DETERMINATION OF ETHANOL FOR PROCESS AND QUALITY CONTROL USING FLOW INJECTION ANALYSIS

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SUMMARY

Flow injection analysis was used to determine ethanol in beverages, pharmaceutical products and fermentation media without the requirement for sample pretreatment or dilution. The sensitivity of the assay was modified by the thickness of a gas diffusion layer. The cost per sample (20 assays) was 1.5 Pfennig (DM 0.015). The frequency of analysis was 120-180 assays per hour, making it suitable for the on-line monitoring of fermentation processes. Samples of wine, beer, spirits, medicine and fermentation broth were tested and a good correlation to the standard methods was obtained.

INTRODUCTION

The use of immobilized enzymes in flow injection analysis (FIA) is increasing rapidly [1]. Immobilization helps to overcome the most serious problems in the use of biocatalysts, especially enzyme instability and high costs per assay. In industry and other areas of chemical analysis large numbers of samples have to be processed in a short time at minimum cost. FIA along with the immobilized enzymes combines rate, sensitivity and precision of the technique with the selectivity, economy and convenience of the immobilized catalyst. This method has been used for the measurement of ethanol in serum [2] and beverages [3] with detection systems such as spectrophotometry, fluorimetry, voltammetry or thermistor probes. But the linear range of the assay was always limited more by the enzyme activity than by the detection system, necessitating sample dilution. This additional source of error could be avoided by using the automated dilution techniques of FIA such as zone sampling [4] or diffusion. In the case of ethanol the volatility of the analyte allows good separation using gas diffusion via hydrophobic membranes.

EXPERIMENTAL

The set-up of the FIA is shown in Fig. 1. The gas diffusion membrane was a silicone modified polypropylene membrane. The donor (H_2O) and the acceptor (potassium phosphate buffer, 100mM, pH 7.5) streams, diffusion unit and enzyme reactor were maintained at 30°C. The immobilized enzyme reactor (20 x 2 mm) contained alcohol oxidase (AOD) from *Pichia pastoris* (Provesta Corp., Bartlesville, USA) covalently bound to controlled pore glass (CPG) (Serva, Heidelberg, FRG) via glutaraldehyde.

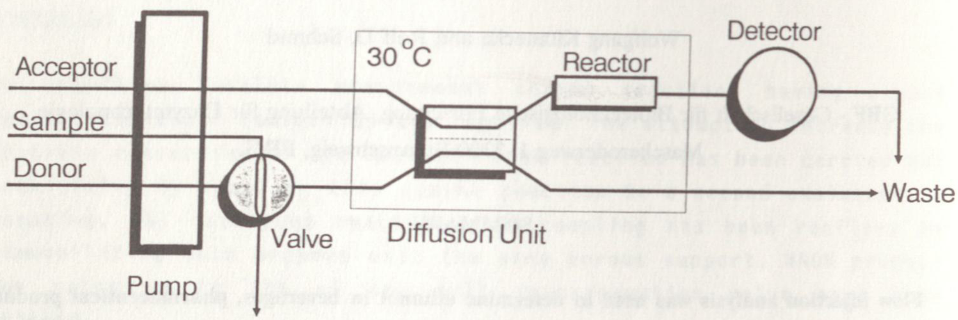

Fig.1: The FIA manifold for ethanol determination using immobilized alcohol oxidase.

The sample (10µl) is injected into the donor stream via the injection valve. An aliquot of the ethanol in the sample passes through the diffusion unit into the acceptor stream and is carried to the enzyme reactor. Ethanol is then converted to acetaldehyde and hydrogen peroxide by the following reaction:

$$\text{ethanol} + O_2 \xrightarrow{AOD} \text{acetaldehyde} + H_2O_2$$

The H_2O_2 is oxidized at an amperometric detector (platinum electrode; 700 mV vs Ag/AgCl). The resulting current is proportional to the ethanol content of the sample.

RESULTS AND DISCUSSION

Linear range and correlation

A linear range of 0 - 0,8 % (v/v) ethanol was obtained with the non-modified membrane. A greater diffusion barrier with a silicone layer on the polypropylene membrane increased the linear range up to 60 % (v/v) ethanol. Correlation to the standard methods is shown in table 1.

Table 1. Linear range of the FIA technique and its correlation to standard methods.

Analyte	Linear range	n	Correlation	Reference method
Fermentation broth	0 - 15 %Vol	24	r=0,9998	Gas chromatography
Beer	0 - 7 %Vol	10	r=0,9901	Distillation
Wine	0 - 20 %Vol	12	r=0,9990	Refractometry
Pharmaceuticals	0 - 40 %Vol	9	r=0,9993	(Manufacturer)
Spirits	0 - 60 %Vol	26	r=0,9981	Distillation

Interference

The major interference was obtained with lower primary alcohols, particularly methanol. However, these compounds did not interfere with the assay at concentrations normally found in food (< 200 ppm). Other substances investigated were: sulphite, acetone, 2-propanol, glycerol, 1,3-propanediol, 1,4-butanediol, 2,3-butanediol and acetic acid (15% vol). These compounds had no influence on the accuracy of the ethanol assay in mixtures nor did they give an independent signal.

Lifetime

The storage stability of the immobilized enzyme was more than 6 months. Under operational conditions with 180 assays per hour and substrate saturation, i.e. maximum conversion, the lifetime was at least 3500 injections (90%).

Analysis of spirits

The ethanol content of spirits was determined using a 60 μm silicone layer on the diffusion membrane. Dilution of the samples was not necessary in the range of 0 - 60% vol ethanol. Different samples of commercial spirits were determined according to the official method (distillation) by Prof. Misselhorn and coworkers (Institut für Gärungsgewerbe, Berlin, FRG). The correlation between FIA and the reference method is shown in Fig. 2.

On-line fermentation monitoring

The sampling line of the FIA was connected to a fermenter in order to investigate its applicability to process control. The ethanol formed during a fermentation of *S. cerevisiae* was measured continuously. The thickness of the silicone layer was about 15 μm with a corresponding range of linearity of 1 - 400mM ethanol. Fig.3 depicts a part of the ethanol profile on the original chart including the calibration. The whole ethanol profile was compared with the glucose concentrations determined with an in situ sensor by Bradley et al. [5].

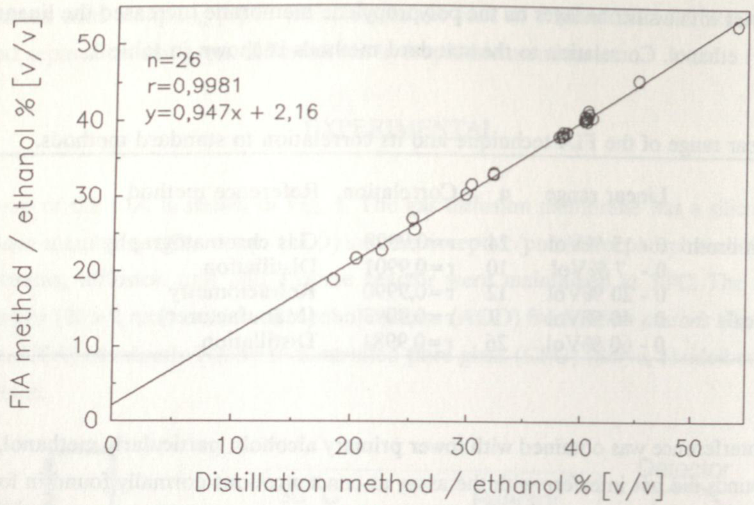

Fig. 2: Correlation of the FIA signals to the distillation method.

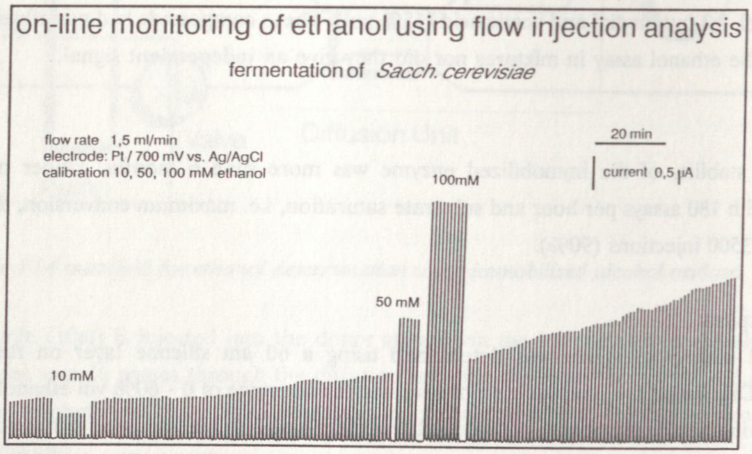

Fig.3: Original chart of an ethanol fermentation

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