Computerized methods for the determination of stability constants

Complexation of crown ethers with organic molecules

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Crown ethers are synthetic, cyclic polyethers with many interesting properties, of which the ability to form complexes with metal salts, with organic salts and with neutral organic molecules is the most prominent one. In theory, it should be possible to design suitable 'host' molecules, based on crown ethers and related molecules, that can form complexes with small organic 'guest' molecules, for example with urea. In order to guide the synthesis of these possible host molecules, it is necessary to be able to determine the (thermodynamic) stability constants of the complexes of crown ethers with the guest molecules. The aim of the work described in this thesis is the development of suitable automatic procedures for this purpose, with emphasis on computerized electroanalytical methods and on the complexation of urea by crown ethers in polar solvents.

There are many applications of crown ethers in analytical chemistry and their (possible) physiological and pharmacological properties include transport of metal ions through biological membranes, antibacterial, antiviral and fungicidal activities, and antidotal applications. There are several (computerized) methods and procedures for the determination of stability constants, including a number of frequently used procedures for non-linear regression.

An indirect polarographic procedure for the determination of stability constants was developed. By means of a competitive reaction, the observed changes in half-wave potential and/or limiting current of the reduction wave of a suitable indicator species upon addition of the crown compound can be used for the determination of stability constants of crown ether complexes. For the evaluation of the experimental data, a modified version of the computer programme 'POLAG' was used. The procedure has been applied to the study of the complexation of urea by some crown ethers in methanol, using alkali metal ions as indicator compounds. The method was evaluated with respect to accuracy and precision using a number of synthetic data sets as the input for the modified 'POLAG' programme. From the results of this study, conclusions can be drawn with regard to the limitations of the (indirect) polarographic procedure.

The polarographic procedure was automated by means of computerization of the experimental procedure and of the evaluation of the data. For the control of the experiments from software, FORTH computer language was employed. The specific properties of this language make it a very suitable language for laboratory automation. As a result of the automation of the procedure, the accuracy was improved and a considerable reduction of the time needed for the total experiment was achieved.

Subsequently, an automated procedure for the conductometric determination of stability constants was elaborated. The software developed for this purpose was written in FORTH as well. Stability constants of the complexes of alkali metal chlorides with some small crown ethers (i.e. 12-crown-4, 15-crown-5 and benzo-15-crown-5) in methanol, water and a mixture of these solvents have been determined by means of this procedure, which has also been applied to the study of the interaction between urea and crown ethers in methanol. In the latter case, an indirect procedure had to be applied as well. The guanidinium ion was found to be a suitable indicator species for this purpose. From the equivalent conductivities of the complex species (which can be determined from the experimental data), information on the solubility characteristics of the ionic species involved may be obtained.

The interaction between urea and some crown ethers in methanol, water and mixtures of these solvents (using the indirect procedures described above) was also studied. The salts of alkali metals, heavy metals and guanidine were used as indicator species. The choice for one of these salts is determined by the combination of solvent, crown ether and experimental methods to be applied. For all the

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crown ethers employed, the stability constants of the complexes with urea were very small, especially for the crown ethers with small ring sizes. The guanidinium ion, however, forms more stable complexes with the larger crown ethers (such as benzo-27-crown-9 or di-benzo-30-crown-10) in methanol.

The computerized electroanalytical methods outlined above have proved to be suitable tools for the study of complexation of ionic species as well as neutral molecules by crown ethers in polar solvents. The binding forces between urea and the crown ethers employed in this study are very weak. More stable complexes may be obtained by using intramolecular binding sites such as proton-donating groups or metal ions.

Results were also presented in the following papers:

Production of L-DOPA by suspension grown cells of Mucuna pruriens


Promoters: Prof. Dr. Th. M. Malingré (Groningen) and Prof. Dr. W.J. Feenstra (Groningen).

L-DOPA is a drug that is used for the symptomatic relief of Parkinson's disease. Several plant species are known to contain L-DOPA. Mucuna pruriens (Leguminosae) is known to contain 4-9% of L-DOPA in the seeds with reference to the dry mass. Therefore, this species was chosen for the establishment of callus and cell suspension cultures. Two approaches were chosen to achieve the production of L-DOPA by these cell cultures: firstly, the endogenous production in cell suspension cultures was studied; secondly, immobilized cells were used for the bioconversion of L-tyrosine into L-DOPA and of N-formyltyrosine into N-formyl-DOPA.

First, callus and cell suspension cultures of M. pruriens were established. In these cultures the presence of L-DOPA could be demonstrated by a number of advanced analytical methods (thin layer chromatography, high pressure liquid chromatography with UV or electrochemical detection, gas chromatography, gas chromatography in combination with mass spectrometry and thermal desorption mass spectrometry). The enantiomeric purity of the L-DOPA was investigated with chiral phase HPLC; no d-DOPA could be detected in the cultures.

A number of cell cultures was established. Although there was some variability in cell growth (14.5-20.0 mg/ml), the variation in L-DOPA content was much greater: 0.2-1.7%. L-DOPA was accumulated intracellularly by the suspension cultures (more than 95% of the total production). Figure 1A shows some growth characteristics of a cell suspension culture of M. pruriens. From Figure 1B it can be read that after six days of cultivation the L-DOPA content of the cells is maximal. Sucrose is the best carbon source for cell cultures of M. pruriens; L-DOPA production was maximal at a sucrose concentration of 3%.

Potassium nitrate is an excellent nitrogen source, both for growth and for L-DOPA production. A decreased phosphate concentration in the medium resulted in decreased cell growth, but in a stimulation of L-DOPA production. Limitation of nitrogen-containing compounds resulted in a decreased accumulation of L-DOPA. It was postulated that only in a case of relative excess of nitrogen-containing compounds, L-DOPA production was stimulated. Also the nature and the concentration of the plant hormones in the medium were important:


Key words: Biosynthesis; Cell culture; Dopa; Mucuna pruriens.