

Analysis of glucose and fetal calf serum in aqueous solution for Multi Organ Tissue Flow (MOTiF) bioreactors using NIR Spectroscopy

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Abstract: Future drug tests will be performed on cell cultures to avoid animal tests. Therefore a Multi Organ Tissue Flow bioreactor was developed. In this paper an approach to detect cell activity via detection of nutrient consumption with Near Infrared Spectroscopy is presented. The ingredients glucose and fetal calf serum were analysed and calibrated.

Keywords: Multi Organ Tissue Flow, bioreactor, glucose, fcs, nutrient solution, Near Infrared Spectroscopy, NIRS

Introduction

Many medications are tested on animals. Current work is about reduction and avoidance of animal suffering. In future new drugs will mainly be tested on cell cultures. In these tests the interrelation of effects and side effects between different cell types is often hidden. To solve this problem a bioreactor which allows the evaluation of effects of medications and metabolism with a high throughput is developed at Jena University Hospital. In this Multi Organ Tissue Flow bioreactor the metabolism of cultures of liver, lung and renal cells can be reproduced and evaluated in a circular flow.

Near Infrared spectroscopy (NIRS) is a fast and non-destructive method to characterise tissue and chemical compounds [1][2]. It allows the analysis of cell density, nutrient and dye concentration as well as the dye uptake in cells. In this paper two ingredients of nutrient solution are studied via NIRS and brought to the limit of detection.

Methods

Glucose and fetal calf serum (fcs) are the main components of nutrient solution used in the Multi Organ Tissue Flow bioreactor. For the analysis of these two components a serial dilution was prepared. The content of glucose in aqueous solution was varied between 0 – 30 g/l in a first setup and 0 – 3 g/l in a second setup, while fcs concentration was varied between 0 – 110 g/l.

The experimental setup consisted of a *Bruker MPA* FT-IR spectrometer with fibre optic transmission extension, a *QUANTUM Northwest* temperature controlled sample compartment and a *Hellma Analytics* absorption cell with 0.2 mm layer thickness. The temperature was adjusted to 37°C.

In order to get the optimal results appropriate wavelength regions had to be chosen. The optimal data preprocessing

has to be determined iteratively. Following options for the iterative approach were tested to provide optimal results:

- no data preprocessing
- subtraction of a constant offset,
- subtraction of a line,
- vector normalisation (snv),
- min-max-normalisation,
- first derivation,
- second derivation,
- first derivation and subtraction of a line and
- first derivation and vector normalisation (snv).

During this iterative procedure the parameters of quality (coefficient of determination R², root mean square error of cross validation RMSECV, residual prediction deviation RPD) were evaluated and consequently the best calibrations chosen.

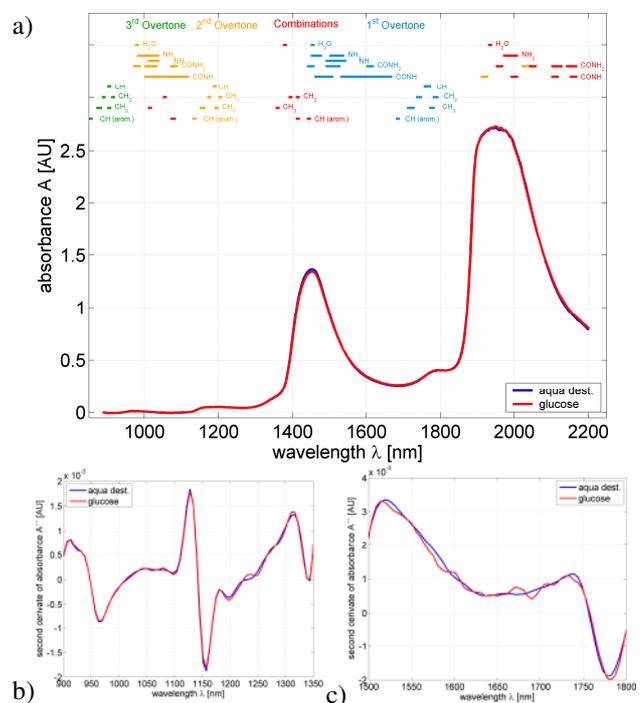


Figure 1: The absorbance of aqua dest. and aqueous solution of glucose as well as assignment to functional groups [3] (a) and second derivate A'' in details (b, c)

The absorbance A of water and aqueous solution of glucose and the second derivatives A'' in details are shown in

Figure 1. The absorption bands of water are dominant and both spectra are approximately congruent (a), but the second derivate details demonstrate differences (b) that can be identified and interpreted via statistical methods.

Results

All spectra of the first setup (glucose 0 – 30 g/l) were used for a calibration shown in Figure 2. The calibration utilises the wavelength ranges of 1124 – 1251 nm and 1887 – 2278 nm. The prediction of one sample (30 g/l) is proposed to be an outlier and became marked red.

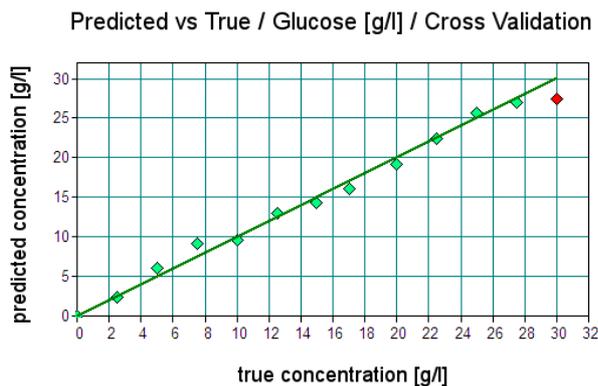


Figure 2: Comparison of true and predicted concentration of glucose in aqueous solution with a concentration range of 0 – 30 g/l

The spectra of the second setup (glucose 0 – 3 g/l) were used for a calibration shown in Figure 3. The calibration, using the wavelength ranges of 801 – 863 nm, 1251 – 1615 nm and 1887 – 2278 nm, offers a more accurate prediction for concentration between 1.4 – 3 g/l than for concentrations between 0 – 1.4 g/l.

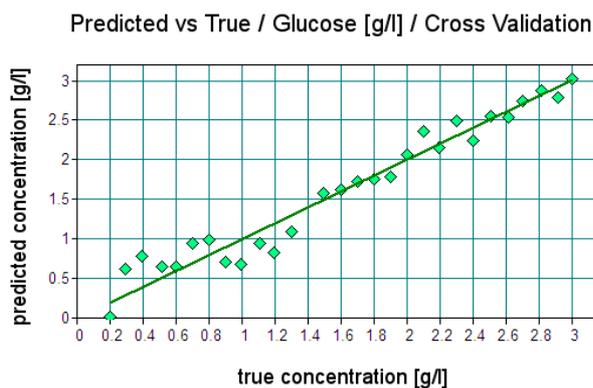


Figure 3: Comparison of true and predicted concentration of glucose in aqueous solution with a concentration range of 0 – 3 g/l

The scatter diagram of the calibration for fcs, which utilises the wavelength ranges of 1124 – 1651 nm and 1887 – 2278 nm and all 15 samples, is shown in Figure 4. The results of the calibrations are listed in Table 1. The calibration of glucose with 13 samples within the range of 0 – 30 g/l is suited for quality assurance in any application. The calibration of glucose within 0 – 3 g/l with 28

samples offers a lower level of determination, but also a lower RMSECV and is suited for quality control, while the calibration for fcs is suited for screening. The absorption bands of peptide bonds are overlaid by water, while the absorption bands of glucose are less influenced by water.

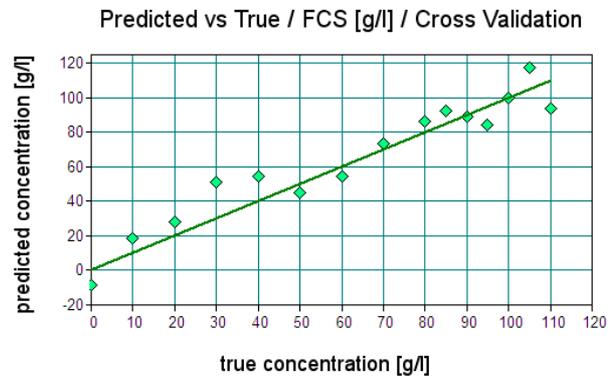


Figure 4: Comparison of true and predicted concentration of fcs in aqueous solution

Table 1: Parameters of quality of the cross validations

Calibration	R ²	RMSECV	RPD
glucose 0 – 30 g/l	98.78	1.03 g/l	9.22
glucose 0 – 3 g/l	95.48	0.181 g/l	4.7
fcs 0 – 110 g/l	91.49	10.2 g/l	3.53

Discussion

One possible approach to detect cell activity is via the measurement of nutrient consumption. The results indicate that the analysis of ingredients in nutrient solution allows the detection of nutrient consumption by cells that are cultivated in the Multi Organ Tissue Flow bioreactor. The absorption bands of peptide bonds and water are superimposed, making fcs hard to detect. Although the concentration of glucose is lower than that of fcs, its prediction is more accurate down to the limit of detection.

Acknowledgement

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