek to characterize the dielectric behavior of interest by means of the Cole-Cole function¹⁵. Whilst it is possible to fit dielectric data to this equation using appropriate nonlinear least-squares methods¹⁶, these methods are computationally rather demanding, and must be undergone, iteratively, for each set of data.

We have found¹⁷ that it is possible to train an artificial neural network with small sets of dielectric data (permittivities measured at various frequencies) as the inputs and the attendant parameters of the Cole-Cole equation as the outputs. The trained net can then give an essentially instantaneous output of the limiting permittivities at frequencies that are both high and low with respect to the characteristic frequency, and thus of their difference, a parameter which, for the so-called β -dispersion, scales with the biomass content of cell suspensions¹⁶.

5. Solution of the Inverse Problem in Pyrolysis Mass Spectrometry of Microbial Cells

Pyrolysis is the thermal degradation of a material in an inert atmosphere, and leads to the production of volatile fragments (pyrolysate) from non-volatile material such as microorganisms. Curie-point pyrolysis is a particularly reproducible and straightforward version of this technique, in which the sample, dried onto an appropriate metal is rapidly heated (0.6s is typical) to the Curie point of the metal, which may itself be chosen and is commonly 530°C. The pyrolysate may then be separated and analyzed in a mass spectrometer¹⁸, and the combined technique is then known as Pyrolysis Mass Spectrometry or PyMS.

Conventionally (within microbiology and biotechnology), PyMS has been used as a taxonomic aid in the identification and discrimination of different microorganisms¹⁹. To this end, the reduction of the multivariate data (150 normalized values in the range m/z 51-200) generated by the PyMS system is normally carried out using Principal Components Analysis (PCA), a well-known technique for reducing the dimensionality of multivariate data whilst preserving most of the variance. Whilst PCA does not take account of any groupings in the data, neither does it require that the populations be normally distributed, i.e. it is a non-parametric method.

The closely related Canonical Variates Analysis technique then separates the samples into groups on the basis of the principal components and some *a priori* knowledge of the appropriate number of groupings. Provided that the data set contains "standards" (i.e. type or centro-strains) it is evident that one can establish the closeness of any unknown samples to a known organism, and thus effect the identification of the former. An excellent example of the discriminatory power of the approach is the demonstration²⁰ that one can use it to

distinguish four strains of *Escherichia coli* which differ only in the presence or absence of a single plasmid.

We have found²¹ that it is possible to train an ANN using the Pyrolysis Mass Spectra as the inputs and the known concentrations of target analytes in standards as the outputs. The trained net can then be tested with the Pyrolysis Mass Spectra of "unknowns", and then accurately outputs the concentration of the target analyte(s), in the case described here the concentration of tryptophan in the growth medium of indole-positive strains of *E. coli*.

We have also been able to effect a rapid distinction between extra virgin and adulterated olive oils using this approach²². It is obvious that this combination of PyMS and ANNs constitutes a powerful technology for the analysis of the concentration of appropriate substrates, metabolites and products in any biological process.

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**QUALITY ASSESSMENT
OF A METABOLIC MODEL
AND SYSTEMS ANALYSIS
OF CITRIC ACID PRODUCTION
BY *ASPERGILLUS NIGER***

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1. Introduction

Citric acid is a chemical of foremost importance in the food, cosmetic and chemical industry. Its annual production amounts up to 350,000 tons, with the carbohydrate fermentation by means of the filamentous fungus *Aspergillus niger* (ascomycetes group) being one of the leading processes for its production worldwide. A great deal of work has been done on the biochemistry of this process¹ and, consequently, information is available on most of its biochemical aspects. However, in spite of the amount of information on the system and its economic importance, no attempt has been made to explain its regulation on a quantitative basis.

In this context the aim of this work is, first to develop a mechanistic model where the information on many aspects of the process is integrated and, then, to translate the mechanistic picture so obtained into an S-system representation², which would allow a quantitative analysis of the control and regulation of citric acid production. Finally, we want to assess the quality of the model by carrying out a sensitivity analysis and studying the predictions of the model against perturbations of the basal steady state.