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Monitoring and control of industrial downstream processing of sugar beet molasses

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Abstract

In present work the determination of several amino acids during the industrial chromatographic desugarisation of molasses is presented. The use of innovative biosensor systems for highly specific detection of serine is described. Using two-dimensional fluorescence spectrometry, a non-invasive method for the determination of several product fractions could be established in an industrial chromatographic procedure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Process monitoring; Biosensors; Molasses; Amino acids

1. Introduction

Sugar beet molasses is a natural source for various products used in daily life, ranging from sucrose to amino acids for pharmaceutical use. The separation of molasses into these high value components is performed at AMINO (Frellstedt, Germany) on a large scale by ion-exclusion chromatography. Six columns, each with 60 m^3 capacity, are used for the separation of molasses. This is one of the world's largest molasses desugarisation plants with an annual capacity of $60 \cdot 10^6$ kg.

Typical products of this process are liquid sugar, nucleic acids, betaines and amino acids. The on-line control of the chromatographic process is based up to now on the monitoring of physical variables like density, conductivity, pH and refractive index. For example the sucrose concentration is estimated on-line by polarimetry: the change in the rotation of light is correlated to the amount of sucrose in the sample. In the presence of other compounds interfering with light rotation (e.g., glucose and other sugars, lactic acid, ethanol), the measurement will not correspond to the actual sucrose concentration in the effluent stream. No direct on-column method is available for the detection of sucrose during the chromatographic cycle.

Also, no analysis is available for the in-time analysis of amino acids like serine so far. The behaviour of the chromatographic columns can change from cycle to cycle (cycle time: 2 h), especially after complete regeneration or changes in the composition of molasses. The effluent stream must be cut in different fractions, each containing a certain compound. Often the fraction cuts for the wanted product are difficult to exactly determine. If the product is collected in the wrong or in different fractions, it might be necessary to rechromatograph the whole fraction.

Within this research project two innovative analy-

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sis methods were developed to be used for the on-column and in-time analysis of different important process parameters. Highly specific biosensor systems enable AMINO to determine the serine fraction in-time for each chromatographic cycle. The analysis of one cycle is performed during 1 h in comparison to 24 h using the off-line high-performance liquid chromatography (HPLC) method.

Another non-invasive method is used with two-dimensional fluorescence spectroscopy. This method enables the on-column detection of fluorescence. Some interesting products of the separation are coeluted with unknown fluorophores. Thus, detection of these components is possible using this very fast method. In general these coeluted substances are downstreamed in a later process stage.

In this article the two innovative analysis systems are described for the detection of several amino acids, which are used for pharmaceutical purposes.

2. Experimental

2.1. Apparatus

All biosensor experiments are carried out with a flow injection analysis system purchased from Trace Biotec, Germany. As detector a commercial photometer (Skalar 6010) with a flow-through cell was used (detection of consumed NADH at 340 nm). The measuring range of this system is 0–0.5 g l^{-1} serine. The flow-rate of the buffer (50 m*M* potassium phosphate, pH 8.0) was 1.5 ml min^{-1}. The measurement was performed as a stopped-flow assay (30 s). The system was used at a constant temperature of 32°C.

For all two-dimensional (2D) fluorescence experiments a commercially available process fluorimeter from Delta Light and Optics, Denmark was used (BioView). The fibre optics were directly coupled into a bypass at the outlet of the chromatographic column. The measurements were performed at the process temperature of 80°C.

2.2. Reagents

All chemicals were purchased in the highest available purity grade from Sigma (Germany). The D-serine dehydratase was isolated from *Klebsiella pneumoniae* using an ammonium sulphate precipitation of the cell debris. Molasses samples from AMINO, were tested.

2.3. Procedures

The enzymes used in the biosensor were immobilised according to the following procedure. A 200-mg amount of VA-Epoxy Biosynth (Riedel-de Haen, Germany) in 1.5 ml potassium phosphate buffer (pH 7.7) was incubated with a solution of 30 U of the partially purified D-serine dehydratase and 360 U of lactic dehydrogenase for 24 h at room temperature. The support polymer was then washed with buffer and free epoxy groups were saturated with 0.01% 2-aminoethanol for 2 h. The immobilised enzymes were used in a flow-through cartridge with a volume of 1 ml (Mobitec, Göttingen, Germany).

3. Results and discussion

3.1. Biosensor analysis

One of the advantages of a biosensor system is the high specificity of the biological component of the sensing system. Using enzymes one is able to select systems which only react with the analyte of interest during the chromatographic separation of sugar beet molasses. Coupled with a flow injection biosensor system an automatic device was set up for the in-time analysis of serine during the molasses desugarisation process. The system consists of an automatic sampling device which is able to dilute the samples and to inject reagents used in this assay (e.g., NADH), and of a commercially available flow injection analysis (FIA) system (Trace Biotec). For the detection of the enzymatic conversion of the analyte a photometer was used as described above.

D-Serine was analysed by converting it using the multi-enzyme system D-serine dehydratase/lactic dehydrogenase. During this reaction D-serine is first converted into pyruvate, which is subsequently converted into lactate (Fig. 1). The NADH consumed stochiometrically during this reaction was determined spectrophotometrically. The method shows good linearity ($R=-0.99899$) in the range of 0.1 to

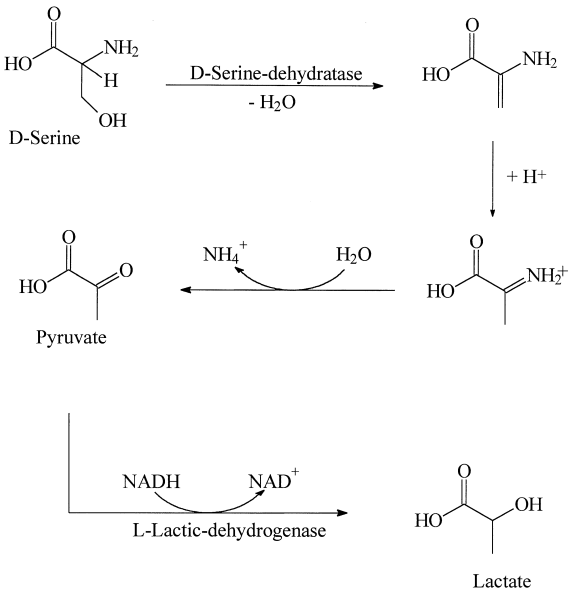

Fig. 1. Reactions in the multi-enzyme system for the detection of D-serine.

0.5 g l^{-1} D-serine. An analysis could be performed within 2–5 min by directly injecting samples from the chromatographic process into the FIA system. The standard deviation for the measurement of D-serine was 1.3% (10 measurements, D-serine concentration of 0.25 g l^{-1}).

The same system was used for the determination of sucrose. In this case the enzyme system invertase–mutarotase–glucose oxidase was used. The oxygen consumed in this reaction was monitored amperometrically and is proportional to the sucrose concentration in the sample [1]. However, the analysis time is too long for real-time control purposes, since the product fraction is eluted within about 5–10 min. For this reason, the biosensor system is used for ''in-time'' analysis: The product fraction is collected in 30-s intervals and analysed one after the other during two chromatographic cycles (cycle time 2 h). Based on the data obtained, the profile of the next product fraction is determined in advance. Thus the best fraction interval t_1 and t_2 (Fig. 4) can be obtained and adjusted to the actual state of the column. The whole process of sampling, analysis and adjusting t_1 and t_2 takes part during two chromatographic cycles and is finished in time, before the next product fraction is collected. The working principle of this system is shown in Fig. 2.

The sensors are stable for at least 1 week under process conditions and the immobilised enzyme can be stored for at least 3 months (−18°C). A biosensor analysis of one complete chromatographic separation cycle is shown in Fig. 3. The knowledge of this process variable leads to a better control of the molasses desugarisation process. The timing for collecting product fractions of interest can be determined more accurately. Thus tremendous ecological and economical advantages were obtained using the intelligent, in-time biosensor system for supervis-

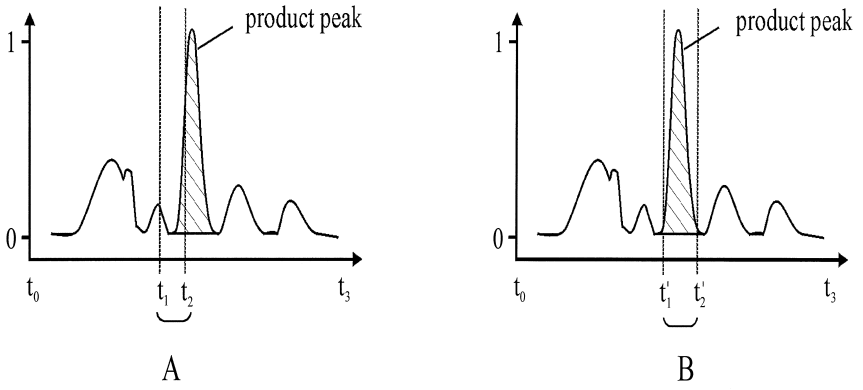

Fig. 2. Set-up of in-time analysis during chromatographic processes. (A) The first chromatographic cycle is collected and measured during column regeneration using the biosensor system described in the text. Thus, the product fraction can be determined (t_1-t_2) (not optimal product fraction). (B) If the product cut is not optimal the fraction cuts are shifted to times t_1' and t_2' (optimised product fraction) for the next chromatographic cycle. Thus, the results of each chromatographic cycle are used to predict the optimal product fraction for the next cycle.

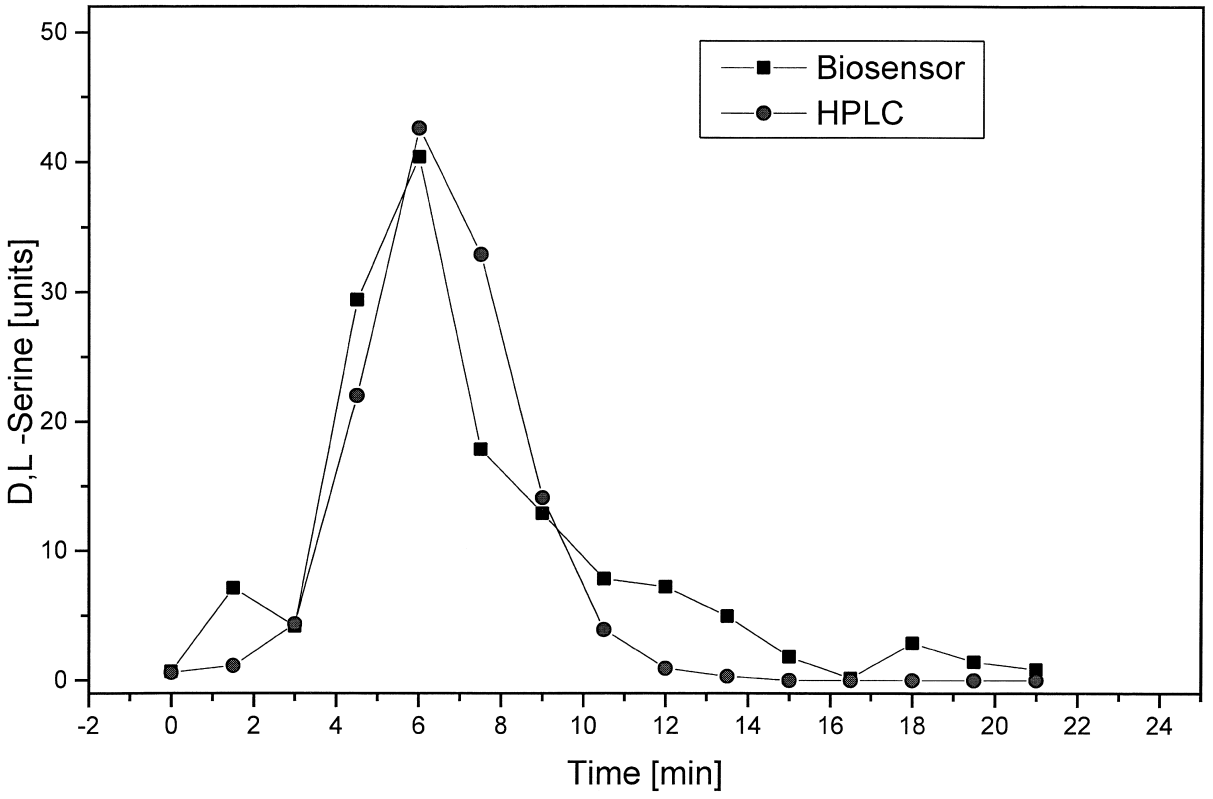

Fig. 3. In-time analysis of D-serine during chromatographic separation of sugar beet molasses using the biosensor system (comparison with HPLC analysis). Concentration of D,L-serine is indicated in relative units (business secret of AMINO).

ing the downstream processing. Serine yield could be increased by 25% with a reduction of 30% in energy needed. On a yearly basis, 3500 m^3 of waste water could be saved. This leads in a reduction of $180 \cdot 10^3$ kg of chemical oxygen demand (COD) per year.

3.2. 2D Fluorescence monitoring

Fluorescence sensors are fibre optical measuring instruments which have been investigated over the last 15 years for different applications in biotechnology, such as biomass concentration determination, bioreactor characterisation and metabolic studies (e.g., aerobic/anaerobic transition) and particularly bioprocess monitoring. With 2D fluorescence, all fluorophores present in the molasses can be monitored simultaneously. On the basis of this technique, a non-invasive sensor was developed which gains information about the process. A 2D spectrofluorometer was used (BioView, Delta Light and Optics) with independent filter wheels for excitation and emission with up to 16 different filters each which can be designed individually [2,3]. Using this instrumentation, measurements in steps of 20 nm are performed (excitation range: 270–550 nm, emission range: 310–590 nm), which was found to be most suitable without any loss of information. At the beginning of the monitoring of a complete 2D fluorescence spectrum, the excitation filter wheel is set to the first filter. The excitation light is guided via the fibre optic into the bioreactor. The backward fluorescent light is monitored via the emission filter which switches from filter to filter for a whole rotation cycle. Afterwards the excitation filter wheel

switches to the next filter and the emission spectrum of the fluorescence produced by this defined excitation wavelength is monitored by one complete cycle of the emission filter wheel. This procedure is repeated until the excitation filter wheel has performed one complete cycle. Afterwards a new spectrum can be measured. The number of measurements for each filter position can be individually chosen. The BioView sensor includes a software package, for sensor control and data analysis, which is able to predict the trend of the further course of the process. A complete spectrum can be collected within 1 min, while the monitoring of a single wavelength only takes seconds. It is possible to determine amino acids like tryptophane, tyrosine and phenylalanine directly. Even non-fluorescent compounds like serine can be detected by correlation of different emission/excitation wavelengths to the serine concentration that was determined off-line by HPLC (see Fig. 4). This effect can be explained by elution of an unknown fluorophore. This fluorophore elutes about 1 min prior to serine in this industrial downstream process of a complex agricultural raw material.

4. Conclusions

Bioanalytics offers interesting features for better chromatographic monitoring. Based on the detailed data obtained, better modelling and control can be achieved resulting in more efficient processes with regard to product quality, product quantity and overall ecological and economical performance. Within the very next future more applications of intelligent bioanalytics must be given, showing the advantages of this sophisticated downstream process monitoring. Researchers and applicants must cooperate to develop modular system units which can be brought together to meet the special needs of individual processes.

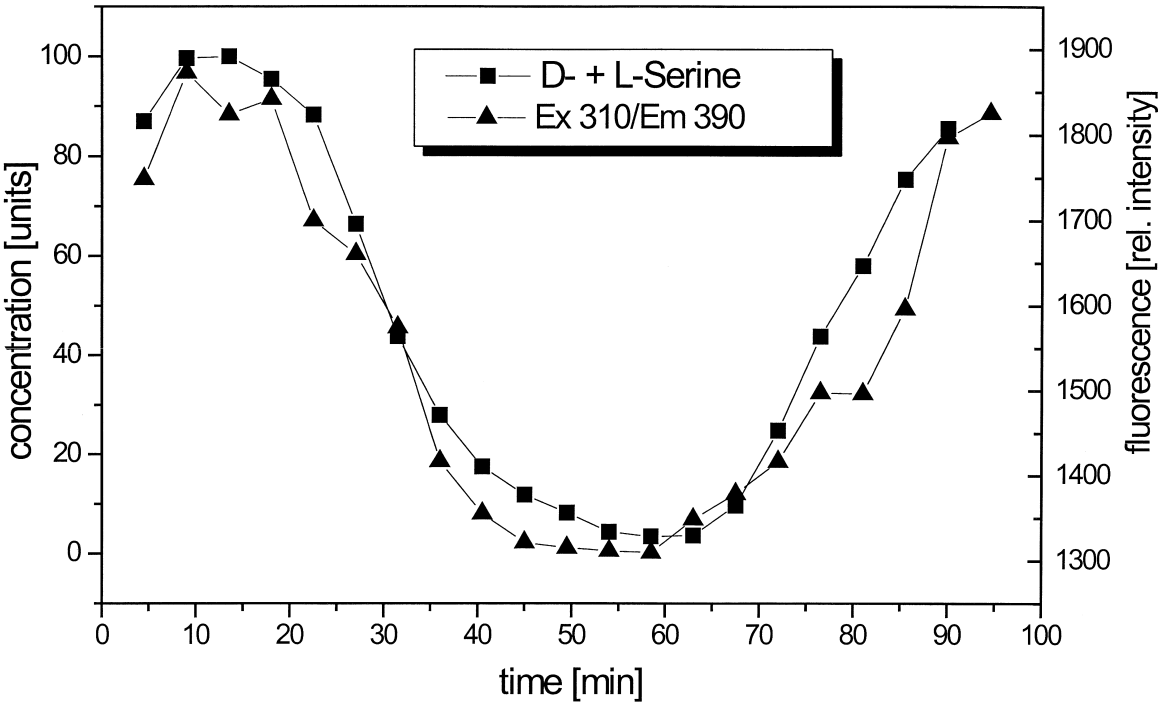

Fig. 4. Fluorescence intensity (excitation 310, emission 390 nm) (▲; right axis) and D,L-serine concentration (determined using off-line HPLC data) (■; left axis). Concentration of D,L-serine is indicated in relative units (business secret of AMINO).

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