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Development of a new method for the performance evaluation of the precision of POC devices according to CLSI standard EP5-A2: stabilizing whole blood

Diploma/Master thesis

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Graz, FEBRUARY 2013

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Acknowledgments

> One, remember to look up at the stars and not down at your feet. Two, never give up work. Work gives you meaning and purpose and life is empty without it. Three, if you are lucky enough to find love, remember it is there and don't throw it away.
>
> Stephen Hawking

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Zusammenfassung

Für die Durchführung einer Performance Evaluierung der Präzision eines *point of care* (POC) Gerätes nach CLSI wird eine Mindestdauer von 20 Tagen empfohlen. Dies setzt eine stabile Probe voraus, die über den Zeitraum der laufenden Evaluierung in Bezug auf die zu untersuchenden Parameter unveränderlich bleibt. Eine Forderung die für biologisch aktive Proben nicht erfüllt werden kann. Das Ziel dieser Arbeit war es eine, für das POC Blutgas Analyse Gerat *cobas b123* optimierte und auf die Parameter Glukose und Laktat abgestimmte Methodenentwicklung zur Durchführung einer Performance-Evaluierung der Präzision, welche möglichst Nahe an der Vorgabe nach der CLSI Richtlinie EP5-A2 ist, bereit zu stellen. Dieses Ziel ist stark mit der Notwendikeit einer Methode zur Präparation einer, in Bezug auf die zu untersuchenden Parameter Glukose und Laktat, stabilen Probe verbunden. Da die eigentliche Messung durch die Probenpräparation nicht beeinflusst werden sollte und somit die Wahl der Methode sehr stark von der Kompatibilität mit dem verwendeten Blutgas Analyse Gerät abhängt, ist die Auswahl an möglichen Stabilisatoren sehr eingeschränkt. Die Kompatibilität der dafür in Frage kommenden chemischen Reagenten (Deoxyglukose, Glyceraldehyd, Natriumfluorid, Quercetin, Isorhamnetin und Bromo-Brenztraubensäure) mit dem Sensor wurde in speziell dafür designten Experimenten untersucht und es konnte gezeigt werden, dass eine ausreichend starke Inhibierung der Glykolyse, und damit eine Verminderung der Abbaurate von Glukose auf unter 0.1 % h zu erreichen, ohne dabei den verwendeten Glukosesensor zu beeinflussen, mit keiner der untersuchten Substanzen erreicht werden konnte. Aufgrund der Komplexität von Vollblut und mangels Sensor-kompatiblen Anti-glykolytischen Wirkstoffen konnte keine neue Methode zur Durchführung der Performance-Evaluierung erstellt werden und es gilt weiterhin die Empfehlung bei dem bisher verwendeten Protokoll zu bleiben.

Abstract

For the performance evaluation of the precision of point of care (POC) devices a standardised evaluation of the precision over 20 days is proposed by CLSI. This evaluation requires a stable sample that does not change in the parameters to be analysed, a requirement that cannot be achieved for biologically active samples such as whole blood. The aim of this work was to establish a measurement protocol optimised for the performance evaluation of the precision of the POC blood gas analyser *cobas b123* for the parameters glucose and lactate concentration that is closer to the recommended CLSI standard. This is strongly linked to the need of a method to stabilize the glucose and lactate concentration in the whole blood sample that is compatible with the measuring device. Due to the fact that the outcome of the precision test depends heavily on the compatibility of the method used to stabilise the glucose concentration, the choice of methods is limited. The compatibility of a selection of chemical reagents characterised by previously done research as anti-glycolytic agents (deoxyglucose, glyceraldehyde, sodium fluoride, quercetin, isorhamnetin, bromopyruvic acid) was tested in specially designed experiments. It could be shown that a sufficient inhibition of the glycolysis, reducing the rate of glucose decomposition below 0,1%/h without compromising the compatibility with the sensor principle in use, cannot be achieved with the investigated anti-glycolytic agents. Due to the complexity of the whole blood sample in combination with the implemented sensor principle and the lack of a feasible anti-glycolytic agent, no new protocol was proposed, but the usage of the established protocol is recommended.

Definitions

anti-glycolytic agent refers to a chemical substance that is used to inhibit the cellular glucose metabolism.

blood gas (BG) parameters originally referred to pO_2 and pCO_2. In the course of this work, BG parameters refer to the whole palette of measured parameters by the *cobas b123* blood gas analyser. This includes additionally to pO2 and pCO2, pH, Hct, Na+, K+, Ca2+, Cl-, Glu, Lac and CO-oxymetry.

point of care (POC) device is a device that is used for laboratory diagnostic testing performed at or near the site where clinical care is delivered, and therefore mostly outside of the well-controlled environment of the traditional, core laboratory [1]. POC testing includes: blood glucose testing, blood gas and electrolyte analysis, screening for abusive drugs, urine strip testing, pregnancy testing, fecal occult blood analysis, food pathogen screening, haemoglobin diagnostics, infectious disease testing and cholesterol screening [2].

glycolytic rate Originally, this term refers to the turnover rate at which glucose is metabolised to lactate during glycolysis. Under physiological conditions this rate should be, per definition negative. In the course of this paper, the term *glycolytic rate* describes the rate at which the blood glucose concentration apparently changes and is used to assess the efficacy of the inhibiting chemical reagent. It can be either negative, or in case of positive interference behaviour, positive.

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Abbreviations

2-DG	2-Deoxyglucose
3-BrPA	3-Bromopyruvic acid
ATP	adenosin triphosphate
BSA	bovine serum albumin
CE	counter electrode
CLSI	Clinical and Laboratory Standards Institute
CP	creatine phosphate
CPD	citrate phosphate dextrose
CPDA	citrate phosphate dextrose adenine
EDTA	ethylenediaminetetraacetic acid
ETC	electron transport chain
G6P	glucose-6-phosphate
G6PDH	glucose-6-phosphate dehydrogenase
GA	Glyceraldehyde
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GOD	glucose oxidase
GTP	guanosine triphosphate
Hct	haematocrit
HK	hexokinase
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
ISE	ion selective electrode
LOD	lactate oxidase
MSS	metabolite sensitive sensor
NaF	Sodium fluoride
PCr	phosphocreatine
POC	point of care
RBC	red blood cell
RE	reference electrode
SAGM	saline-adenine-glucose-mannitol
STD	standard deviation
TCA	tricarboxylic acid cycle
WE	working electrode

> Perhaps it would be simpler if you just did what you're told and didn't try to understand things.
>
> Terry Pratchett, Sourcery

1. Introduction

1.1. Point of care testing

Providing rapid results and reliable information on the status of a patient has always been an important issue in health care institutions where therapeutic decisions are based on this informations. Modern blood gas (BG) analysers provide data on critical parameters such as blood gas, electrolytes, metabolites and CO-oximetry parameters [3]. Traditionally these tests have been performed on stationary, bulky analysers in central laboratories. Advances in the field of sensor technology and improved design of the devices, it is now possible to move these measurements closer to patient or so-called point of care (POC) environment. POC devices try to manage the span between providing fast and high quality analytical performance while at the same time minimizing their footprint, keeping turnaround times low and operation and maintenance simple [4]. With diabetes becoming an increasingly important issue in health care, fast and accurate measurement of blood glucose with POC BG analysers has also become a prominent topic. Especially when it comes to maintaining tight glycaemic control in critical care units, POC measurement of glucose is favourable to central laboratory diagnostic due to their low turnaround times [5].

1.2. Precision testing according to CLSI

For a POC device to be legally sold on the Northern American market it needs to be approved by the American food and drug agency (FDA). To receive this approval the manufacturer needs to declare that the device meets the requirements specified by corresponding standards. For analytical medical devices such as the POC BG Analyser *cobas b123* by Roche characteristics that describe the quality of the measurement are of special interest. Trueness, accuracy and precision are such characteristic parameters. In order to prove these performance claims in respect to the previously named parameters several standards and guidelines are provided by institutions such as the *Clinical and Laboratory Standards Institute* (CLSI) and the *International Federation of Clinical Chemistry and Laboratory Medicine* (IFCC) [6, 7]. The *accuracy* describes the closeness of agreement between a measured quantity value and a true quantity value of a measurand while *trueness* relates to the closeness of agreement between the average of an infinite number of

replicate measured quantity values and a reference quantity value [8]. Furthermore, the term *precision* refers to the closeness of agreement between independent test or measurement results obtained by replicate measurements under stipulated conditions [6]. This leads to the term *intermediate precisions*. It describes the precision under conditions where measurement results are obtained under the same test set-up, such as; a) time, calibration, operator, equipment or b) between-run, within-day, between-day, within-device, *within-laboratory* (=former *total precision*). A *run* defines an interval within which it is expected that trueness and precision do not change and is usually shorter than 24 hours. The *repeatability* refers to the precision under a set of conditions, which include the same measurement procedure, same operators, same measuring system, same operating conditions and the same location or replicate measurements on the same or similar objects over a short period of time.

To determine these parameters a so-called precision evaluation experiment needs to be conducted.

1.2.1. CLSI method

In order to evaluate the precision of a system the CLSI recommends conducting the experiment over a minimum of 20 operating days [6]. The first 5 operating days should serve as a familiarisation period, for the operational staff to become familiar with the experimental protocol. For every different type of sample *random access analyser*, two repeat determinations per day and level are necessary and should be timed such that at least two hours are between the individual measurements. Moreover, each type of sample should be analysed at no less than two different levels of concentration ,i.e. high and low. Furthermore, a minimum of 4 devices on which the precision test is performed is required. The required minimum of 20 days in combination with the familiarisation period is to ensure that only the imprecision of the device itself leads to the final precision result, and sampling, handling or preparation errors can be safely excluded from further considerations. Nevertheless, it is vital that for the whole duration of the experiment the study is performed under the previously mentioned conditions for repeatability (see 1.2). This also applies to the properties of the analysed sample itself, which leads to the need of a sample of which its behaviour in respect to the to-be-measured analyte concentrations is exactly known at all times for the total duration of the experiment. Otherwise it can not be differentiated between possible imprecisions as a result of a false measurement or due to actual changes in the analyte concentrations.

1.2.2. Current FDA conform method

Due to the complexity and associated difficulties in regard to living tissue samples, a reduction from 20 to 10 day precision was accepted by the FDA. However, whole blood

cannot be kept constant over more than a few minutes or hours at the maximum for most of the blood gas (BG) parameters. One way to work around the lack of stability of whole blood is to simulate the 10 or 20 day precision test on one single day by using multiple devices instead of one. The simulation is performed by using one device for each run and analysing 2 aliquots of one previously prepared whole blood sample on all devices simultaneously. All other variations demanded by the CLSI standard, such as variation of calibration cycles, multiple consumable lots, order of analysis, different runs per day and different operators can be provided for this set-up. In the case of the FDA-approved shortened version of a 10-day simulated precision test, a minimum of 20 devices is needed. From statistical analysis point of view, it makes sense to use more than the minimum required number of devices in case unexpected problems occur during the course of measurement and some devices have to be excluded from the analysis. Each device is assigned to one specific day and run. Then the precision test can be performed within a short enough time interval in order to guarantee that the concentrations of the analytes have not changed significantly. However, due to the high number of devices needed this method is associated with a high financial effort. Not only the acquisition costs have to be considered but also arising expenses for maintenance and laboratory staff have to be taken into account.

1.2.3. Motive of this work

The declared objective of this work was to develop a strategy to carry out a performance evaluation of the precision of a POC device i.e. the *cobas b123* that is closer to the original method suggested by the CLSI guideline EP5-A2 [6]. One major criterion for success was to find a way to keep a certain set of clinical relevant blood gas parameters of a whole blood sample at a constant level for a minimum of 5 hours, or more. By keeping the whole blood sample stable for at least 5 hours the number of devices could be reduced by 50%. In order to be able to attribute possible imprecisions solely to measurement errors of the sensor or the system and not to changes in the analysed sample a threshold of 0.1% change per hour for the corresponding parameter was postulated [1]. Furthermore, the method used to stabilise a certain parameter should have as little influence as possible on the physiological characteristics of whole blood. This means that ideally other parameters should be unbiased by the reagent. Due to the complexity of whole blood and the fact that glucose and lactate were considered to be two of the parameters with the highest rate of change and extremely difficult to get under control by established methods, this work was strongly tied to the need of producing a whole blood sample that allows the performance of a precision test for glucose and lactate simulated for a duration of ten days with only 50% of the previously necessary number of devices, i.e. 10.

[1] This threshold value is based upon the specified precision of the cobas b123, see specification in the user manual [9]. A more elaborate exemplification can be found in B.3.

1.3. Whole blood

In the course of this work the term whole blood refers to untreated collected human whole blood. Whole blood is a non-newtonian fluid which consists of two components; blood plasma and blood cells. With an essential part of 92 % water and 8% protein, plasma can be considered an aqueous fluid.

The liquid medium of the blood accounts for 55% of the total blood volume. Besides proteins which are mainly fibrinogenes, albumins, globulins and other clotting factors, plasma transports blood gases, e.g. O_2 and CO_2, various nutrients, e.g. lipids, carbohydrates, vitamins and nucleic acids, numerous hormones, electrolytes as Na^+, K^+, Ca^{2+}, Mg_2^+ and Cl^-, metablites and waste products such as lactate or nitrogenous wastes [10, 11, 12].

Usually, 44% of the blood volume is made up by blood cells, the so called haematocrit. Lymphocytes, thrombocytes and erythrocytes form the cellular part of whole blood. With 95%, erythrocytes are the predominant component of the haematocrit. The number of erythrocytes varies in average from 4,7 x 10^6 to 6,1 x 10^6 per μL for males and from 4,2 x 10^6 to 5,4 x 10^6 per μL for females. Between the blood plasma and the red blood cells (RBC) a brisk exchange of particles and substances prevails. Due to diffusion processes, small molecules and ions can pass from the plasma through the cell membrane into the cytoplasm and vice versa, depending on the diffusion gradient (see figure 1.1).

In order to counter the natural diffusion process and to keep up the necessary cell membrane potential, but also to allow the controlled exchange of metabolites, hormones or waste products, various transporter and channels are embedded into the cell membrane of the RBCs. Through these, the previously mentioned components of the plasma can be transported into the cell, and vice versa components of the cytoplasm of the RBC can be transported out of the cell. The transport of ions against the diffusion gradient implies the consumption of energy. So for example the Na^+/K^+-pump requires ATP in order to transport Na^+ out of the cell and K^+ into the cytoplasm. An overview of all relevant transporter, co-transporter and ion-channels is given by figure 1.2.

In the case of the RBC, the membrane is virtually impenetrable for high energy molecules such as ATP, NADH / NAD^+ or creatin phosphate (CP). Therefore the energy is mainly provided by anaerobic glycolysis, where glucose is metabolised via numerous steps to pyruvat and lactate. Figure 1.3 depicts the entire metabolic pathway for glucose in a RBC. The complete glycolysis yields a net gain of 2 ATPs per glucose molecule. The aerobic citric acid cycle or tricarboxylic acid cycle (TCA) would yield another 2 ATPs. In combination with the subsequent oxidative phosphorylation during the electron transport chain (ETC), the aerobic pathway then would yield a total of 36 ATP. However, the RBC only relies on the anaerobic metabolism. The anaerobic metabolic pathways TCA and ETC are not present in the RBC. Therefore glucose is the main energy source for the

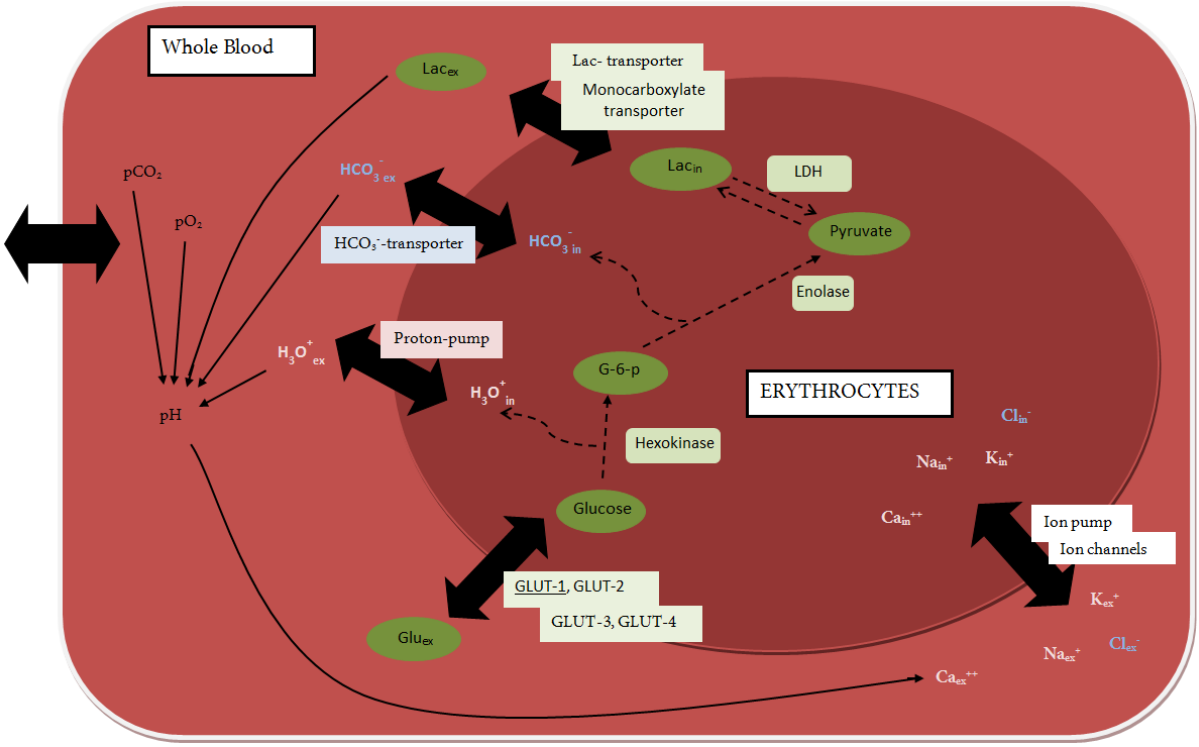


Figure 1.1.: Model of interaction between extracellular whole blood and human red blood cell depicting the most relevant intracellular metabolic pathways and their influences on BG parameters - based on [13], [14] and [15].

RBC [13, 14].

While glucose is eventually metabolised to lactate the glucose level decreases and, as a direct consequence of the ongoing glycolysis the lactate level increases. Furthermore lactate and several side products of the glycolysis such as H_3O^+ and HCO_3^- have an direct influence on other BG parameter. The most critical parameter is without doubt the pH value. The number of protons H_3O^+ and the concentration of HCO_3^- but also the lactate concentration, the partial oxygen and carbon dioxide pressure have a major influence on the pH level of whole blood.

1.3.1. Inhibition of glycolysis

Based on the results of the literature research a model was established (see figure 1.1). The RBC membrane is considered to be virtually impermeable for high energy models such as ATP, guanosine triphosphate (GTP) and CP [16], [17]. Therefore, glucose is the major energy source for RBCs and enters the cell with the help of specific transporters, mainly by GLUT-1 but also to some extent by GLUT-3 and GLUT-4 [15]. In order to stabilize the extracellular glucose level, the inhibition of the glycolytic pathway was considered to be the essential key. This can be achieved in various ways, either by changing the ambient conditions that effect the activity of the involved enzymes or by interfering at a specific point in the metabolic pathway. By cooling down the whole blood sample to

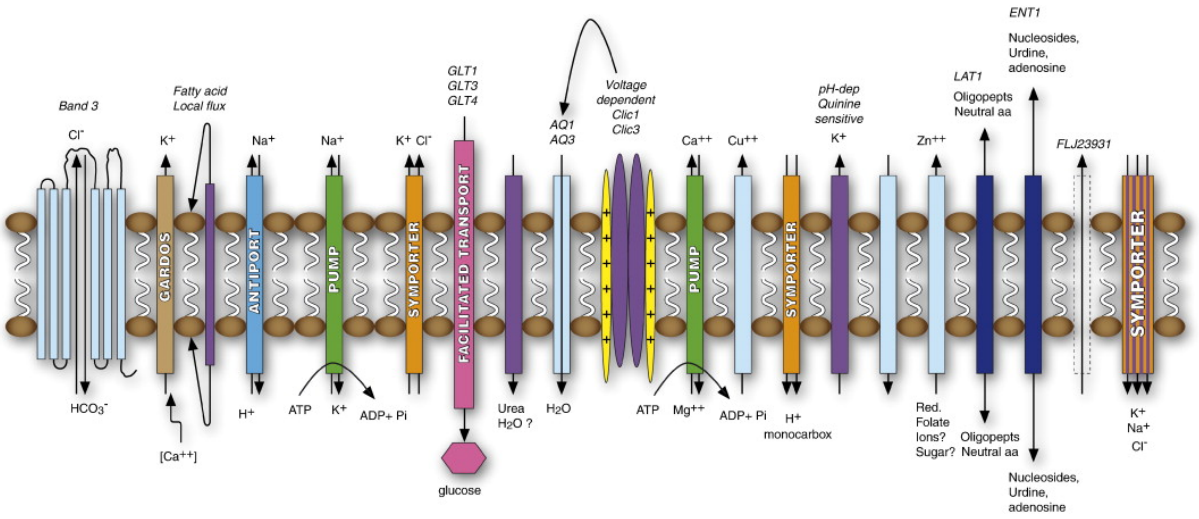


Figure 1.2.: Model of the red blood cell membrane depicting all anchored ion channels, transporter and co-transporter anchored in the membrane. Cited from Pasini et al. [15].

temperatures i.e. 2 - 8°C the metabolic activity of the enzymes is reduced drastically and the decomposition of glucose and corresponding increase in lactose concentration can be slowed down by the 3,5 to 7 fold (compare 3.1, 3.2 and [18]). Furthermore, the activity of the enzymes can be slowed down by acidification. An environment with pH considerably lower than the physiological pH level of 7.2-7.4 also has a negative effect on the performance of glycolytic enzymes [19, 20, 21]. Gambino et. al [20] were able to show that with acidification of whole blood with citrate buffer, the rate of glycolysis can be slowed down to 0,15% per hour. However, slowing down or stopping the metabolic activity of the RBCs by acidification was not considered as an option since one major requirement for the whole sample preparation and initially the measuring protocol was to guarantee that the procedure has as little impact on the rest of the measured BG parameters as possible. Due to this requirement well known preanalytical procedures such as treatment with EDTA or CPD, CPDA and SAGM had also to be eliminated as a putative option.

Another way to stabilize the extracellular glucose level is to inhibit the key enzymes in the metabolic pathway such as hexokinase (HK), glucose-6-phosphate isomerase (GPI), glucose-3-phosphate dehydrdrogenase (GAPDH) and enolase, respectively. One of the longest known glucose stabilisers is sodium fluoride. Already in the thirties of the previous century the effect of NaF on blood was studied. Yoshimaru [22] in 1935 and later Siggard-Andersen et al. [23] in 1961 already discussed the effects of NaF on the acid-base values of whole blood. Nevertheless, it had been discovered that the inhibition of glycolysis with NaF has a major drawback. As an antiglycolytic agent NaF targets enolase, an enzyme at the end of the glycolytic pathway. Until the competitive inhibition of enolase by the fluoride ion binding to the active side of the enzyme [24] becomes effective and a constant glucose level can be observed it can take up to three hours [25, 26]. Therefore, over the

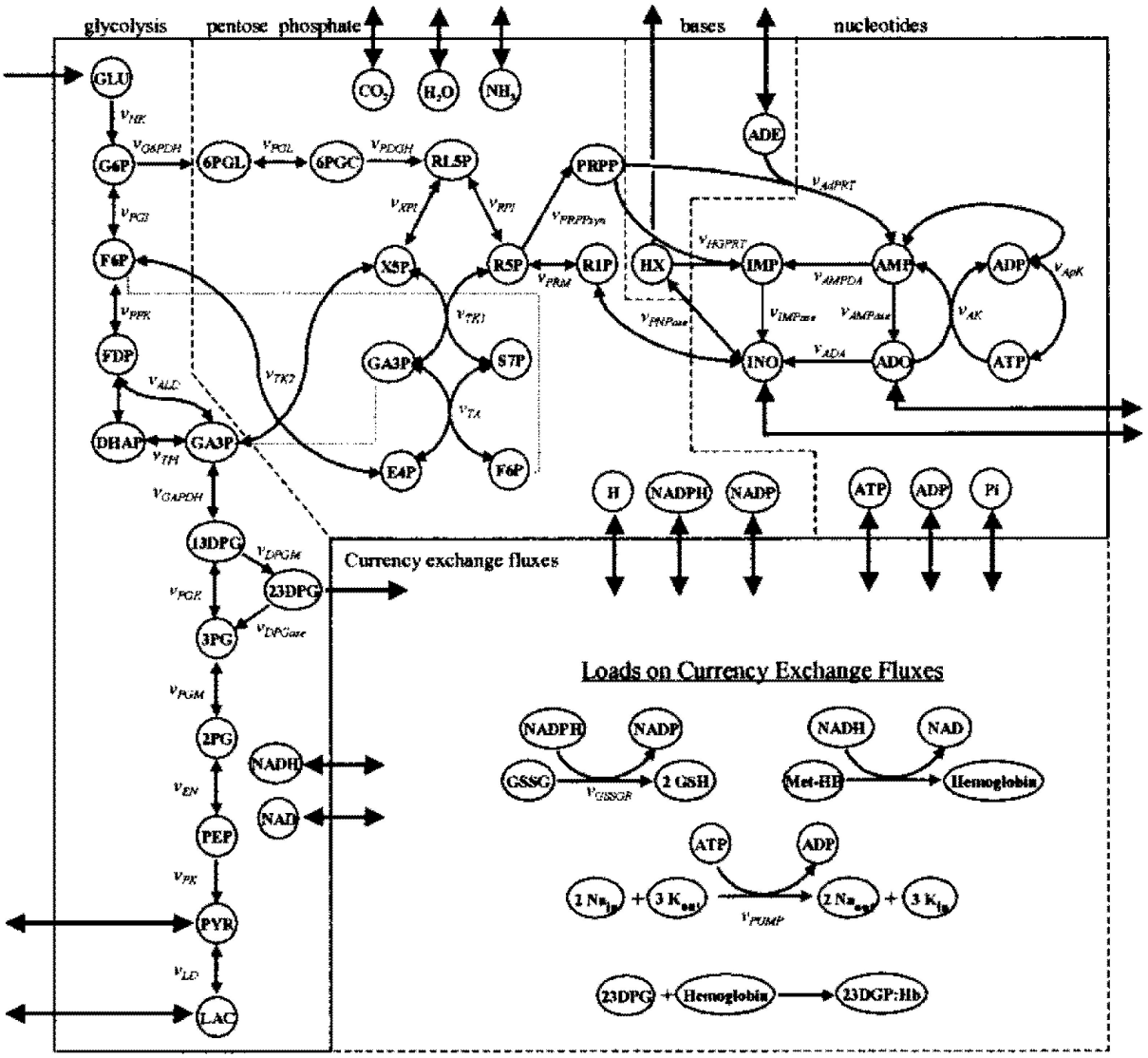


Figure 1.3.: Model of the metabolic pathways that are present in a normal human RBC, i.e. glycolysis, pentose phosphate pathway, base and nucleotide exchange and currency exchange fluxes. Cited from Wiback et al. [13].

first three hours glucose is still decomposed and the initial glucose concentration cannot be maintained.

In the course of the search for new anti-cancer treatments several other anti-glycolytic agents were determined that target key enzymes in the glycolysis such as the HK, GPI or GAPDH [27, 28, 29]. 2-Deoxy-D-Glucose and lonidamine are known to effectively inhibit the HK enzyme by reversible competitive inhibition. 3-BrPA was shown to inhibit HK activity in cancer cells due to selective alkylation of sulfhydrul groups of the enzyme. However, in non-tumor cells 3-BrPA seems to have a higher potency as an inhibitor of GAPDH than of HK [30]. Different sugar derivatives, such as D-mannose [31] or glyceraldehyde also inhibits the HK and due to its favourable characteristics (nontoxic, relatively stable at room temperature and inexpensive) they are considered to be ideal anti-glycolytic agents [32].

Another approach to keeping the extracellular glucose level constant is by making sure that extracellular glucose molecules are hindered from entering the RBC from the start. This can be achieved blocking the glucose transporter, which ships glucose into the RBC and therefore is responsible for the decrease of the extra cellular glucose concentration in the first place. The mycotoxin Cytochalasin B for example, can either act as competitive inhibitor or as non-competitive blocker of GLUT1, depending on the side of inhibition [33]. Vera et al. [34] were able to show that several flavones and isoflavones are capable of inhibiting hexose transporter GLUT1 in human erythrocytes. Especially the tyrosine kinase inhibitors quercetin, rhamnetin and isorhamnetin seem to have a very strong inhibitory effects by binding to one of the functional regulatory binding sites of GLUT1.

If you're not part of the solution,
you're part of the precipitate.

Henry J. Tillman

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals, reagents and buffers

Substance	Company	Kat. nr
- 2-Deoxy-D-Glucose	Sigma Aldrich GmbH	D8375-1G
- 3-Bromopyruvic acid	Sigma Aldrich GmbH	16490-10G
- Aqua Bi-Dest	Fresenius Kabi	
- D-(+)-Glucose	Sigma Aldrich GmbH	
- Calciumcloride dihydrate	Merck KGaA	
- HEPES	Sigma Aldrich GmbH	
- human whole blood	Baxter Plasmazentrum Graz	
- Potassium Cloride	Merck KGaA	
- Sodium Carbonate	Merck KGaA	
- Sodium Cloride	Merck KGaA	
- Titrisol®NaOH 1M	Merck KGaA	
- Ouabain octahydrate	Sigma Aldrich GmbH	O3125-1G
- Quercetin	Sigma Aldrich GmbH	Q4951-10G
- DL-Glyceraldehyde	Sigma Aldrich GmbH	G5001-1G
- Sodium fluoride	Sigma Aldrich GmbH	201154-100G
- Isorhamnetin	Sigma Aldrich GmbH	17794-5MG

Buffer solutions

Table 2.1.: **Dialysis buffer: ISE/GLU nat V2** - recipe for standard dialysis buffer solution for medium glucose level. For dialysing 45 mL whole blood 2 L of buffer solution were prepared with H_2O-BiDest.

	concentration		
	g/mM	g/L	g/Vol
Na_2CO_3	17.85	1.892	3.7838
NaCl	99.3	5.803	11.6062
KCl	5.1	0.380	0.7605
HEPES	62.5	14.894	29.7875
$CaCl_2$	1.45	0.213	0.4264
NaOH (1M Titrisol)	10	10.000	20.000
Glu	6.5	1.171	2.3421

Table 2.2.: **Dialysis buffer: ISE/GLU nat V3** - recipe medium glucose level, standard ISE levels but reduced Na-level. For dialysing 45 mL whole blood 2L of buffer solution were prepared with H_2O-BiDest.

	FW	concentration		ion balance					g/Vol
	g/mol	mM	g/l	Na	K	Ca	Cl	F/Br	
Na_2CO_3	105.99	17.85	1.892	35.7					3.7838
NaOH (1M Titrisol)	1000	10	10.000	10.0					20.0
NaCL	58.44	70	4.091	70.0			70		8.1816
KCL	74.56	5.1	0.380		5.1		5.1		0.7605
Glukose	180.16	6.5	1.171						2.3421
Glyceraldehyd	90.08	10.0	0.901						1.8016
HEPES	238.3	55.5	13.226						26.4513
Calciumchlorid	147.02	1.45	0.213			1.45	2.9		0.4264
NaF	41.99	65.0	2.729	65.0				65.0	5.4587
	total ion balance			145.7	5.1	1.45	78	65.0	

Stock solutions

Table 2.3.: **Stock solution: Glucose 50x** - Vol: 500 mL stock with H_2O−Bidest

	FW	concentration		
	g/mol	mM	g/L	g/Vol
Glucose	180.16	1000	180.160	9.0080

Table 2.4.: **Stock solution: DG 5M** - Vol: 5 mL with H_2O−Bidest

	FW	concentration		
	g/mol	M	g/L	g/Vol
DG	164.16	2.5	410.4	20.52

Table 2.5.: **Stock solution: DG 5M** - Vol: 5 mL with H_2O−Bidest

	FW	concentration		
	g/mol	M	g/L	g/Vol
DG	90.06	2.5	225.2	1.126

Table 2.6.: **Stock solution: GA 5M** - Vol: 0.5 mL with H_2O−Bidest

	FW	concentration		
	g/mol	M	g/L	g/Vol
GA	90.08	5	450.3	0.225

Table 2.7.: **Stock solution: quercetin 0.05M** - Vol: 1.25 mL with 0.2M NaOH

	FW	concentration		
	g/mol	M	g/L	g/Vol
GA	338.28	0.05	16.91	0.021

Table 2.8.: **Stock solution: isorhamnetin 5mM** - Vol: 1 mL with H_2O

	FW	concentration		
	g/mol	M	g/L	mg/Vol
GA	316.26	0.005	1.58	1.58

Table 2.9.: **Stock solution: Na-3-BrPA 2.5M** - Vol: 2 mL with H_2O-Bidest

	FW	concentration		
	g/mol	M	g/L	g/Vol
3-BrPA	166.96	5	0.83	
Na_2CO_3	105.99	2.5	0.265	
H_2O-bidest				

Table 2.10.: **Stock solution: NaF 5M** - Vol: 5 mL with H_2O-Bidest

	FW	concentration		
	g/mol	M	g/L	g/Vol
NaF	41.99	5	209.95	1.05

2.1.2. Devices

cobas b123	Roche Diagnostics	blood gas analyzer
cobas b221	Roche Diagnostics	blood gas analyzer
Hitachi 902	Roche Diagnostics	chemical analyzer
XS205 Dual Range	Muttler Taledo	analytical balance
Centrifuge 5702	Eppendorf	centrifuge

2.1.3. Kits and other material

OmniFix®10ml	B.Braun	10ml plastic syringe
OmniFix®-F	B.Braun	1ml plastic syringe
Cobas 200 μL cappilary tubes	Roche	glas capillary tubes
RCT basic safety control IKAMAG®	IKA	magnetic stirrer
ZelluTrans/Roth V-series 38mm	Carl Roth	dialysis membrane MWCO: 1000 Da
ZelluTrans/Roth V-series 45mm	Carl Roth	dialysis membrane MWCO: 1000 Da

2.2. Methods

2.2.1. Amperometric glucose sensor

Both the glucose and lactate sensors that are integrated in the *Roche* sensor cartridge in the *cobas b123* POC BG analyser, as well as the sensors used by the reference device *cobas b221* blood gas analyser, operate according to the same amperometric sensor principle. The sensor is based upon a 3-electrode array. A model of the 3-electrode array is shown in figure 2.1. The sensor consists of a working (WE), a reference(RE) and a counter electrode (CE).

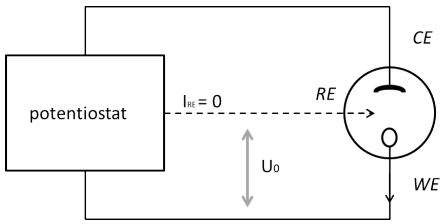


Figure 2.1.: Model of a 3-electrode setup: WE... working electrode, CE... counter electrode, RE... reference electrode.

At the WE an enzyme (in the case of the b123 and b221 *glucose oxidase* (GOD) and *lactate oxidase* (LOD) respectively are put to use) is immobilised on a membrane [9].

The enzyme catalyses a reaction:

$$\beta\text{-}D\text{-}Glucose + O_2 \xrightarrow{\text{GOD}} \text{glucono acid} + H_2O_2 \tag{2.1}$$

$$\text{L-lactic acid} + O_2 \xrightarrow{\text{LOD}} \text{pyruvic acid} + H_2O_2 \tag{2.2}$$

The thereby produced H_2O_2 is then oxidised at the WE and an electrical current results which is, in theory, directly proportional to the concentration of the analyte.

$$H_2O_2 \longrightarrow 2\,e^- + O_2 + 2\,H^+ \tag{2.3}$$

In order to completely oxidise H_2O_2, a potential difference of 600 mV is recommended [35]. By substituting the platinum layer for a manganese dioxide/carbon coating for the electrode material the required potential of 600 mV can be further reduced to 350 mV (vs. Ag/AgCl).

When measuring glucose concentration in a non ideal sample such as whole blood, the resulting amperometric signal u_s[1] is of course biased by interfering signals. These interfering signals have their origin in the limited specificity of the enzyme and the sensor design. While interfering reactions between the GOD on the WE and other substances other than glucose are monitored by the so-called BSA-sensor, other interfering incidents such as pO_2 and pH values in the sample have to be considered as well, resulting in a corrected glucose signal

$$u_{s_{corr}} = u_s * c_{corrO2} * c_{corrpH} + u_{corrCl} - C * u_{glu_{corrBSA}} \tag{2.4}$$

where the influence of pO_2 and pH are taken into consideration by the multiplicative factors c_{corrO2} and u_{corrCl}, and further influences of the chloride concentration and interference incidents on the WE are taken into account additively by u_{corrCl} and $C*u_{glu_{corrBSA}}$. The influence of the BSA sensor is discussed in detail in the following section. A detailed analysis of the chloride influence can be found in B.2.

2.2.2. BSA sensor and BSA correction algorithm

In addition to the glucose and lactate sensor, another biosensor based on the amperometric sensor principle is integrated on the sensor cartridge. Instead of an active enzyme, bovine serum albumin (BSA) is immobilised on the sensor tip in order to detect and compensate unspecific electrochemical interferences such as when confronted with samples containing paracetamol, ascorbic acid or uric acid. If a interference signal is detected, which exceeds a threshold BSA_{min} a correction algorithm, based on a weighted compensation model kicks in [35, 36]. Equation 2.5 shows the influence of incorporated BSA correction algorithm. The algorithm corrects the measured voltage from the glucose sensor by a weighted correction factor and is applied to its fullest effect for measurements where BSA equivalent levels above the upper threshold z_1 are detected. Below this threshold the influence of the BSA correction algorithm has only minor effects.

$$u_{glu_{corrBSA}} = u_{glu} - b_p * w_{c_{BSAs}} * c_{BSAs} * u_{D2BSAn} \tag{2.5}$$

where c_p is unit less, specific batch parameter that is obtained during calibration states Cal-1 and Cal-2 and can be approximated by

[1] the resulting amperometric signal is a current in mA but for easier comparison and further processing, the current is transformed to a corresponding voltage in mV.

$$c_p = \frac{\frac{u_{D1n}}{Kint}}{u_{D2BSAn}} \approx 0,3 \tag{2.6}$$

and

$$u_{D2BSAn} = BSA_{Slope} \tag{2.7}$$

where BSA_{Slope} is another batch specific parameter and the weighting function w_{BSA} is a simple ramp function with a lower ($z_0 = 1$) and upper ($z_1 = 2.5$) threshold value

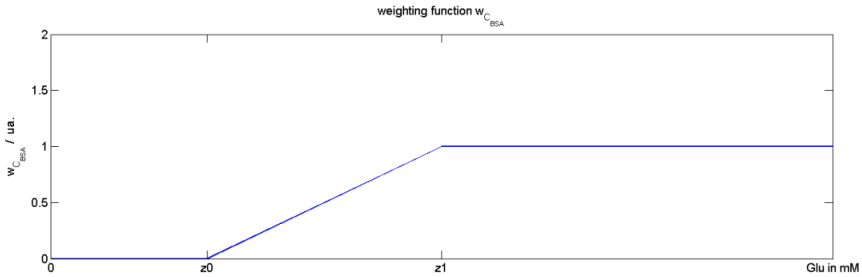


Figure 2.2.: For BSA equivalents higher ($z_0 = 1$) the glucose concentration is corrected, taking the BSA signal into account. The influence of the BSA signal on the glucose signal is governed by the weighting function $w_{c_{BSA}}$ - see equation 2.8.

$$w_{c_{BSA}} = \begin{cases} 0 & \text{if } c_{BSA_s} \leq z_0 \\ \frac{c_{BSA_s} - z_0}{z_1 - z_0} & \text{if } z_0 < c_{BSA_s} < z_1 \\ 1 & \text{if } c_{BSA_s} \geq z_1 \end{cases} \tag{2.8}$$

and c_{BSA_s} is the BSA equivalent in mM. Calculations that quantify the influence of the BSA-correction algorithm can be found in the appendix B.1 and a detailed analysis of the influence of the chloride correction algorithm is presented in B.2.

2.2.3. Optical glucose sensor

Besides detecting glucose and lactate with electrochemical means several optical methods have been established as well. Most methods make use of an enzyme catalysed reaction in combination with measuring a change in the absorption characteristics of the medium. As reference to the amperometrically obtained measurement results a Hitachi 902 Analyser was used. This analyser makes use of the hexokinase method. The glucose in serum or plasma is first transformed under the consumption of energy in form of ATP into glucose-6-phosphate (G6P):

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{G-6-P} + \text{ADP} \tag{2.9}$$

The resulting G6P together with NAD^+ is then transformed by the G6P dehydrogenase (G6P-DH) to gluconat-6-phosphate, NADH and one proton:

$$\text{G-6-P} + \text{NAD}^+ \xrightarrow{\text{G6P-DH}} \text{D-Gluconat-6-phosphate} + \text{NADH} + \text{H}^+ \tag{2.10}$$

The rate of change of the NADH concentration is directly proportional to the glucose concentration present in the sample and can be determined at a wavelength of 340nm.

Furthermore, the Hitachi 902 Analyzer measures the glucose concentration in plasma instead of whole blood.

2.2.4. Implementation of the Experiments

Experimental Setup Experiment 1 (MR001)

For each run, fresh heparinised whole blood of one individual donor was used. The whole blood sample ($\approx$ 45ml) was dialysated over night (>12h) in order to re-establish the values for ISE (Na, K, Cl, Ca and pH) and MSS (Glu) within a physiological range. For this purpose a puffer solution (2 L) was created according to table 2.1. Before the start of the measuring series the individual samples were prepared. The dialysed blood was then divided into aliquotes of 10 mL. Each aliquote was treated with a different anti-glycolytic agent. In addition, one aliquote was left untreated and served as control sample. For the duration of the experiment, all samples were kept at room temperature. Furthermore, each sample was rolled on a roller mixer for at least 20 minutes in order to guarantee homogeneous distribution and mixture of the substance in the blood. Thereafter, the ISE and MSS levels were monitored over the next 6 hours by taking samples in a 30 to 60 minutes interval. The samples were taken via 200 μL glass capillaries and analysed on the devices (*cobas b123* and *cobas b221*) in capillary mode. Figure 2.3 illustrates the previously described course of the experiment.

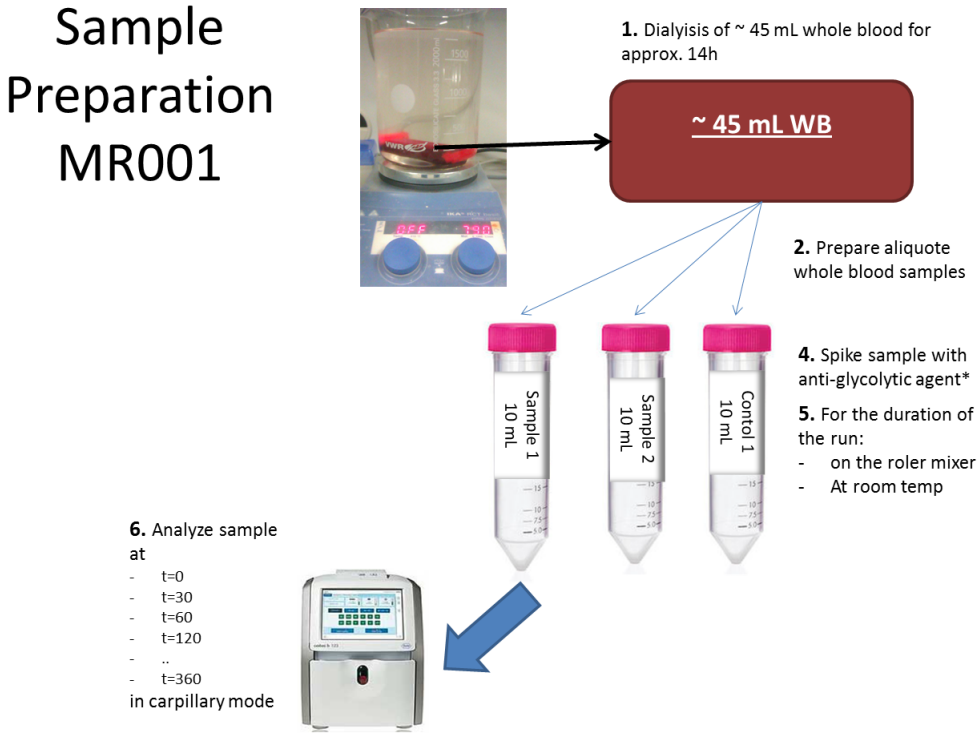


Figure 2.3.: Design and course of experiment MR001.
*) the exact concentrations depended on the anti-glycolytic agent.

Experimental Setup Experiment 2 (MR002)

In figure 2.4 the experiment design for the second experiment MR002 is presented. For each run heparinised whole blood of one individual donor was used. The blood was received around noon of the previous day. The experiments were started in the morning of the following day. Over night the blood was kept in the refrigerator at 8 $°C$. Before the start of the measurement, the whole blood was first spiked with a glucose spike solution (50x) - see table 2.3 - to raise the glucose level to its native concentration. After 15 minutes on a shaker, the total volume of 90 mL whole blood was separated into aliquotes of 23 mL per sample. Each sample was then treated with the same anti-glycolytic agent, but with different concentrations. After spiking the respective concentration, each sample was rolled on a roller mixer for 10 minutes. Then each sample was aliquoted again into 6 subsamples to 3.5 mL each, whereby each subsample represented one measuring point. This was to ensure that each individual measurement was unbiased by the previous measurements. The first aliquot of each sample was analysed at beginning of the experiment (t=0). Thereafter measurements were taken in an interval of 60 - 90 minutes. The last aliquot was analysed after approximately 6 hours (t = 360 min). For the ongoing of each individual run all aliquotes were kept in the refrigerator at 8$°C$ on a roller mixer. The analysis of the samples was performed with 6 *cobas b123*, one *cobas b221* and 1 Hitachi 902 analyser. Therefore 7 capillaries were filled simultaneously and inserted into the devices (7 x 200 μL). The remaining volume of the aliquot was centrifuged and the resulting plasma sample was stored in the refrigerator for later analysis with the Hitachi 902 analyser.

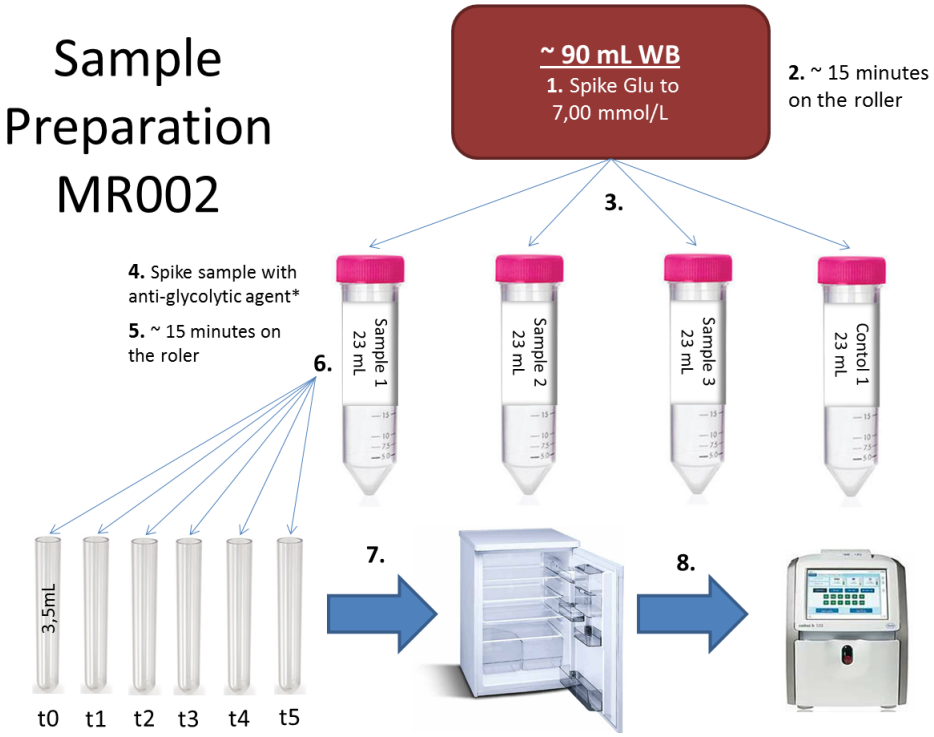


Figure 2.4.: Design and course of experiment MR002.
*) the exact concentrations depended on the anti-glycolytic agent.

GA sample preparation: For the run with GA, 4 samples were prepared. S221 to S223 were each treated with a different concentration of GA. S221 contained a concentration

of 10 mM, sample S222 contained 30 mM and Sample S223 50 mM GA. Additionally Sample C220 was kept untreated and functioned as control sample.

NaF sample preparation: For this run 4 samples were prepared: Sample S721, S921, D921 und C921. Sample S721 was spiked with glucose to a medium glucose level of approximately 7 mM and contained 65 mM NaF. The glucose level of sample S921 was also adjusted to $\approx$ 7 mM by spiking. Furthermore, 65 mM and 10 mM GA were added to the sample. The sample D921 was prepared by means of dialysis according to table 2.2. C920 was kept untreated and functioned as control sample.

Na-3-BrPA sample preparation: In order to keep the pH level of the whole blood sample roughly at a physiological level, 3-BrPA needs to be buffered and neutralised before the substance can be added to the whole blood sample. This was done by solving 3- BrPA in aqua bidest and neutralising it with Na_2CO_3. For this purpose a 2.5 M stock solution $Na-3-BrPA$ was prepared on the previous day. From this stock solution 0.24 mL were added to 20 mL whole blood resulting in a concentration of 30 mM. Pelicano et al. [28] suggest that bromopyruvic acid is already effective at the concentration of 100 μM while Ko et al. claimed that 5 mM of 3-BrPA are sufficient to inhibit glycolsis completely in VX2 tumor cells [37]. Since the first experiments with a concentration of 10 mM did not show the expected slowdown of glycolysis, the concentration was raised to 30 mM, which was considered to be the maximum concentration of bromine that can be measured with this setup, without damaging the sensor.

We must conduct research and then accept the results. If they don't stand up to experimentation, Buddha's own words must be rejected.

Dalai Lama XIV

3. Results

3.1. Results in experiment 1 - MR001

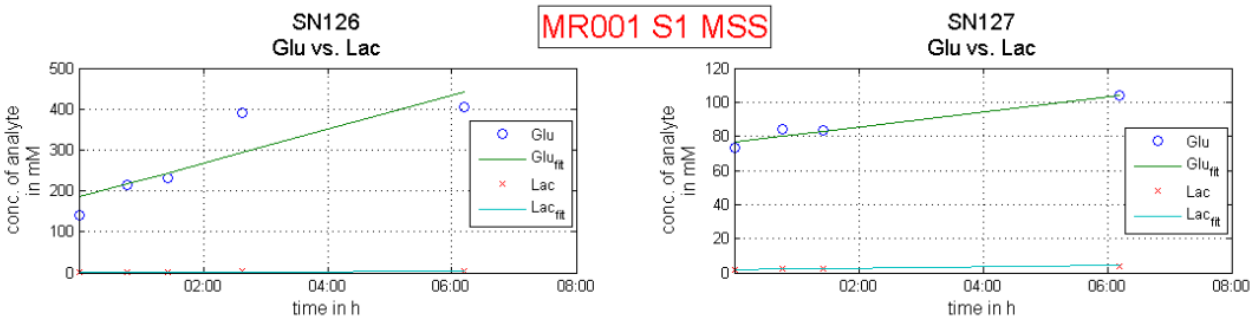
Each single measurement included a full blood gas analysis where pO_2, pCO_2, pH, concentrations of the electrolytes Na^+, K^+, Ca^{2+}, Cl^-, glucose and lactate levels and haematocrit were determined. All measurement results were saved in the in-house database (LogMonitor) and managed by the database management tool (LogDB-Analyzer). In order to identify the efficacy of a substance with respect to its anti-glycolytic properties the glucose and lactate levels were analysed. The in-depth analysis was done in Excel™ and MatLab™.

As already pointed out in the introductory section 1.2.3, the actual aim of this work, the development of an optimised method for the performance evaluation of the precision of POC devices according to CLSI EP5-A2, is strongly tied to the need for a stable whole blood sample. Therefore, a major part of the research focused on finding a practicable way of stabilizing glucose and lactate levels in the whole blood sample. The following sections present the analysis of the measurement results for glucose and lactate. Further in-depth analysis of the efficacy of each anti-glycolytic agent and its possible effects on other blood gas parameters are shown in the appendix A.1. An overview of all results for glucose in the experiment MR001 can be found in the table 3.1. Since the increase in lactate concentration is indirectly proportional to the decrease in glucose concentration, in favour of simplicity and readability, the matrix only shows the results for the glucose concentrations (ΔGlu_{tot} and $\Delta Glu/h$). ΔGlu_{tot} states the rate at which the glucose concentration decreased (-) or increased (+) over the time of the experiment in percent and was calculated with the first and the last measurement result as reference value. The normed rate (decrease or increase per hour) is represented by $\Delta Glu/h$. Results that promise a reasonable improvement in terms of stability of glucose level are highlighted according to their nearness to the aspired rate of 0.1% per hour (orange: 1% - 2%; yellow: $\leq$ 1%; green $\leq$ 0.1%). The following sections present the detailed results for each anti-glycolytic agent, depicting the trend for glucose and lactate for the anti-glycolytic and its corresponding control sample.

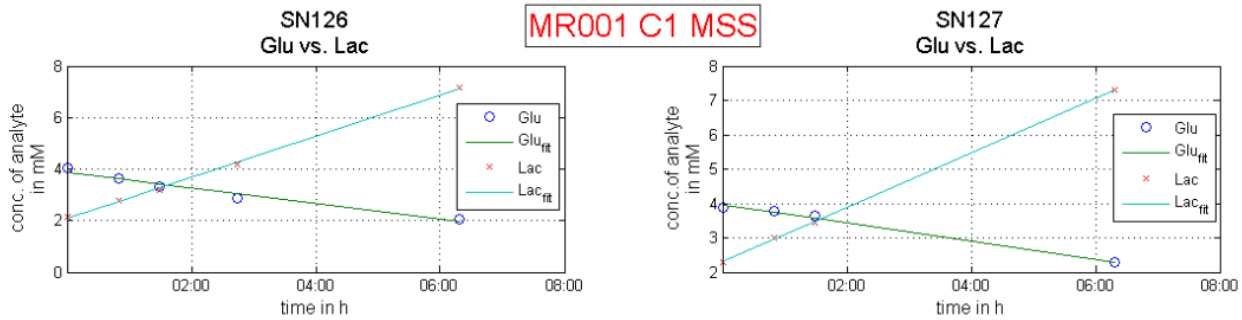
MR001 - Glu													
		SN126				SN127				SN5213 (b221 - ref)			
		ΔGlu tot	ΔGlu / h	GluSTART	GluEND	ΔGlu tot	ΔGlu / h	GluSTART	GluEND	ΔGlu tot	ΔGlu / h	GluSTART	GluEND
run 1	C1:control	-48.60%	-7.69%	4.03	2.07	-41.20%	-6.54%	3.88	2.28	-48.19%	-7.17%	4.42	2.29
	S1:2DG	189.63%	30.59%	139.6	404.2	41.87%	6.75%	73.08	104.2	2.89%	0.46%	80.63	76.54
	S2a:3-BrPA	-24.51%	-4.00%	3.38	2.55	-11.56%	-1.88%	2.82	2.49	-76.33%	-11.77%	4.90	1.16
run 2	C2:control	-43.71%	-7.21%	4.47	2.51	-36.73%	-6.07%	4.83	3.05	-23.76%	-4.31%	4.42	3.37
	S2b:3-BrPA	9.80%	1.62%	3.54	3.89	13.59%	2.24%	3.60	4.09	32.54%	5.64%	2.95	3.91
	S3a:GA	-12.02%	-1.98%	4.60	4.05	2.02%	0.33%	4.71	4.81	14.03%	2.54%	4.49	5.12
run 3	C3:control	-35.61%	-5.56%	4.20	2.70	-32.32%	-5.06%	4.48	3.03	-20.18%	-3.11%	4.46	3.56
	S3b:GA	-16.90%	-2.30%	4.22	3.51	0.63%	0.09%	4.24	4.27	-4.28%	-0.66%	4.91	4.70
	S4:Ouabain	-34.24%	-5.39%	4.21	2.77	-26.57%	-4.27%	4.48	3.29	-25.10%	-3.87%	4.94	3.70
run 4	C4:control	-43.25%	-5.60%	4.78	2.71	-39.97%	-5.18%	4.88	2.93	-43.12%	-5.61%	5.38	3.06
	S5:Quercetin	-58.38%	-7.57%	4.72	1.97	-56.22%	-7.25%	4.90	2.15	-61.23%	-7.97%	5.21	2.02
run 5	C5:control	-20.54%	-3.99%	5.33	4.24	-18.04%	-3.49%	5.57	4.56	-9.01%	-2.21%	5.33	4.85
	S6:Quercetin	-24.14%	-5.05%	5.17	3.92	-21.82%	-4.51%	5.43	4.24	-13.23%	-3.24%	5.14	4.46
run 6	C6:control	-45.69%	-6.23%	4.61	2.50	-42.73%	-5.83%	4.79	2.74	-40.23%	-5.41%	5.22	3.12
	S7:NaF	-15.01%	-2.04%	4.62	3.92	-11.34%	-1.54%	4.81	4.27	-9.72%	-1.31%	5.04	4.55
	S8:Isorham	-40.08%	-5.45%	4.71	2.82	-38.11%	-5.19%	4.91	3.04	-37.09%	-4.99%	5.15	3.24
	legend:	very strong interference				ΔGlu/h: 1 - 2%				ΔGlu/h <1,00			ΔGlu/h <0,1

Figure 3.1.: The matrix lists all results of the first experiment MR001. The decrease (-) and increase (+) of glucose concentration in percent over time for all analysed samples were calculated based on linear approximation. Each run was performed on a different day. ΔGlu_{tot} describes the overall difference in concentration between start and end of the experiment in %. $\Delta Glu/h$ is the rate at which glucose concentration decreased/increased per hour.

3.1.1. Inhibition of glycolysis with deoxyglucose



(a) S1 (**2-deoxyglucose**): Sample was prepared as described in section 2.2.4. Deoxyglucose (20 mM) was added by spiking 80 μL of a 2.5 M stock solution to the whole blood sample.



(b) C1: control sample for run with deoxyglucose. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added to the sample.

Figure 3.2.: Sample 1 (deoxyglucose and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.

Figure 3.2 depicts the trend for glucose and lactate concentrations over 6 hours and shows the results obtained for the run with 2-DG as anti-glycolytic agent. At the beginning of the measurement (t=0 min) glucose concentrations varying from 73.08 mM (SN127) to 139.6 mM (SN126) were determined. After approximately 6 hours (t= 372 min) blood glucose concentrations of 104.2 mM (SN127) and 404.2 mM were measured. This means an overall increase of glucose concentration of 41.87 %, or 6.75 %/h on the device SN127 and an overall increase of 189.63 % or 30.59% per hour on the device SN126, respectively (see 3.2a). For the control sample (Figure 3.2b) a glucose starting concentration of 4.03 mM (SN126) and 3.88 mM (SN127) was measured. An end concentration of 2.07 mM (SN126) and 2.28 mM (SN127) was determined, which results in a decrease of 7.69% per hour (SN126) and 6.54%/h (SN127), respectively.

Due to the striking difference in results of the two devices (compare $[Glu_{\ t=0,\ SN126}]$ = 139.6 mM and $[Glu_{t=0,SN127}]$ = 73.08 mM) the glucose sensors were analysed in further detail. Figure 3.3 compares the glucose sensors used in the devices SN126 and SN127 in regard to their calibration curve, taking the linearity and the slope response of the sensors into consideration. The implications of this analysis will be discussed further in the discussion section (see 4.2).

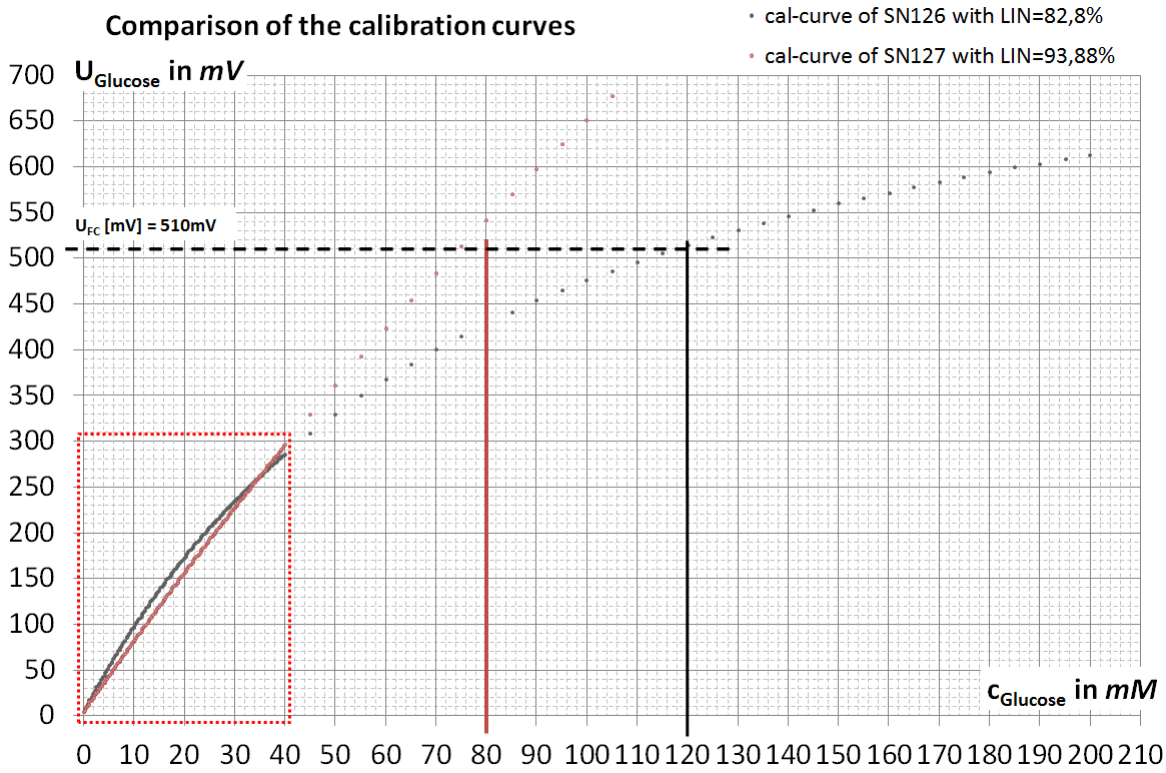


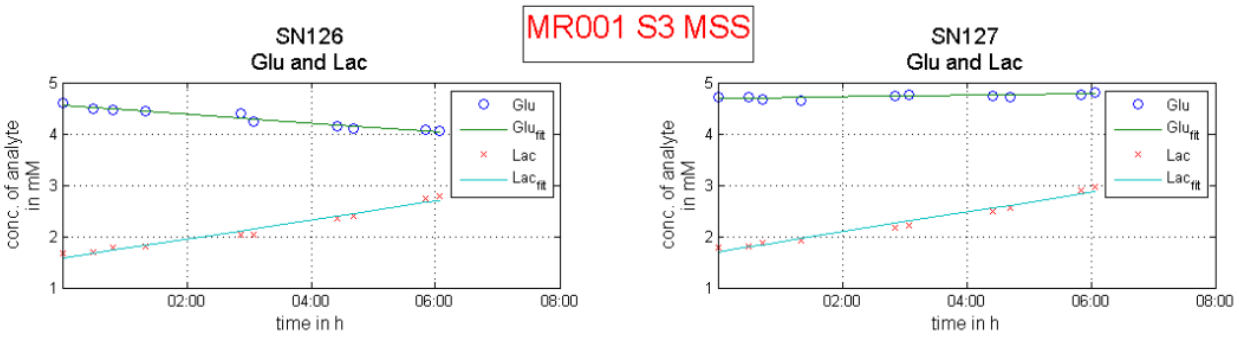
Figure 3.3.: shows a comparison of the calibration curves of the two sensors that have been in use in the devices SN126 and SN127 for the experiments with the deoxyglucose sample. The area enclosed by the dashed red box marks the range of concentrations with the corresponding sensor signal for which the sensor is specified.

3.1.2. Inhibition of glycolysis with glyceraldehyde

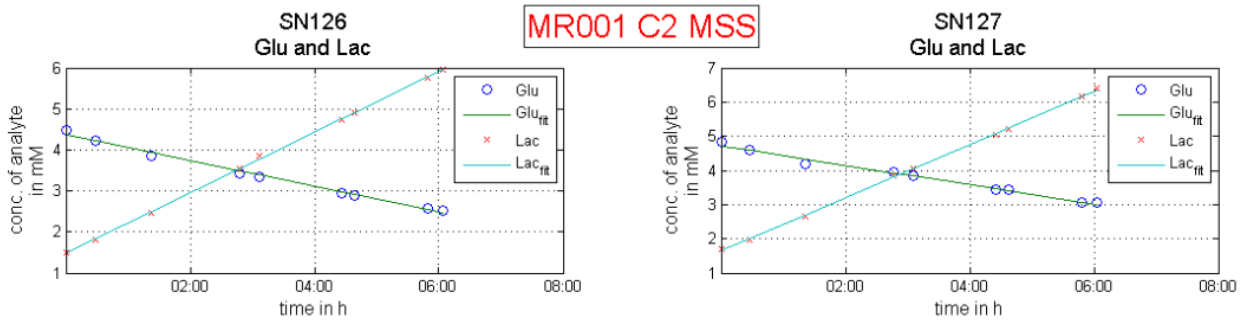
For the first experiment with glyceraldehyde as glycolytic agent (see figure 3.4a), the following results were obtained: At beginning of the experiment (t=0) 4.60 mM (SN126) and 4.71 mM (SN127). After 6 hours (t=364 min) glucose concentrations of 4.05 mM (SN126) and 4.81 mM (SN127) were measured. This results in an overall decrease of -12.02% or -1.98%/h (SN126) and an overall increase of 2.02% or 0.33%/h (SN127), respectively. The trend is depicted in figure 3.4 in a concentration vs. time diagram. A second run was conducted a day after the first experiment under the same conditions (see figure 3.4c). At beginning of the experiment (t=0) 4.22 mM (SN126) and 4.24 mM (SN127). After 6 hours glucose concentrations of 3.51 mM (SN126) and 4.27 mM (SN127) were measured, which leads to a decrease of -16.90% or -2.30%/h (SN126) and a very slight increase of -0.63% or -0.09%/h.

3.1.3. Inhibition of glycolysis with 3-bromopyruvic acid

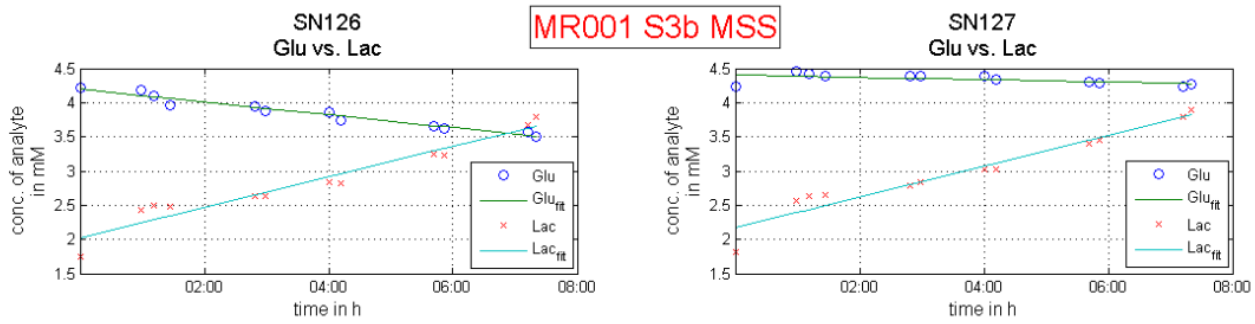
In the run with 3-BrPA as anti-glycolytic agent, the following glucose concentrations were measured: At beginning of the experiment (t=0) 3.54 mM (SN126) and 3.60 mM (SN127). After 6 hours (t=364 min) glucose concentrations of 3.89 mM (SN126) and 4.09 mM (SN127) were measured. This results in an overall increase of glucose of +9.80% or +1.62%/h (SN126) and an overall increase of +13.59% or +2,24%/h (SN127), respectively.



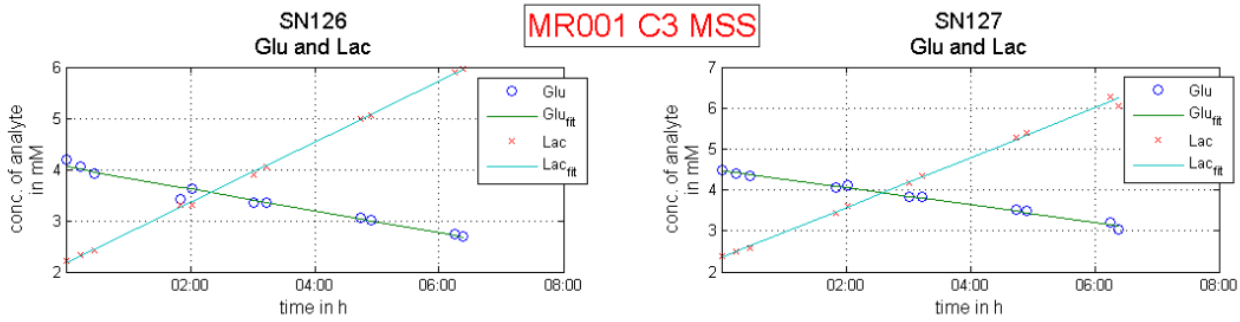
(a) S3 (**glyceraldehyde**): Sample was prepared as described in section 2.2.4. Glyceraldehyde was added by spiking 80 μL of a 2.5 M stock solution to the whole blood sample resulting in a final concentration of 20 mM.



(b) C2: control sample for run with glyceraldehyde. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added to the sample.

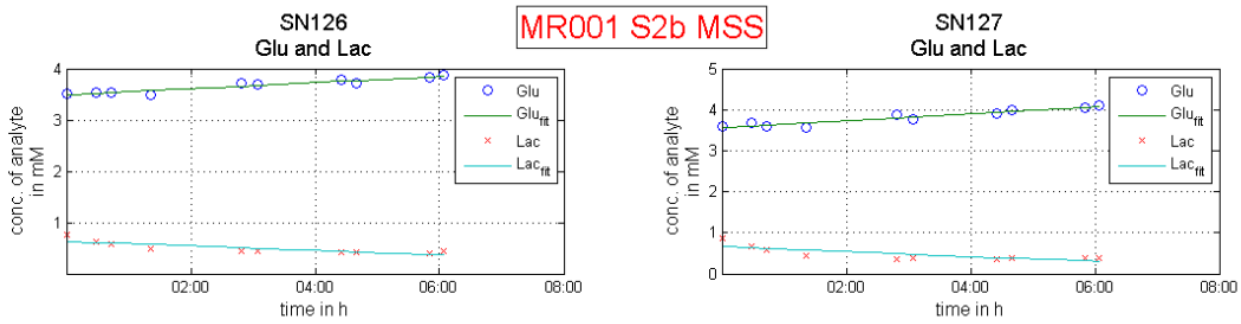


(c) S3b (**glyceraldehyde**): Experiment with Glyceraldehyde was repeated on the next day under the same conditions as in the first run. Sample was prepared as described in section 2.2.4. Glyceraldehyde was added by spiking 80 μL of a 2.5M stock solution to the whole blood sample resulting in a final GA concentration of 20 mM.

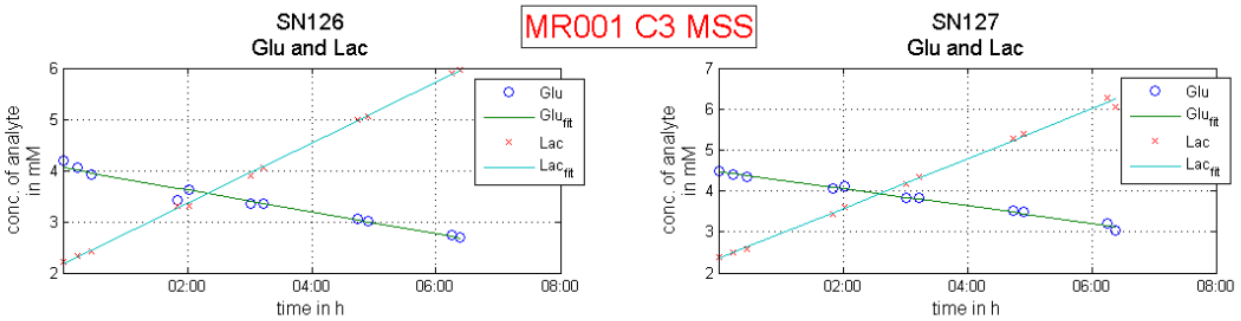


(d) C3: control sample for the second run with glyceraldehyde. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added to the sample.

Figure 3.4.: Sample 3 (glyceraldehyde and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.



(a) S2b (**bromopyruvic acid**): Sample was prepared as described in section 2.2.4. Bromopyruvic acid was added by spiking 80 μL of a 2,5M stock solution to the whole blood sample resulting in a final 3-BrPA concentration of 20 mM.

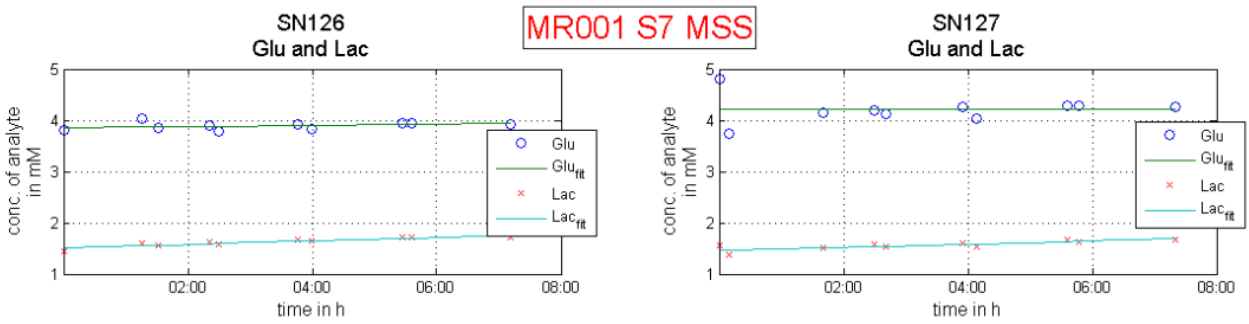


(b) C3: control sample for run with 3-PrPA. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added to the sample.

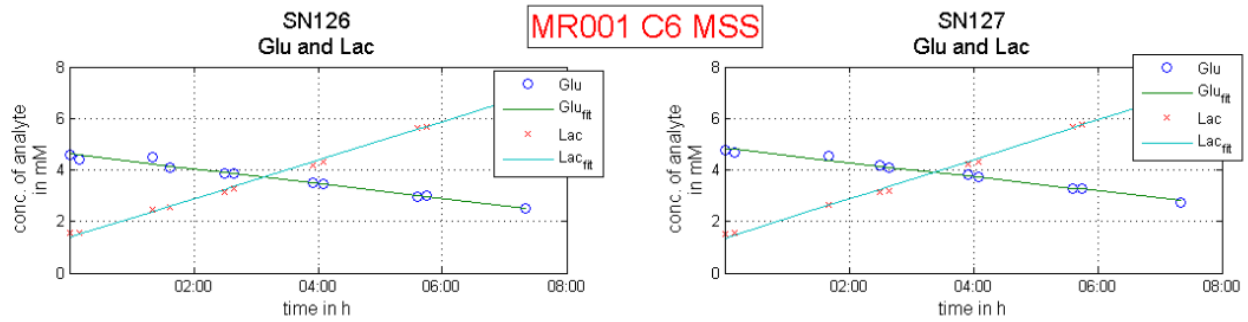
Figure 3.5.: Sample 2b (bromopyruvic acid and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.

The results are depicted in figure 3.5 in form of a concentration vs. time diagram.

3.1.4. Inhibition of glycolysis with sodium fluoride



(a) S7 (**sodium fluoride**): Sample was prepared as described in section 2.2.4. Sodium fluoride was added by spiking 0.26 mL of a 5M stock solution to the whole blood sample resulting in a final concentration of 130 mM of NaF in the sample.



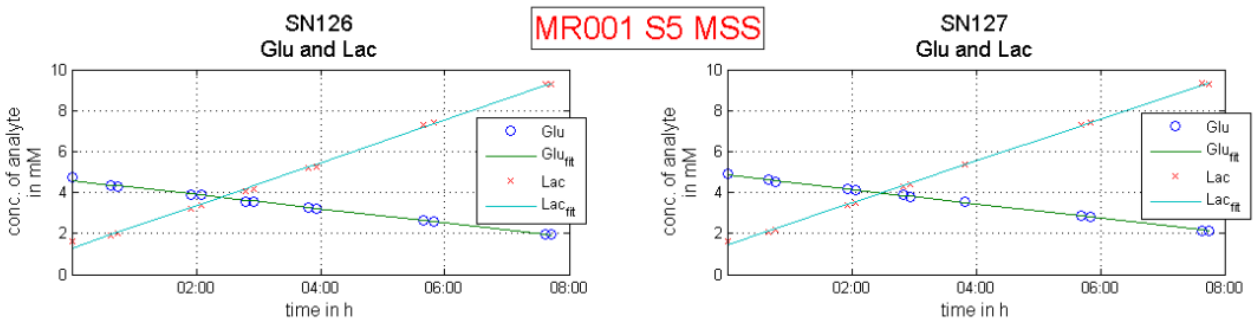
(b) C6 (**control sample**: Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added to the sample.

Figure 3.6.: Sample 7 (sodium fluoride and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.

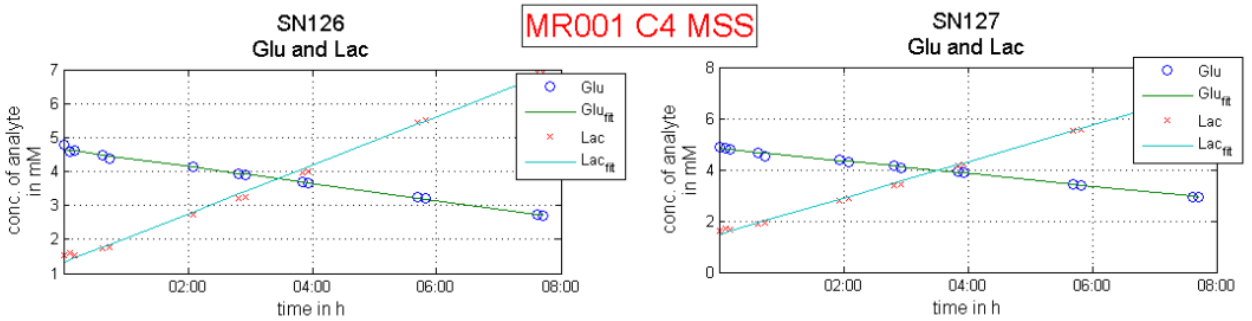
The experiment with NaF resulted in the following concentrations for glucose: At the beginning of the experiment, (t=0) 4.62 mM (SN126) and 4.81 mM (SN127) were measured. After 6 hours glucose concentrations of 3.92 mM (SN126) and 4.27 mM (SN127) were measured. This results in an overall increase of glucose of 15,01% or 2.04%/h (SN126) and 11,34% or 1.54%/h (SN127), respectively. The results are depicted in figure 3.6 in form of a concentration vs. time diagram.

3.1.5. Inhibition of glycolysis with quercetin

For quercetin as anti-glycolytic agent glycolytic rates ranged from -58,8% (SN126) and -56.22% (SN127) over a total time span of 6 hours, resulting in a rate of -7.57% per hour (SN126) and -7.25% per hour, respectively. In the sample S5 containing a concentration of 20 mM of quercetin, the starting concentration of glucose was determined to be 4.72 mM (SN126) and 4.90(SN127). After 6 hours the glucose concentration was 1.97 mM (SN126)



(a) S5 (**quercetin**): Sample was prepared as described in section 2.2.4. Quercetin was added by spiking 125 μL of a 0.05 M stock solution to the whole blood sample resulting in a final quercetin concentration of 50mM.

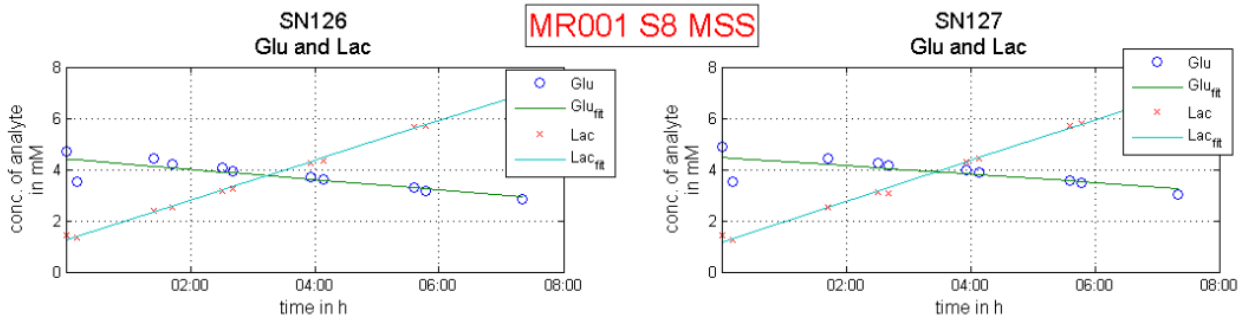


(b) C4: control sample for run with Quercetin. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added.

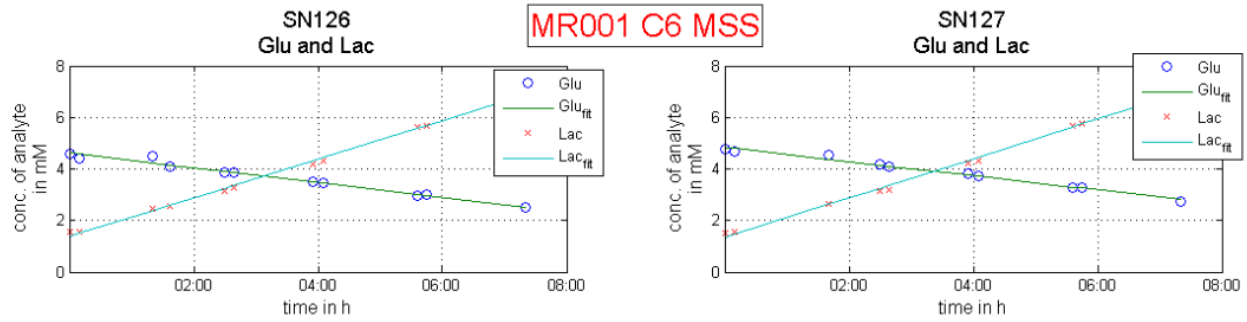
Figure 3.7.: Sample 7 (sodium fluoride and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.

and 2.15 mM (SN127), respectively. In comparison, in the control sample glycolysis proceeded with a rate of -43.25% (SN126) and -39,97%, resulting in a normed rate of -5,60% per hour and -5,18% per hour, respectively. In the control sample, glucose concentrations of 4.78 mM and 4.88 mM were measured at the beginning of the experiment and 2.71 mM and 2.93 mM after 6 hours.

3.1.6. Inhibition of glycolysis with isorhamnetin



(a) S8: Sample was prepared as described in section 2.2.4. Isorhamnetin was added by spiking 0,1 ml of a 5 mM stock solution resulting in a final isorhamnetin concentration of 0.05 mM.



(b) C6: control sample for run with Isorhamnetin. Sample was prepared as described in section 2.2.4 but no anti-glycolytic agent was added.

Figure 3.8.: Sample 7 (sodium fluoride and control sample): the elapsed time of the experiment is shown on the x-axis and glucose and lactose concentration plotted on the y-axis.

In the second experiment with a flavon as anti-glycolytic agent, isorhamnetin, glycolytic rates ranged from -40.08% (SN126) and -38.11% (SN127) over a total time span of 6 hours, resulting in a rate of 5.45% per hour (SN126) and 5.19% per hour, respectively. In the sample S8 which contained a concentration of 0.05 mM of isorhamnetin, the starting concentration of glucose was determined to be 4.71 mM (SN126) and 4.91(SN127). After 6 hours the glucose concentration was 2.82 mM (SN126) and 3.04 mM (SN127), respectively. These results were compared to the control sample where glycolysis proceeded with a rate of -43.25% (SN126) and -39.97%, resulting in a normed rate of -5.60% per hour and -5.18% per hour, respectively. In the control sample glucose concentrations of 4.78 mM and 4.88 mM were measured at the beginning of the experiment and 2.71 mM and 2.93 mM after 6 hours.

3.2. Results in experiment 2 - MR002

In experiment 2 (MR002), all BG parameters were measured and analysed. Since the focus was primarily on glucose, this section only presents results of the detailed evaluation of the data for glucose concentrations. The results of the other parameters are shown in the appendix A.2.

Figure 3.9 gives an overview of all results for the experiment MR002. ΔGlu_{tot} states the percentage by which the glucose concentration decreased (-) or increased (+) over the time of the experiment. These results were normed in regard to time and are represented by $\Delta Glu/h$. Results that are close or below the specified critical value of 0.1% per hour are highlighted according to their nearness (orange: 1% - 2%; yellow: $\leq$ 1%; green $\leq$ 0.1%). Detailed analysis of the glucose concentrations is presented in the following sections 3.2.1 - 3.2.3. The experimental setup for experiment MR002 included 6 b123 devices. Therefore, further statistical analysis such as calculation of mean, standard deviation and an error estimation were performed.

In order to examine the influence of possible ongoing interferences between the anti-glycolytic agent and the sensor or the whole blood components, the BSA interference signal of the sensor was analysed as well.

MR002 - Glu												
		GlycerAldehyde				Na-3-BrPA			NaF			
		S221	S222	S223	C220	D322	S322	C320	S721	S921	D921	C920
SN9999	ΔGlu tot	-0.79%	-1.10%	-0.16%	-14.10%	4.75%	3.05%	-11.23%	-4.28%	2.20%	0.96%	-5.04%
(Hitachi-Ref)	ΔGlu / h:	-0.13%	-0.17%	-0.03%	-2.23%	0.79%	0.51%	-1.87%	-0.72%	0.37%	0.16%	-0.85%
SN127	ΔGlu tot	14.21%	16.17%	17.33%	-3,63%	15.80%	2.05%	-6.57%	-19.05%	13.06%	34.61%	-24.68%
	ΔGlu / h:	2.36%	2.69%	2.89%	-0,58%	2.62%	0.34%	-1.09%	-3.83%	2.76%	8.27%	-5.12%
SN126	ΔGlu tot	13.26%	17.21%	16.80%	-2.66%	6.58%	7.30%	-6.04%	22.51%	13.83%	28.46%	-0.24%
	ΔGlu / h:	2.20%	2.86%	2.79%	-0.42%	1.09%	1.22%	-1.00%	3.84%	2.90%	5.05%	-0.04%
SN125	ΔGlu tot	15.24%	24.25%	28.57%	-6.40%	24.51%	6.78%	-10.05%	-8.84%	-2.85%	26.10%	-7.27%
	ΔGlu / h:	2.53%	4.03%	4.75%	-1.01%	4.06%	1.13%	-1.67%	-3.01%	-0.60%	4.63%	-1.51%
SN124	ΔGlu tot	12.12%	16.12%	16.27%	-6.51%	8.95%	5.38%	-6.56%	-14.98%	10.45%	45.78%	-2.33%
	ΔGlu / h:	2.01%	2.68%	2.70%	-1.03%	1.48%	0.89%	-1.09%	-3.02%	2.21%	8.13%	-2.45%
SN123	ΔGlu tot	5.05%	7.62%	9.14%	-11.01%	10.63%	4.49%	-6.22%	43.10%	30.65%	45.03%	-10.93%
	ΔGlu / h:	0.84%	1.27%	1.52%	-1.74%	1.76%	0.75%	-1.03%	7.35%	5.20%	7.99%	-1.87%
SN122	ΔGlu tot	11.40%	12.51%	15.57%	-5.15%	13.41%	9.61%	-8.64%	-19.52%	4.51%	38.17%	-27.45%
	ΔGlu / h:	1.90%	2.08%	2.59%	-0.81%	2.22%	1.60%	-1.44%	-3.93%	0.95%	6.78%	-5.72%
SN5639	ΔGlu tot	x	x	x	x	-3.19%	-5.38%	-9.74%	-4.12%	3.52%	0.84%	-5.73%
(b221-Ref)	ΔGlu / h:	x	x	x	x	-3.31%	-0.90%	-1.56%	-0.70%	0.59%	0.15%	-0.98%
	Legend:	ΔGlu/h ≈ 1,00%	ΔGlu/h < 1,00 %	ΔGlu/h < 0,1 %	x ... no measurement							

Figure 3.9.: Matrix listing decrease (-) / increase (+) of glucose levels over time for all analyzed samples. ΔGlu_{tot} describes the overall difference from start to end of the experiment in %. $\Delta Glu/h$ gives the rate at which glucose decreased/increased per hour. While the measurements for SN122-SN127 and SN5639 were obtained by the amperometrical measurement principle described in 2.2.1 the results for SN9999 were obtained by optical glucose measurements in plasma with the Hitachi 902 Analyser, see 2.2.3.

3.2.1. Inhibition of glycolysis with glyceraldehyde

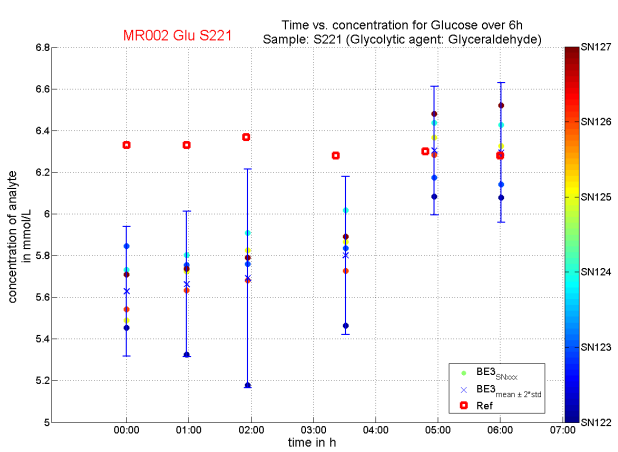
In figure 3.10 the result for the second trial of inhibition of glycolysis with glyceraldehyde can be seen. For the data obtained by the 6 *cobas b123*, mean and standard deviation were calculated. Figure 3.10a shows the result for the sample containing 10 mM glyceraldehyde. The mean rate of glycolysis was 11.83% over 6 hours or 2.83 % per hour, with a mean glucose concentration of 5.63 mM and corresponding standard deviation of 0.16 mM at the beginning of the measurements. After 6 hours, a mean glucose concentration of 6.115 and 0.142 STD was determined. The reference measurement of the plasma with the Hitachi 902 analyzer resulted in a rate of - 0.79% and - 0.13% per hour, respectively.

For a glyceraldehyde concentration of 30 mM (see 3.10b), a mean rate of 2.57% per hour was determined. At the beginning of the measurement, a mean glucose contentration of 5.40 mM with a standard deviation (STD) of 0.23 mmo/L was measured. After 6 hours a mean glucose concentration of 6.23 mM with a STD of 0,12 was determined.

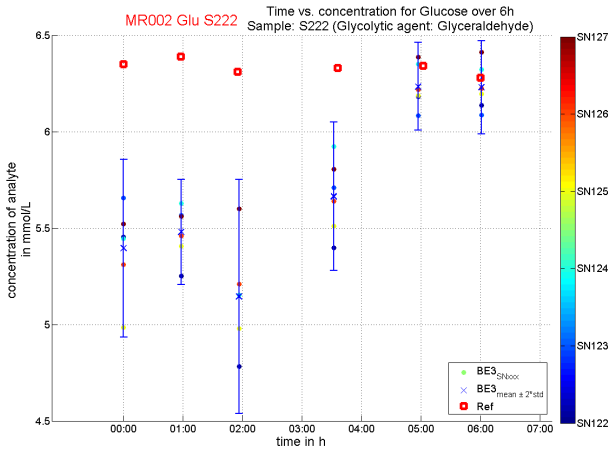
In the sample with 50 mM glyceraldehyde (see 3.10c), a mean starting concentration of 5.23 mM with a STD of 0.296 mM was measured. After 6 hours a mean glucose concentration of 6.11 mM with a STD of 0.14 mM was determined. This results in a mean glycolysis rate of 2.834% per hour.

Figure 3.10d shows the results of the corresponding control sample containing no anti-glycolytic agent. The mean rate of the glycolysis was 0.94% per hour.

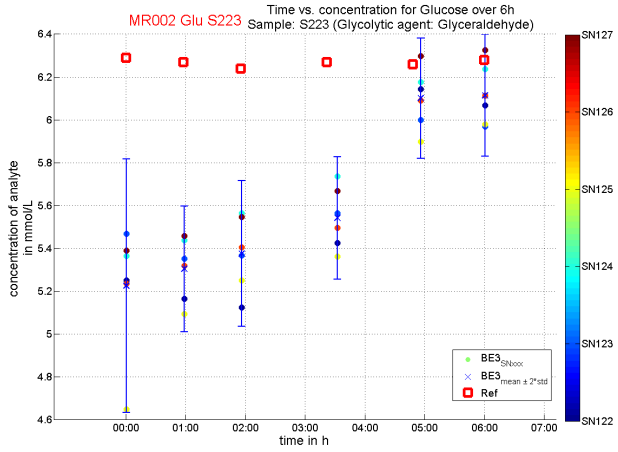
In order to better assess the influence that glyceraldehyde has on the sensor, for all 6 *cobas 123* sensors the number of incidents and their equivalent strength were determined. In figure 3.12 the statistical analysis of the measured BSA-equivalents is shown in form of a histogram. Figure 3.11 depicts the trend of the BSA-equivalent signals over the course of the experiment in a time vs. BSA level plot. Due to the facts pointed out in section 2.2.2, only the number of detected interference signals with a strength greater than 2 BSA equivalents were considered to have a significant influence on the results. In the run with glyceraldehyde as anti-glycolytic agent, the highest BSA level was found to be 2.5 with only one occurrence. Most of the six sensors showed at least one or more incidents where the BSA level was equal or greater than 2. All in all the analysis of the BSA levels showed that the BSA correction algorithm had only a partial influence on the above presented results of the glucose concentration measurements. A BSA level of 2 would result in a deviation of the measured glucose concentration of -0.39 mM or 6.5% and a BSA level of 3 leads to an error of -0.87 mM or 14.5%.



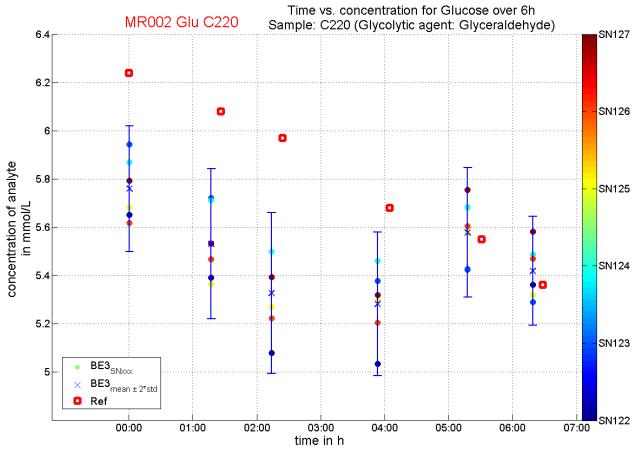
(a) Sample S221: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, GA: 10 mM.



(b) Sample S222: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, GA: 30 mM.



(c) Sample S223: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, GA: 50 mM.



(d) Sample C220: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, GA = 0.0 mM:

Figure 3.10.: Time series for glucose measurements with glyceraldehyde as anti-glycolytic agent under the conditions mentioned in the corresponding diagrams above. a) - d) present results from all 6 b123 devices, the mean (×) and error estimation (⊥,⊤; ±2 * STD) for each measuring point and compares them to the results from the reference device (□).

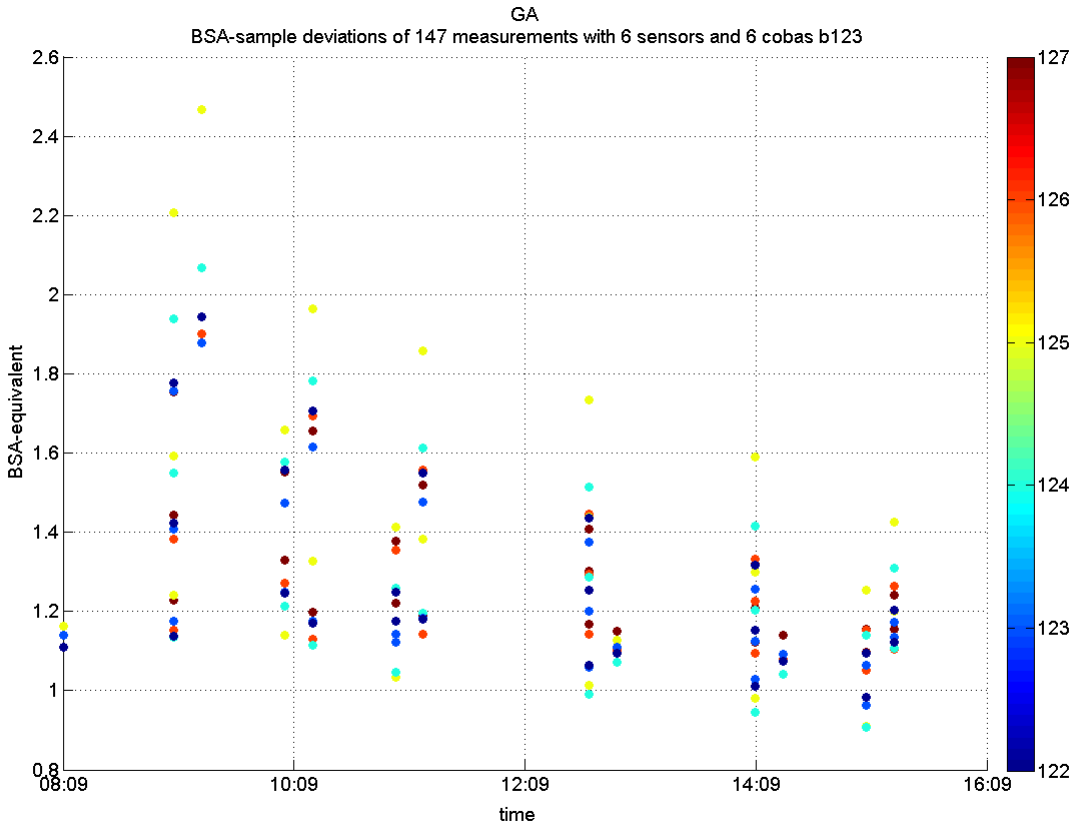


Figure 3.11.: Trend of the BSA equivalents measured by the *cobas b123* devices during the run with glyceraldehyce in a time vs. level of the BSA equivalent signal.

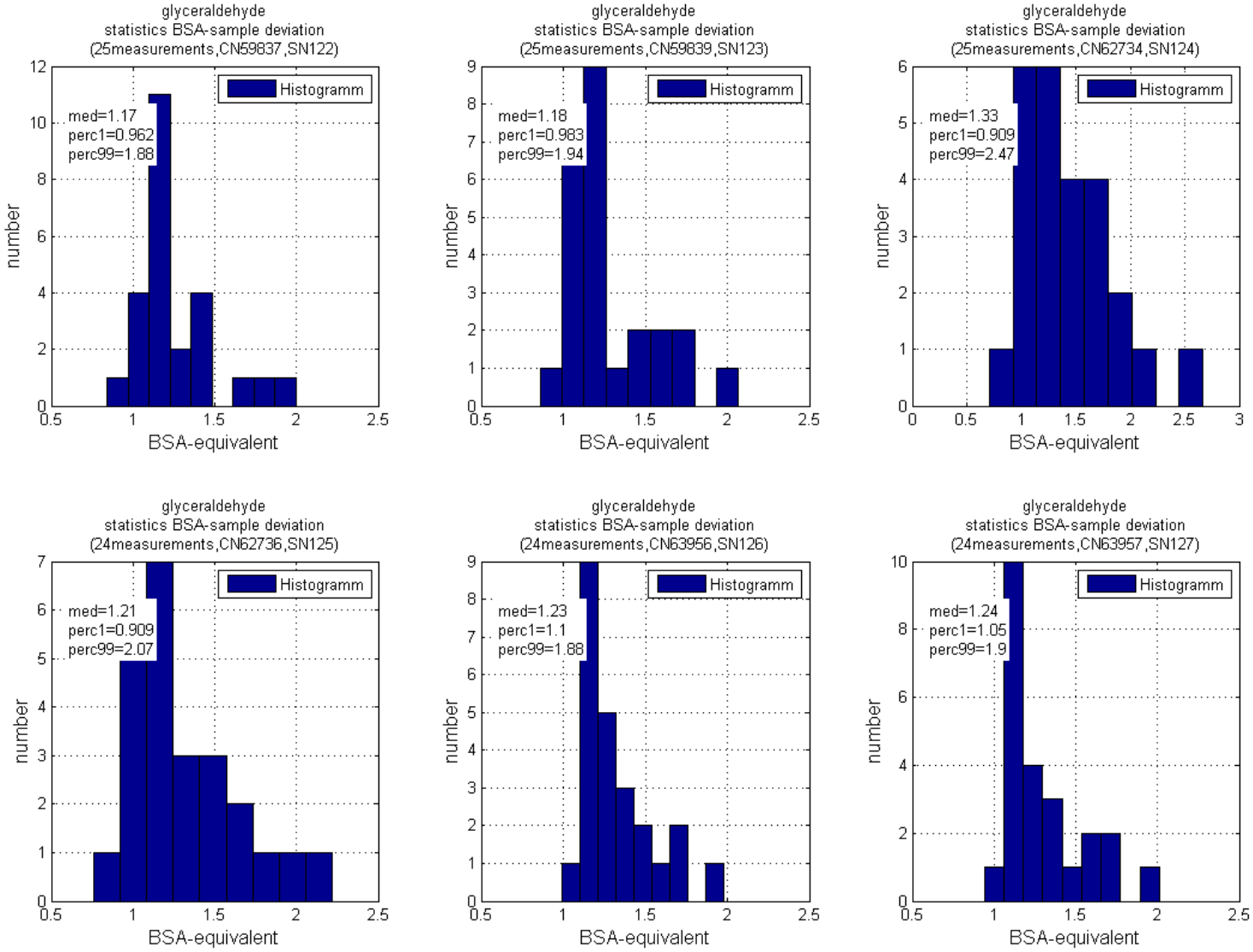


Figure 3.12.: Shows the statistical analysis of the BSA equivalents measured by the *cobas 123* devices during the run with glyceraldehyde. The histogram depicts the strength of the BSA signal on the x-axis and the number of incidents with the according strength on the y-axis.

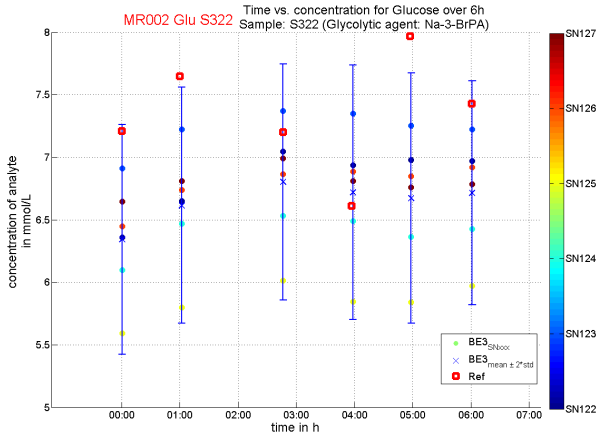
3.2.2. Inhibition of glycolysis with Na-3-BrPA

Figure 3.13 shows the detailed results for the second experiment with bromopyruvic acid as anti-glycolytic agent. For the obtained data from the 6 *cobas b123* devices mean and standard deviation were calculated. Figure 3.13b shows the result for the dialysed sample containing 30 mM Na−3−bromopyruvicacid. A mean rate of glycolysis of 2.11% per hour was determined. The mean glucose concentration at the beginning of the measurement was 5.97 mM and corresponding standard deviation of 0.78 mM. After 6 hours a mean glucose concentration of 6.73 with 0.61 mM standard deviation were determined. The reference measurement of the plasma with the Hitachi 902 analyser resulted in a rate of 0.79% per hour.

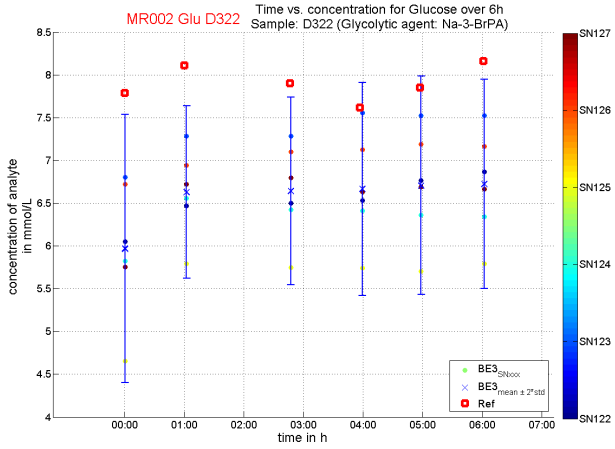
In Figure 3.13a the results for Sample S322 which was spiked with a concentration of 30 mM bromopyruvic acid (see 2.2.4) can be seen. A mean rate of 0.98% per hour was determined. At the beginning of the measurement a mean glucose contentration of 6.34 mM with a standard deviation of 0.46 mM was measured. After 6 hours a mean glucose concentration of 6.73 mM with a standard deviation of 0.61 was determined. The reference measurement of the plasma with the Hitachi 902 analyser resulted in a rate of 0.51% per hour.

Figure 3.13c shows the results of the corresponding control sample containing no anti-glycolytic agent. The mean rate of the glycolysis was 1.87% per hour.

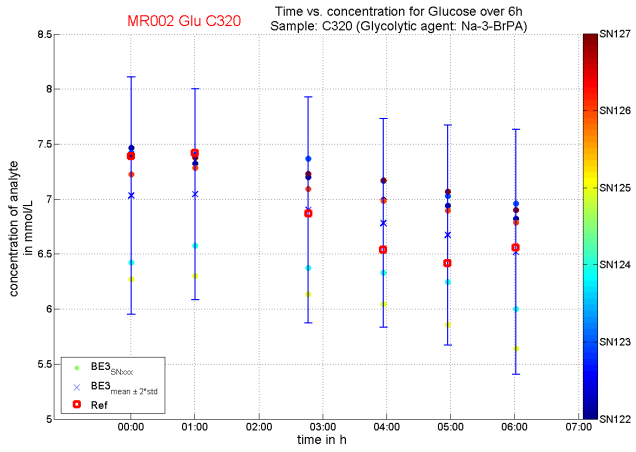
In order to assess the influence of possible interfering effects that bromopyruvic acid could have on the blood gas sensor the BSA levels were analysed as well. In figure 3.15 the statistical analysis of the measured BSA equivalents is shown in form of a histogram. Figure 3.14 depicts the trend of the BSA equivalent signals over the course of the experiment in a time vs. BSA level plot. Especially the histogram illustrates that every sensor except SN123 detected a number of interfering incidents with a BSA level equal or higher than 2. Therefore, it was expected that the presented results were influenced by the BSA correction algorithm to a higher degree than, for example, in the previous run with glyceraldehyde.



(a) Sample S322: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, Na−3−BrPA: 30 mM (spiked).



(b) Sample D322: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, Na−3−BrPA: 30 mM (dialysed).



(c) Sample C320: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 7 mM, Na−3−BrPA: 0 mM

Figure 3.13.: Time series for glucose measurement with bromopyruvic acid as anti-glycolytic agent under the above mentioned conditions. a) - c) Results from all 6 b123 devices, the mean (×) and error estimation (⊥,⊤; ±2 * STD) for each measuring point and compares them to the results from the reference device (□).

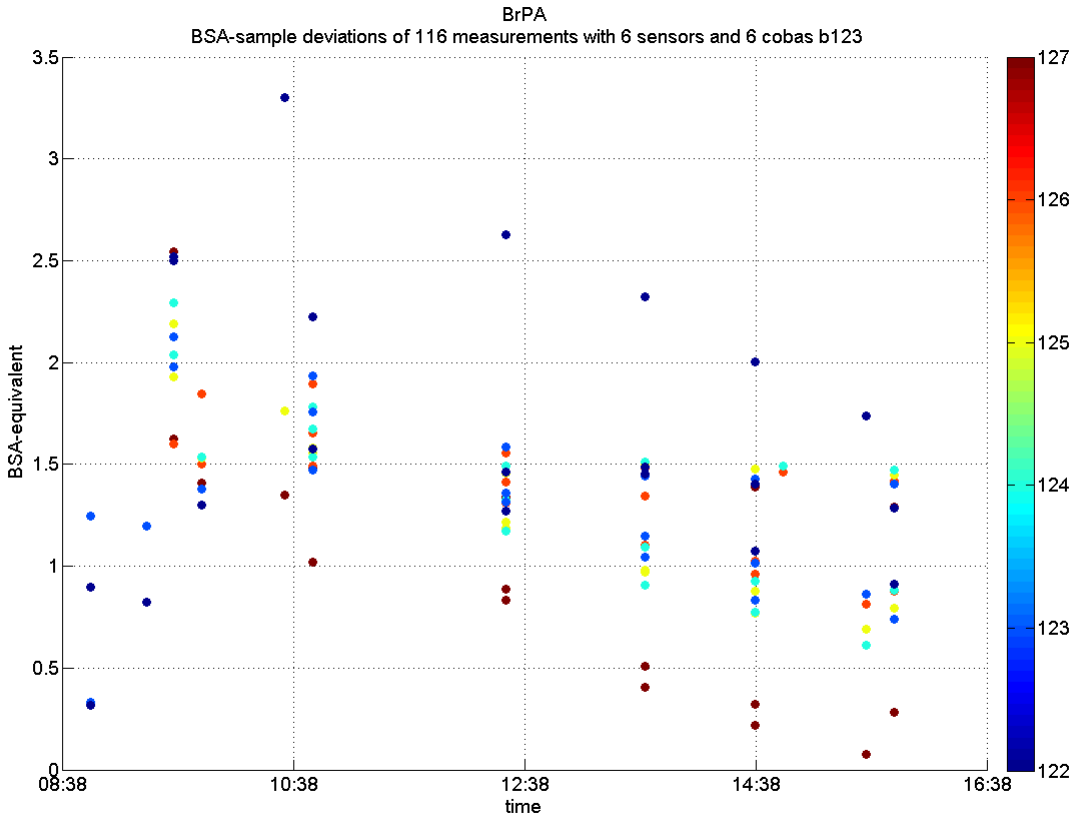


Figure 3.14.: Trend of the BSA equivalents measured by the *cobas b123* devices during the run with bromopyruvic acid in a x-y plot of time and the BSA equivalent signal.

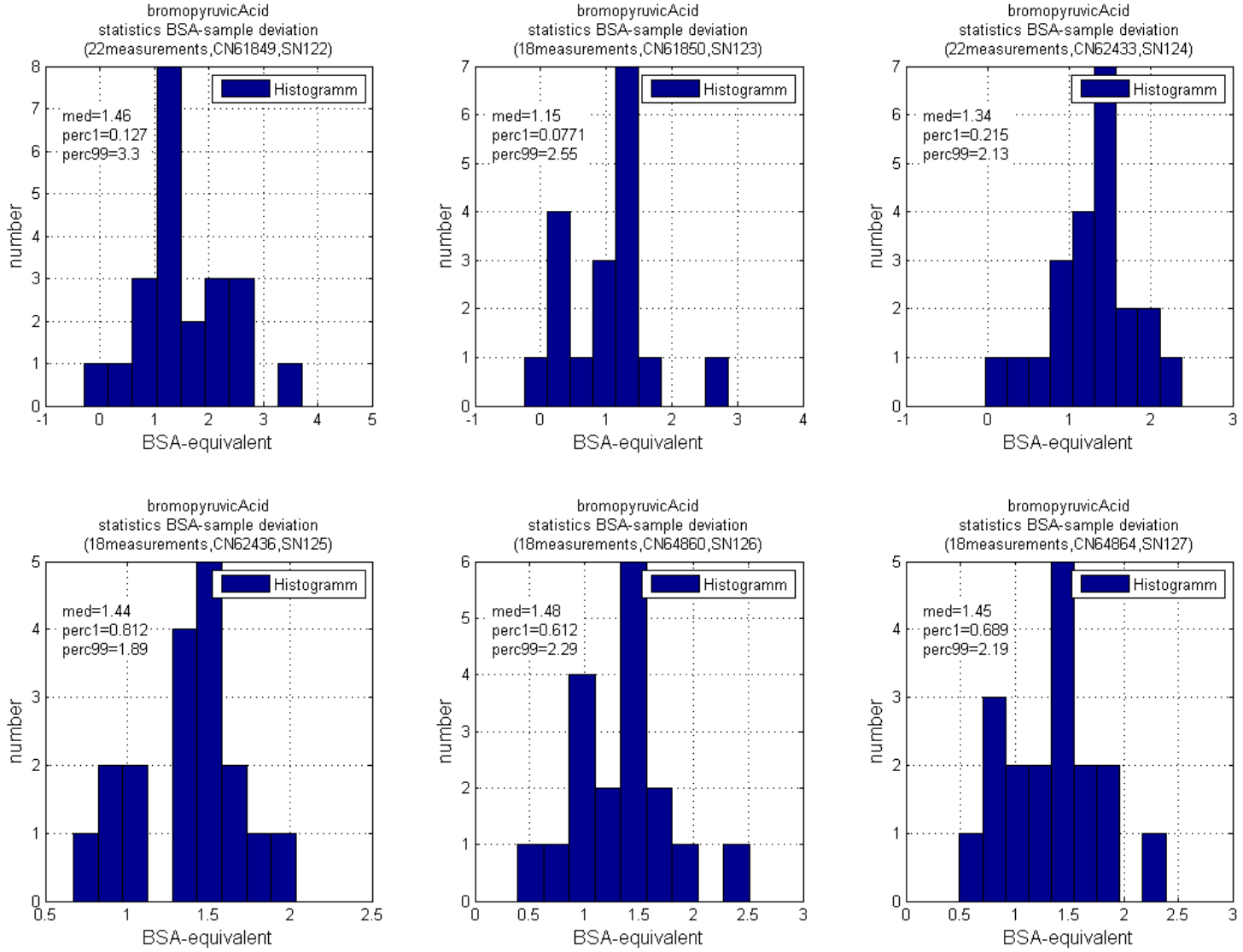


Figure 3.15.: Shows the statistical analysis of the BSA equivalents measured by the cobas 123 devices during the run with bromopyruvic acid. The histogram depicts the strength of the BSA electrode on the x-axis and the number of incidents with the according strength on the y-axis.

3.2.3. Inhibition of glycolysis with sodium fluoride

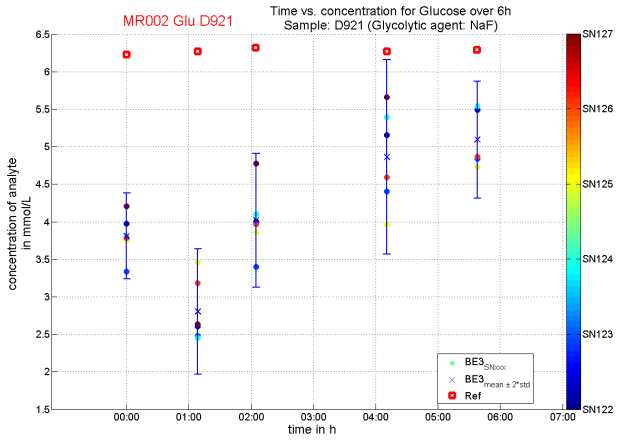
Figure 3.16 shows the detailed results for the second trial with sodium fluoride and the combination of sodium fluoride and glyceraldehyde as anti-glycolytic agent. For the obtained data from the 6 *cobas b123* devices, mean concentrations and standard deviation were calculated. Figure 3.16a shows the result for the dialysed sample containing 65 mM NaF and 10 mM glyceraldehyde. The mean rate of glycolysis was 5.99% per hour, with a mean glucose concentration of 3.81 mM and corresponding standard deviation of 0.28 mM at the beginning of the measurements. After 6 hours, a mean glucose concentration of 5.09 with 0.39 mM standard deviation were determined. The reference measurement of the plasma with the Hitachi 902 analyser resulted in a rate of 0.16% per hour.

For the sample spiked with 65 mM NaF and 10 mM glyceraldehyde (see 3.16b) a mean rate of 5.18% per hour. At the beginning of the measurement a mean glucose contentration of 3.51 mM with a standard deviation of 0.58 mM was measured. After 6 hours a mean glucose concentration of 4.59 mM with a standard deviation of 0.31 was determined. The reference measurement of the plasma with the Hitachi 902 analyser resulted in a rate of and 0.37% per hour.

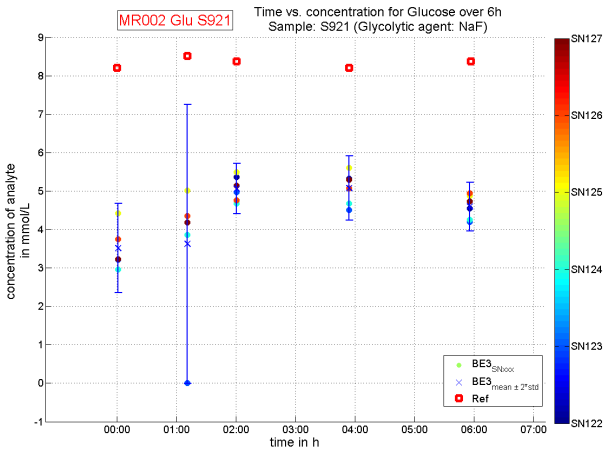
In the sample S721, containing solely sodium fluoride (see 3.16c) a starting concentration of 3.05 mM with a standard deviation of 0.74 mM was measured. After 6 hours, a mean glucose concentration of 3.79 mM with a standard deviation of 0.34 mM was determined. This results in a mean glycolysis rate of 4.14% per hour. The reference measurement of the plasma with the Hitachi 902 analyser resulted in a rate of and -0.72% per hour.

Figure 3.16d shows the results of the corresponding control sample containing no anti-glycolytic agent. The mean rate of the glycolysis was 0.94% per hour.

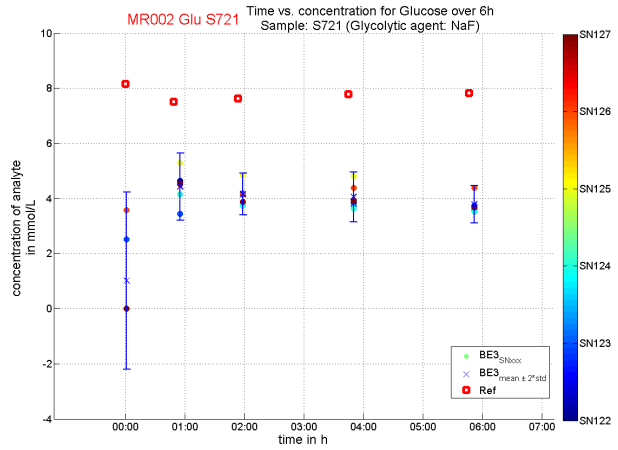
Moreover, the BSA levels were analysed, in the same manner as previously for glyceraldehyde and bromopyruvic acid. In figure 3.18 the statistical analysis of the measured BSA-equivalents is shown in form of a histogram. Figure 3.17 depicts the trend of the BSA-equivalent signals over the course of the experiment in a time vs. BSA-level plot.



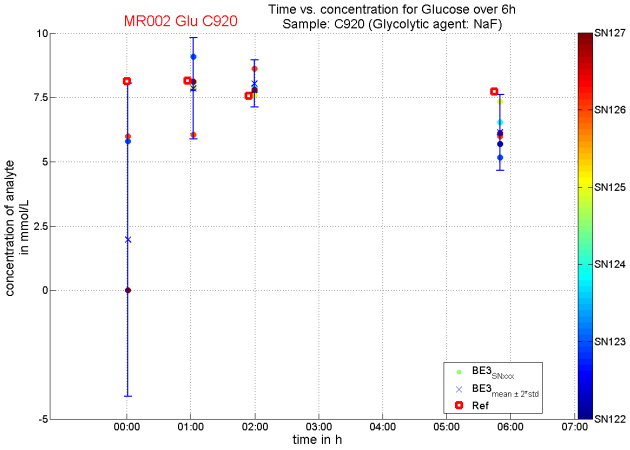
(a) Sample D921: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 6,5 mM (dialysed), NaF: 65 mM (dialysed), GA: 10 mM (dialysed).



(b) Sample S921: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 8 mM (spiked), NaF: 65 mM (spiked), GA: 10 mM (spiked).



(c) Sample S721: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 8 mM (spiked), NaF: 65 mM (spiked), GA = 0 mM (spiked).



(d) Sample C920: The sample was prepared according to the protocol described in 2.2.4. Glucose level: 8 mM (spiked), NaF = 0 mM, GA = 0 mM:

Figure 3.16.: Time series for glucose measurement with bromopyruvic acid as anti-glycolytic agent under the above mentioned conditions. a) - c) Results from all 6 b123 devices, the mean (×) and error estimation (⊥,⊤; ±2 * STD) for each measuring point and compares them to the results from the reference device (□).

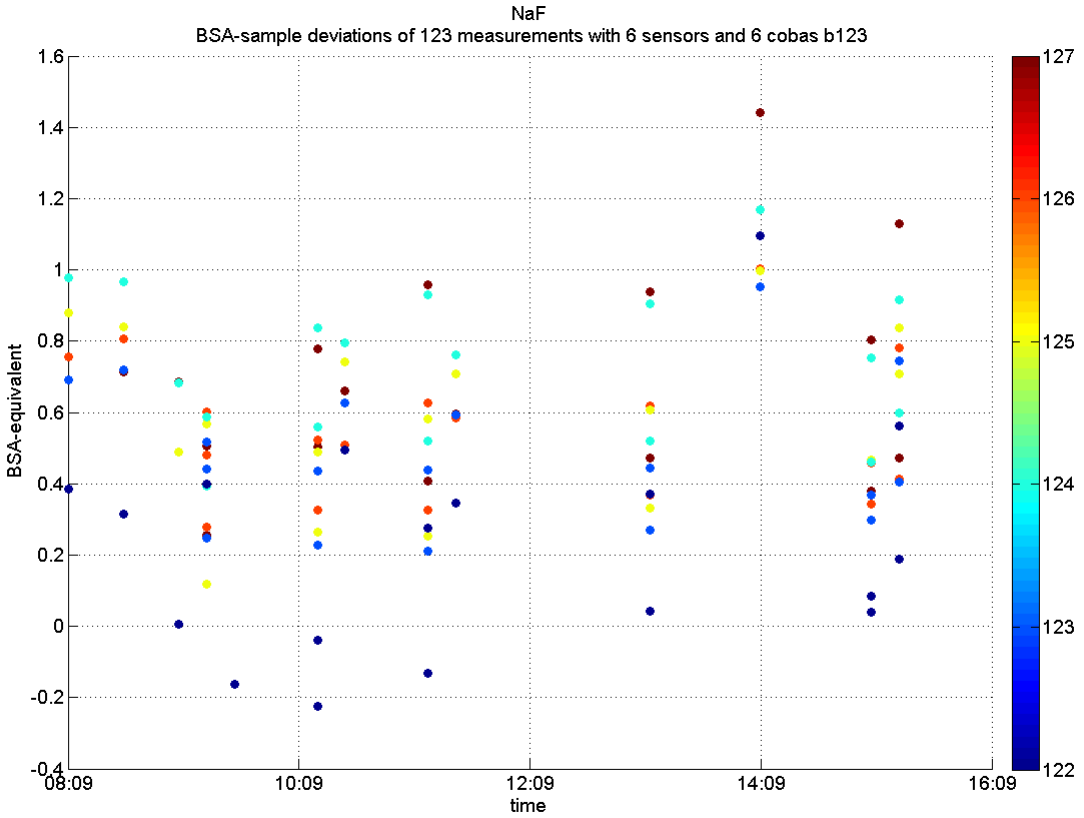


Figure 3.17.: Trend of the BSA equivalents measured by the cobas 123 devices during the run with sodium fluoride alone and in combination with glyceraldehyde in a x-y plot of time and the BSA-equivalent signal.

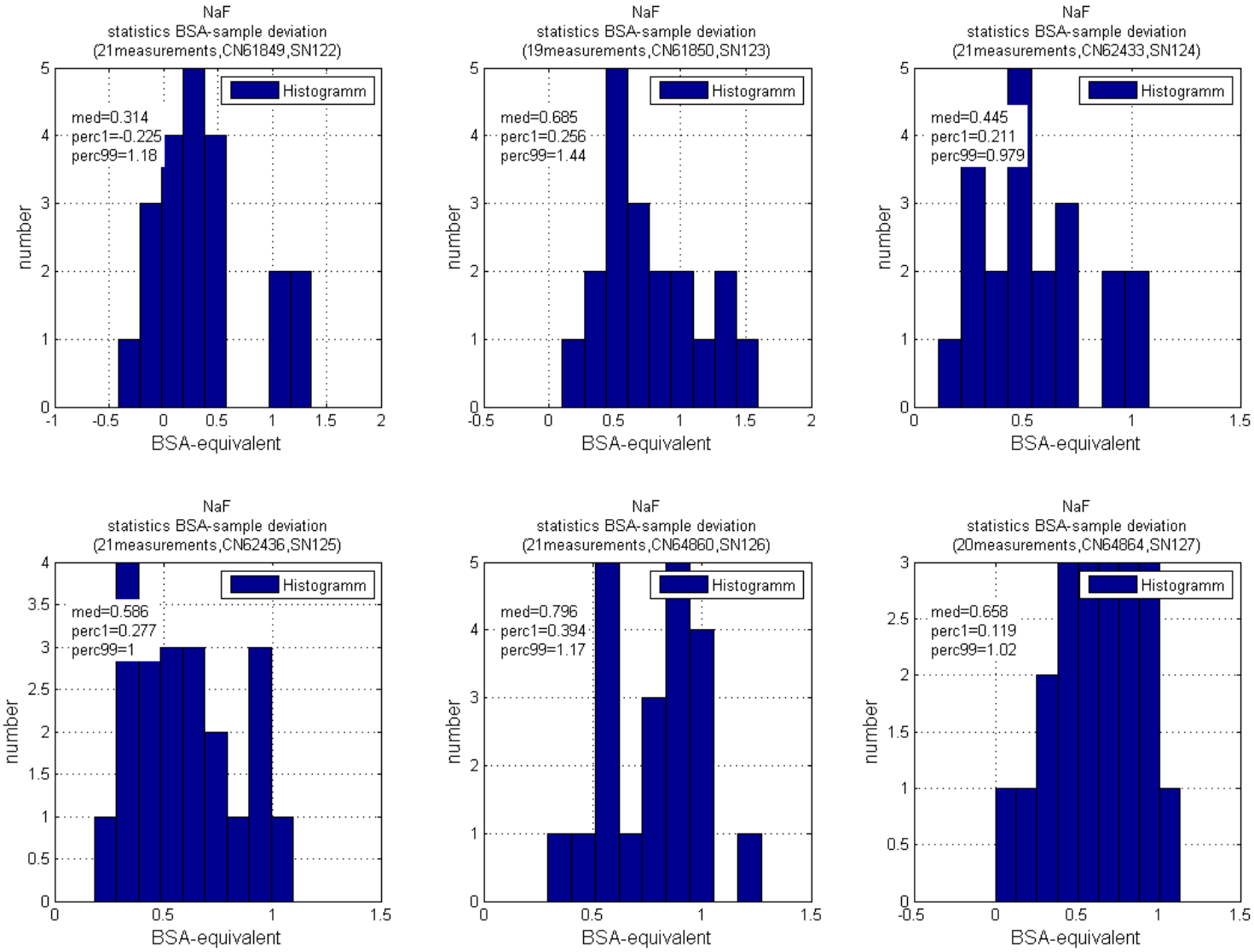


Figure 3.18.: Shows the statistical analysis of the BSA equivalents measured by the cobas 123 devices during the run with glyceraldehyce. The histogram depicts the strength of the BSA-electrode on the x-axis and the number of incidents with the according strength on the y-axis.

The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

William Lawrence Bragg

4. Discussion

4.1. Methods

The setup of the second experiment MR002 was slightly changed compared to the first experiment MR001. Whereas in the setup of the first experiment the samples were kept at constant room temperature ($24°C$) and on the roller mixer for the duration of the whole experiment, the samples of the second experiment were kept in the refrigerator at $8°C$. The aim was to evaluate the efficacy of the anti-glycolytic agents in combination with cooling the sample down to $8°C$. According to previous studies and literature [38] a significant increase in performance was expected compared to the results obtained at room temperature. Since pO_2 in particular and, to an almost equal extent pCO_2, have a major influence on the whole blood sample and the sensor performance [9], the setup was also optimised in such a way that the bias of pO_2 was limited. Although the samples in experiment MR001 were stored in a air-tight container, every time the capillaries were filled in order to perform a analysis, native pO_2 and pCO_2 were biased by exchange with O_2 and CO_2 in the surrounding air. In order to circumvent this problem, the setup of experiment MR002 was adapted such that each sample was aliquoted into six aliquots stored in air tight containers. For each measurement, a new aliquot was used making thereby sure that each new measurement was unbiased by the previous measurements.

The O_2 and CO_2 parameter are very good indicators for ongoing enzymatic activities within the whole blood sample and within the RBCs, respectively. Furthermore, pO_2 and pCO_2 have a considerable influence on the pH value and on the performance of the glucose and lactose sensors, therefore an increase in performance in regard to the stabilizing effect agent was expected. Nevertheless, a considerable improvement of the performance due to the changed setup could not be verified.

It could be demonstrated that the choice of the principle by which the glucose and lactate is measured, i.e amperometrically (*cobas b123* and *cobas b221*) or photometrically (*Hitachi 902 Analyzer*), has a considerable influence on the type and the performance of the anti-glycolytic agents, and therefore also on the stability of the whole blood sample with regard to its glucose and lactate concentrations. The results obtained with the photometric method applied in the *Hitachi 902 Analyzer* demonstrated that inhibition of the glycolysis is in fact possible while the amperometrically obtained results showed to be very sensitive in terms of interference incidents caused by the anti-glycolytic agents (see results in 3.2.1). Nevertheless, one has to be careful when comparing the results obtained by the devices mentioned above. When analysing the data set of the Hitachi it has to kept in mind, that these results were in fact obtained from measurements in plasma and not in actual whole blood (see sections 2.2.3 and 2.2.4). By transforming the whole blood sample to a plasma sample also the complexity of the matrix of the sample is changes considerably and the chance for interferences is reduced. The deviations observed in the data sets from the *cobas b123* are caused by the complex composition of the whole blood matrix. Since the sensor is not in direct contact with the sample, another advantage of

optical measurement of the glucose concentration is that the system is relatively stable with regard to the interference effects of the anti-glycolytic agent. The combination of these interferences and the complexity of the whole blood sample are considered to be the major sources for the considerable discrepancies between the results obtained with the *cobas b123* and the Hitachi and are further discussed in the following sections (see 4.3).

4.2. Discussion of the results of experiment 1 (MR001)

As already pointed out in the methods part in 2.2, the first experiment was designed to give a first impression and further information on the performance of the substances described in the referred literature [19, 20], [26],[31, 32],[34],[39] as anti-glycolytic agents and their compatibility with the sensor cartridge of the *cobas b123*.

Deoxyglucose A closer look at the results with deoxyglucose as an anti-glycolytic agent reveals that deoxyglucsose seems to cause serious positive interferences. A positive rate of glycolysis is a reliable indicator for interference behaviour, since no glucose was added after the start of the experiment. In the case of the run with deoxyglucose, the results were rather drastic. Unfortunately, deoxyglucose is an inhibitor of the GOD enzyme used by the biosensor [40]. A measured glucose concentration of 140 - 200 mM in a sample that should contain only $\approx$ 5 mM (compare figure 3.2b) shows that the measuring system (biosensor, electronic and software) is not capable of handling deoxyglucose. Due to the inhibition of GOD, the sensor cannot produce a reliable signal and the downstream signal interpreting electronic circuit, as well as the software interpret the signal without valid reference to the actual glucose concentration present in the sample. Figure 3.3 already illustrates the reason for the great deviation between the glucose concentration measured by devices SN126 and SN127. Due to the great difference in the calibration curves, which take the sensor specific parameters such as linearity and slope into account, the concentration calculated on base of the mV-value may differ considerably. Within the specified, medically relevant range for the glucose concentration of 0-40 mM, the differences in linearity have little to no influence on the measured concentrations but at higher voltages such as i.e. 510 mV the influence of the non-linearities have a considerable influence on the outcome. Due to this, a measured signal of 510 mV may lead to a corresponding glucose concentration of $\approx$ 80 mM or $\approx$ 120 mM.

Nevertheless, analysis of the lactate concentration in A.1 indicated that glycolysis was slowed down, but not effectively enough since the lactate concentration still increased over time. The high glucose concentration is a result of correction algorithms that interpret this false signal, producing these totally out of range results. For this reason, deoxyglucose was eliminated as a possible option for producing a stable whole blood sample.

Glyceraldehyde The first results with glyceraldehyde as anti-glycolytic agent showed promising results. In particular the results for SN127 (compare figure 3.1 and 3.4a) showed the potential of glyceraldehyde. Compared to the control sample, the rate of glycolysis was slowed down by glyceraldehyde 3.6 fold in the case of SN126 resulting in a rate of -1.98% per hour. SN127 showed even better results. With a resulting rate of +0.33 % per hour the glucose level changed 18 times slower than the control sample. Although the analysis of the data of device SN127 resulted in a slightly positive rate,

the presumed positive interference of glyceraldehyde was not assessed as problematic as in the case of the previously discussed deoxyglucose. Together with the fact that there was still potential for optimizing the conditions for the sample preparation in terms of concentration of the anti-glycolytic agent, sample cooling and handling, glyceraldehyde was still considered to be effective as a potential stabilizer of glucose in a whole blood sample.

Sodium fluoride The analysis of the results for the first run with sodium fluoride as anti-glycolytic agent showed that it might work well in combination with the roche-sensor as stabilizer of the glucose level in whole blood samples. By applying 110 mM sodium fluoride, glycolysis could be slowed down to rates of -2.04% and -1.54%, respectively compared to the control sample with rates of -6.23% and -5.83%, respectively. According to the user manual of the *cobas b123* [9], sodium fluoride was tested as a possible interfering substance. However, this test included only concentrations at approximately 0.1 mM. The concentration that was used in this experiment was with 110 mM approximately by a factor 10^3 higher. Nevertheless, no signs of a possible interference were determined.

Bromopyruvic acid Besides glyceraldehyde and sodium fluoride, the run with bromopyruvic acid resulted in the third best outcome in terms of slowing down the rate of glycolysis. With a rate of +1.96% and +2.24% per hour, bromopyruvic acid seemed to slow down glycolysis in a sufficient manner, although as in the case of glyceraldehyde, a slightly positive rate indicated positive interference of the bromopyruvic acid with the glucose sensor. Due to these results, bromopyruvic acid was also taken into consideration for detailed evaluation of its efficacy as potential glucose stabiliser.

Quercetin The flavone Quercetin is a known inhibitor of the hexose transporter GLUT1. The results that were presented by Vera et al. [34] in red blood cells made Quercetin a logical choice for the first experiment. Unfortunately, these results could not be reproduced with the present setup in use for these measurements and no inhibitory effect of Quercetin could be observed. With -5.05% and -4.51% per hour the glycolysis rate of the sample containing Quercetin was even almost 1% higher than in the control sample. It appears that the presence of Quercetin actually increases the rate at which glucose is transformed to lactose. Signs for other interference behaviour of Quercetin in combination with the sensor were not observed. Due to the outcome, a second run was performed leading to the same results. Therefore, Quercetin was eliminated from the list of possible stabilizers.

Isorhamnetin The anti-glycolytic agent isorhamnetin is a synthetic flavone with similar characteristics to Quercetin. According to Vera et al. [34], the inhibitory effect of isorhamnetin is supposed to be slightly less effective than that of Quercetin. The run was performed in order to test the hypothesis that flavones, in general, seem to have a negative bias in combination with the Roche-sensor. The outcome of the run showed that its effect is not as drastic as compared to quercetin, but the rate of glycolysis only slowed down insignificantly. Therefore isorhamnetin was also excluded from further experiments.

Based on these results it was decided to start another series of measurements assessing the potential of those substances that proved to be most compatible with the measuring system; glyceraldehyde, bromopyruvic acid and sodium fluoride. Moreover, since the first series of measurements were performed under the 'worst case' environmental conditions,

the goal of slowing the rate of glycolysis down to -0.1% was still considered to be achievable by improving the experimental setup in regard to temperature and O_2 and CO_2 exposure.

4.3. Discussion of the results of experiment 2 (MR002)

The results presented in 3.2 showed that for none of the anti-glycolytic agents or any tested combination of them the rate of glycolysis could be decreased to 0.1% per hour when analysed on the *cobas b123* system. A comparison between the results obtained from the reference device, the Hitachi 902 Analyser and the data from the *cobas b123* revealed that an inhibition of glycolysis can be achieved in principle but the particular Roche-sensor module in the *cobas b123* is not able to handle the anti-glycolytic agents used.

Especially the run with glyceraldehyde and the analysis of the reference data might be proof for that hypothesis. By studying the reference data from the glucose measurement in plasma in figures 3.10a-3.10b, it can be observed that, in fact, the glycolysis rate decreased with the increasing amount of glyceraldehyde that was added to the whole blood sample. While a concentration of 10 mM glyceraldehyde was already sufficient to slow the rate down to 0.13% per hour a concentration of 50 mM improved the result to a rate of 0.09% per hour. This rate would be slow enough to declare the whole blood sample stable with regard to its glucose level. However, these results were achieved in plasma and by photometric glucose measurement. When comparing the data obtained by the *cobas b123* with the data set of the Hitachi it is obvious to the observer that some interferences must occur during the measurement procedure. An obvious hint for the presence of interference signals is the fact that the glucose concentration seems to increase instead of decrease, which is impossible since no glucose was added to the sample after the start of the measurements. These interference signals can have several origins, such as reaction with the active enzyme on the working electrode or reactions between the anti-glycolytic agent and the reference electrode. Both would have a direct influence on the measured blood gas parameters and the results obtained for the glucose concentration. Another source for error would be a series of reactions within the blood sample or directly within the RBC, catalysed by the anti-glycolytic agent that cannot be observed directly.

In order to determine the presence and assess the influence of assumed interfering incidents, the measured results of the BSA sensor were analysed as well. In fact, the analysis of figure 3.10 and 3.11 reveals that, as the mean glucose signal of the *cobas b123* tends to approach the glucose level measured by the reference device, the corresponding BSA levels generally decrease over time. This is a strong indicator for interferences caused by possible direct reactions of the anti-glycolytic agent with the active enzyme immobilised on the sensor. Nevertheless, the analysis of the BSA signals (compare section 3.2) has shown that, depending on the anti-glycolytic agent and its concentration, some but only minor interfering events happened at the WE of the glucose sensor. Furthermore, the influence of the concomitant correction algorithm was shown to be only fractionally responsible for the difference in results. A detailed analysis of the influence that the BSA correction algorithm has on the glucose concentration is presented in the appendix B.1

Therefore, the huge difference in results between the measurements executed by the *cobas b 123* devices in whole blood and the results the measurements of the Hitachi cannot be explained by the BSA correction algorithm alone. Further interfering events

were either not detected by the BSA sensor or were of different origin.

Due to the arrangement of the electrodes (principle of a three electrode elctrochemical cell setup - see section 2.2.1), the potential of the Ag/AgCl RE can be influenced by high concentrations of ions of the halogen family such as F^- and Br^-. Since Br^- has a lower electronegativity than Cl^- its influence is not as crucial as the bias due to a high concentration of F^--ions, which have a higher electronegativity than Cl^-. Therefore, the difference between the measured glucose concentration can be explained to a major part by interfering sodium and bromide ions that interact with the Ag/AgCl reference electrode implemented in the amperometric sensor setup. Due to the adsorption of F^- and Br^-, respectively, the potential is no longer kept constant at 350 mV and therefore the measured signal no longer corresponds to the actual glucose concentration present in the blood sample.

4.4. Conclusional statement

The performance evaluation of the precision of a POC blood gas analyser, such as the *cobas b123* by Roche according to CLSI EP5-A2 is inevitably linked to the need for a stable whole blood sample. Producing a stable whole blood sample is all but trivial. Due to the numerous cellular activities and highly interdependent processes, in combination with a multitude of influencing coefficients that have to be taken into consideration, the production of one single whole blood sample is rather complex and probably not accomplishable. Since the high rate at which glucose decreases and lactate increases, stabilizing the whole blood sample in regard to its glucose and eventually lactate concentration was considered to be essential. Therefore, the focus was on finding a practical method to stabilize the glucose and lactate concentrations in a whole blood sample to a sufficient extent. While it was possible to show that the inhibition of glycolysis can be principally accomplished when measured optically, it was not possible to establish a practical method for the the sufficient inhibition of glycolsis in combination with amperometrical measurement of glucose and lactate, respectively. The necessary concentration of anti-glycolytic agents needed to reduce the rate of glycolysis to a reasonable level of 0.1% per hour and thereby sufficiently stabilizing the glucose concentration, led to a couple of interfering incidents. These incidents had such great influence on the measurement results, that an evaluation of the precision of the POC device was considered to be pointless. Therefore, a new protocol for the performance of an precision test and evaluation according to the CLSI standard EP5-A2 could not be established. In combination with the POC blood gas analysing device *cobas b123*, it is recommended to stick with the established method (described in 1.2.2). Nevertheless, the usefulness of a cost effective method for evaluating the precision of POC devices or glucose meter that is closer to the CLSI standard is still given and therefore further research is recommended. Especially based on the fact that due to the focus of modern anti-cancer research on the inhibition of glycolysis, the development of more powerful anti-glycolysis agents is to be expected. Additionally, not only glucose and lactate need to be kept on a constant level, but the remaining BG parameters, i.e. electrolytes or pH need to be addressed, too. While for some parameters, such as chloride, this seems to be quite trivial (compare chloride concentrations of the control samples i.e. A.22, A.28 and A.36) other parameters, such as pH might turn out to be as much of a challenge as glucose and lactate, or even greater.

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2.2. Dialysis buffer: ISE/GLU nat V3 13
2.3. Stock solution: Glucose 50x 14
2.4. Stock solution: DG 2.5M 14
2.5. Stock solution: GA 2.5M 14
2.6. Stock solution: GA 5M 14
2.7. Stock solution: quercetin 0.05M 14
2.8. Stock solution: isorhamnetin 5mM 14
2.9. Stock solution: Na-3-BrPA 2.5M 15
2.10. Stock solution: NaF 5M 15

B.1. Components contributing to the imprecision 99

A. Appendix: charts and figures

A.1. Charts and figures for experiment 1 (MR001)

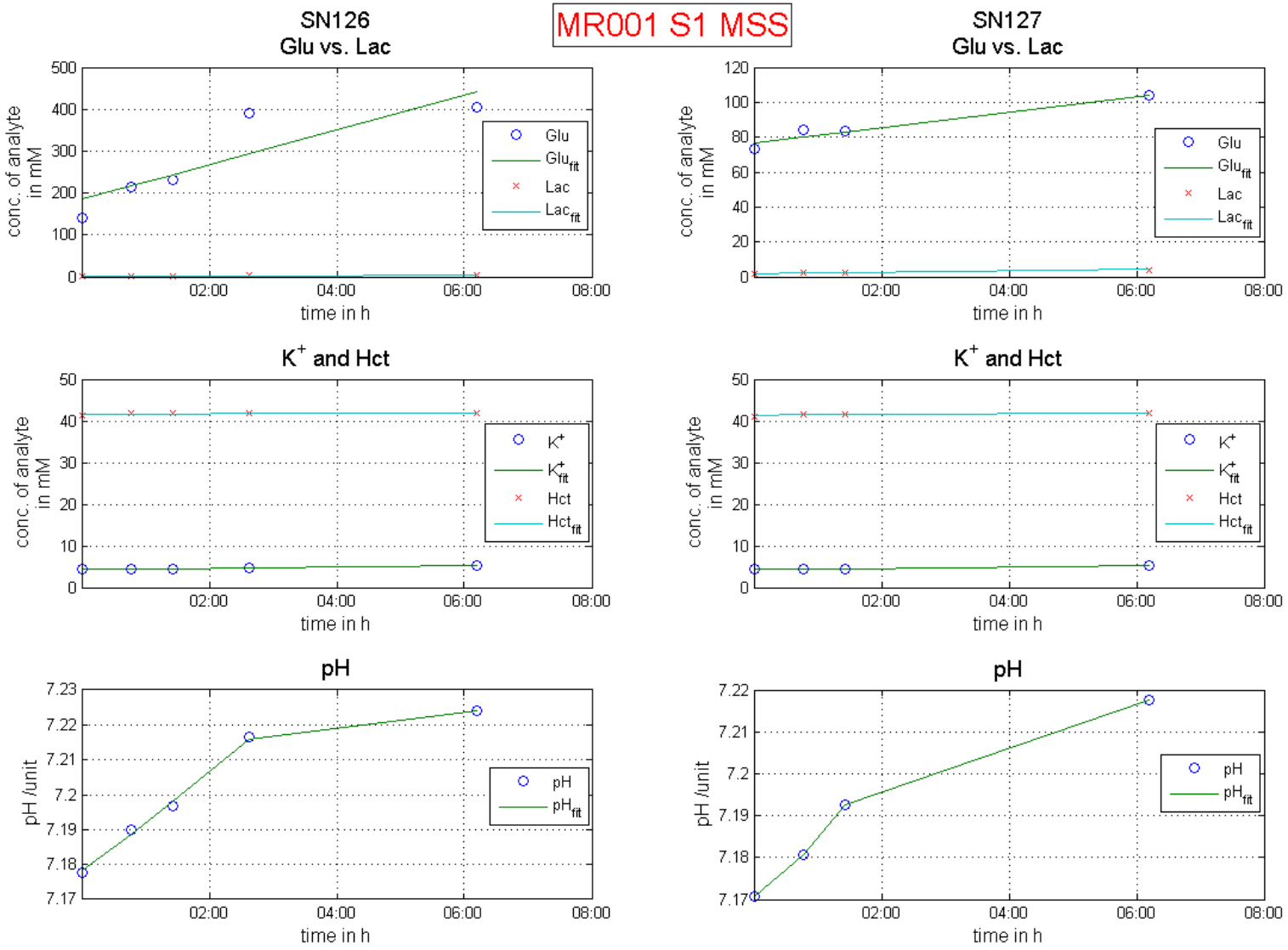

Figure A.1.: **S1 2-DG**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

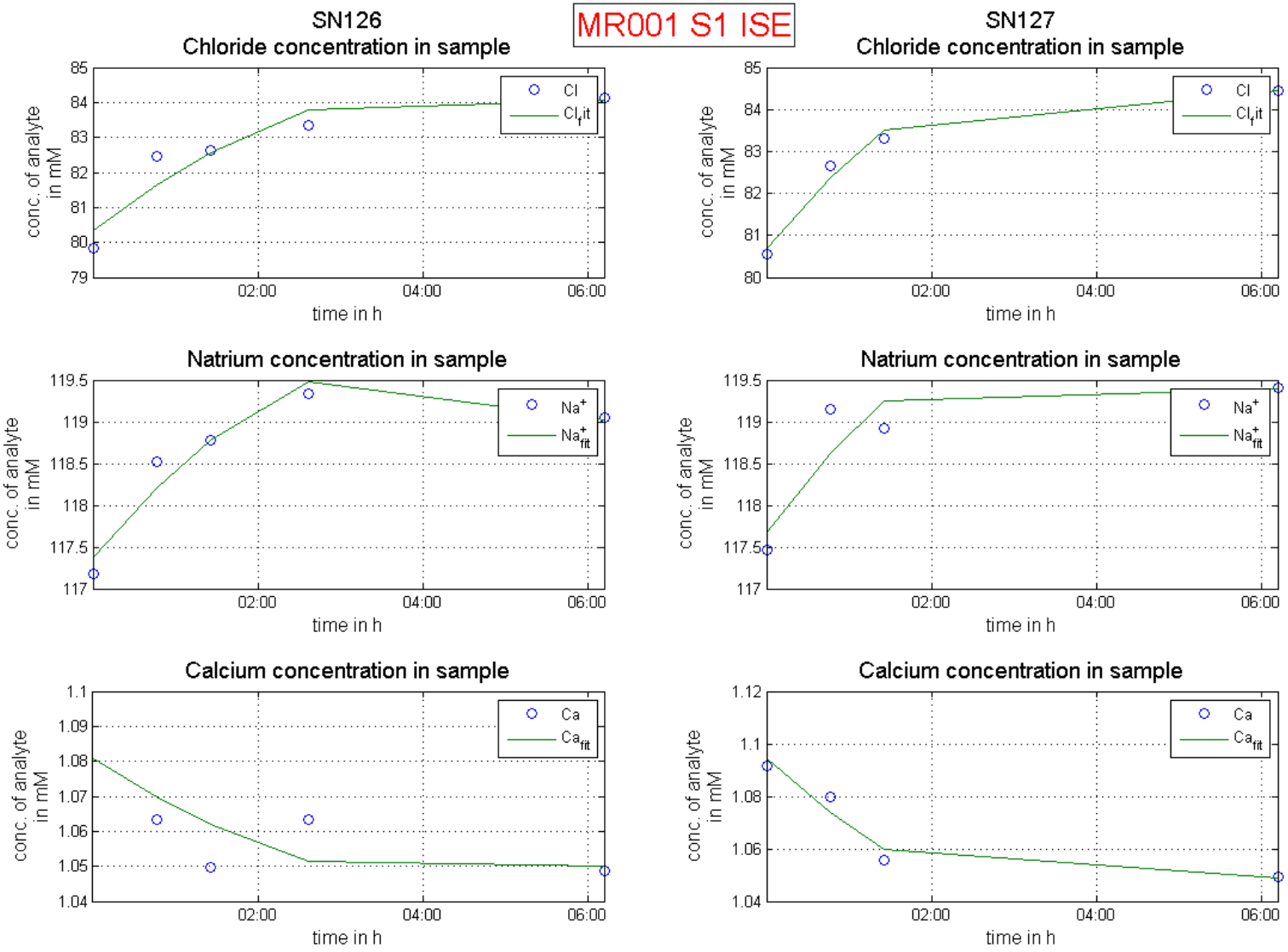

Figure A.2.: **S1 2-DG**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

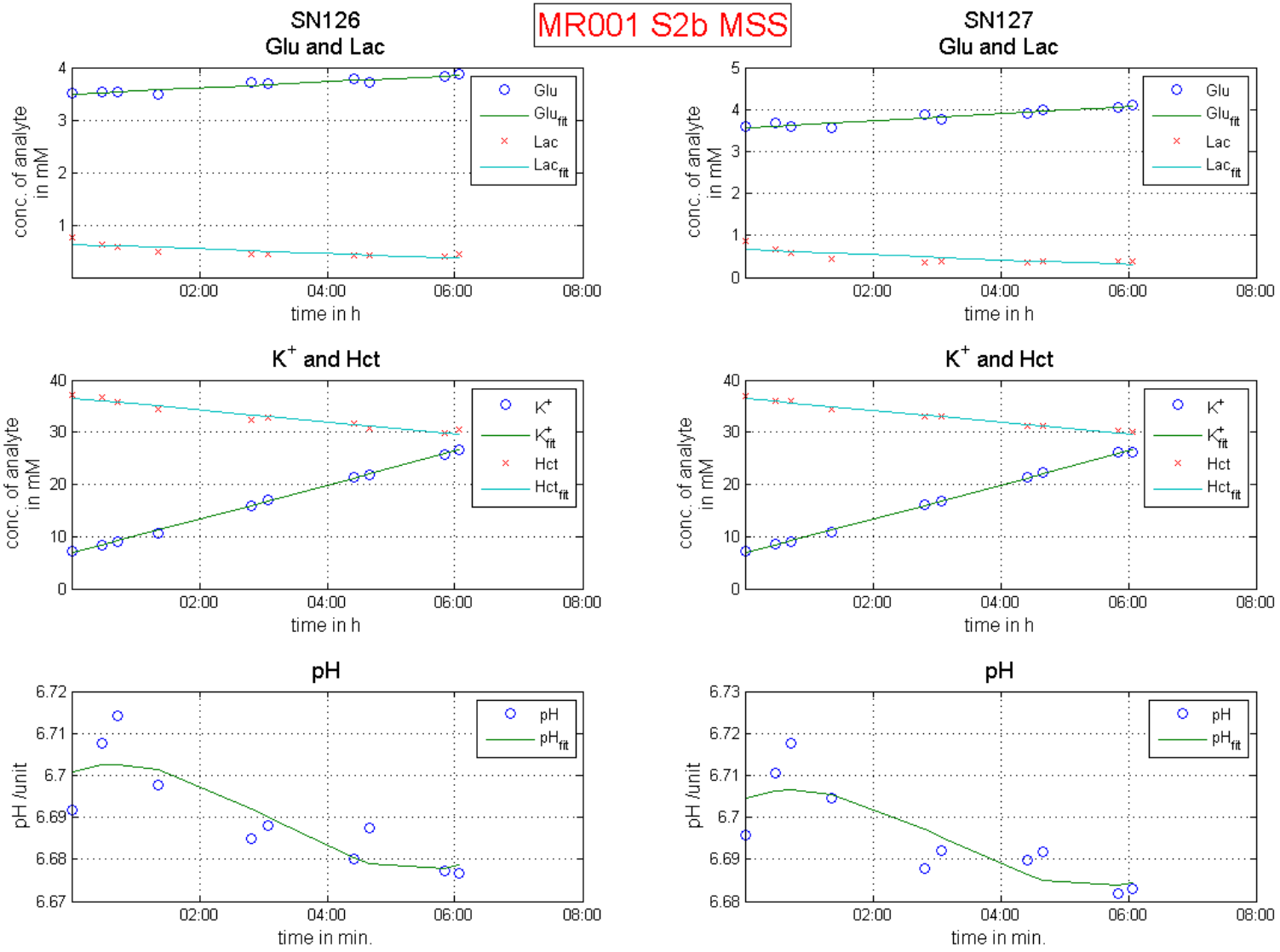

Figure A.3.: **S2 3-BrPA**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

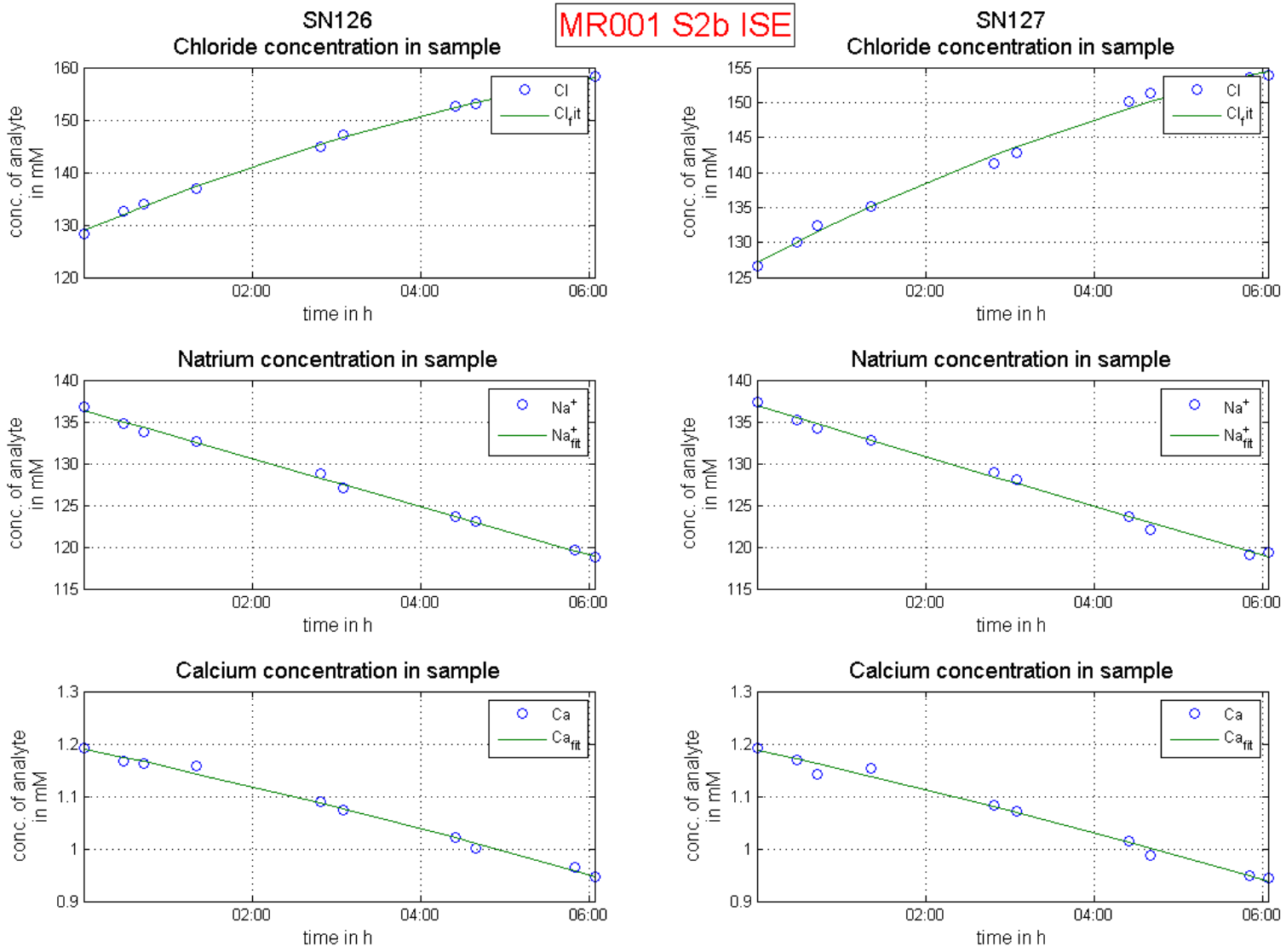

Figure A.4.: **S2 3-BrPA**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

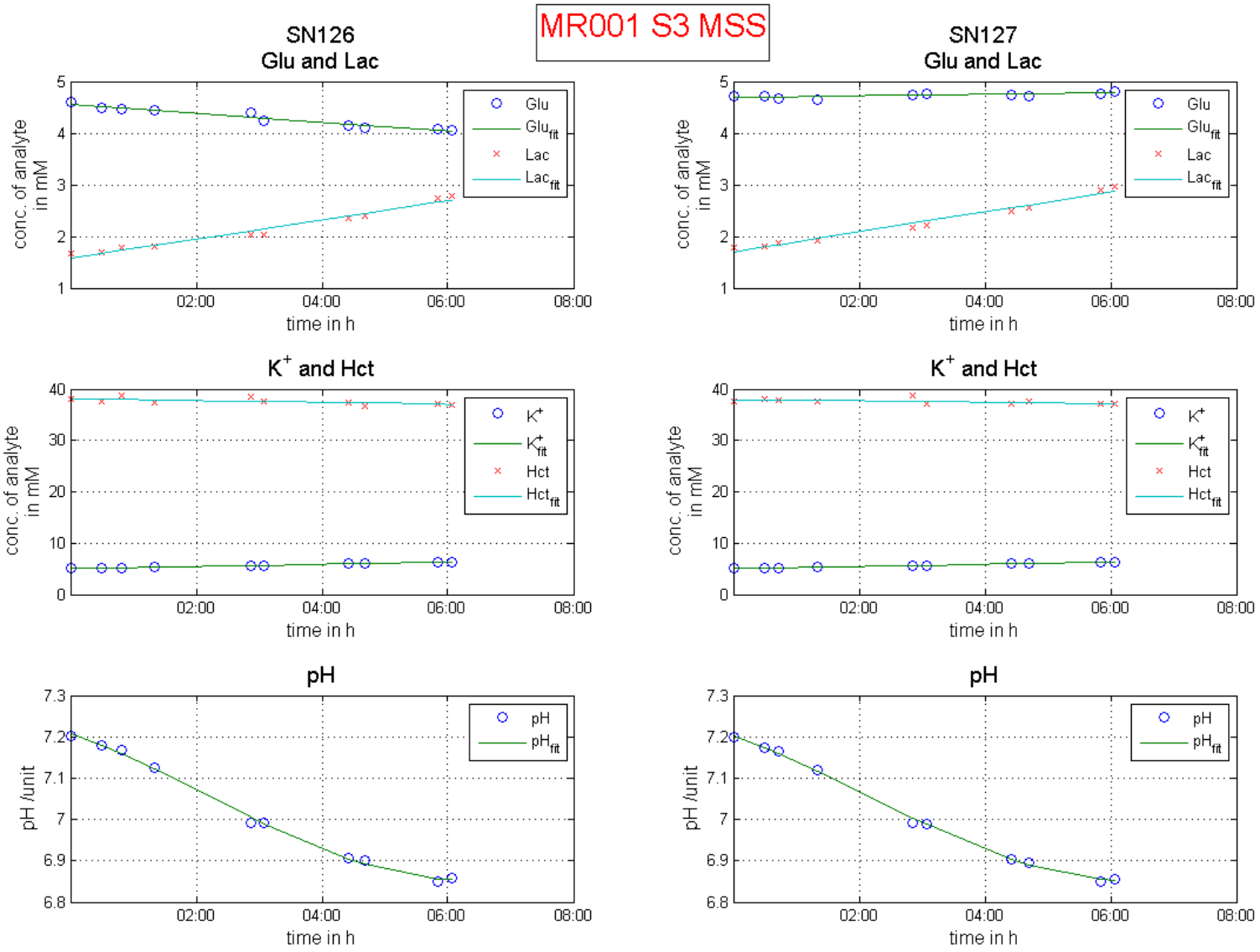

Figure A.5.: **S3 GA**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

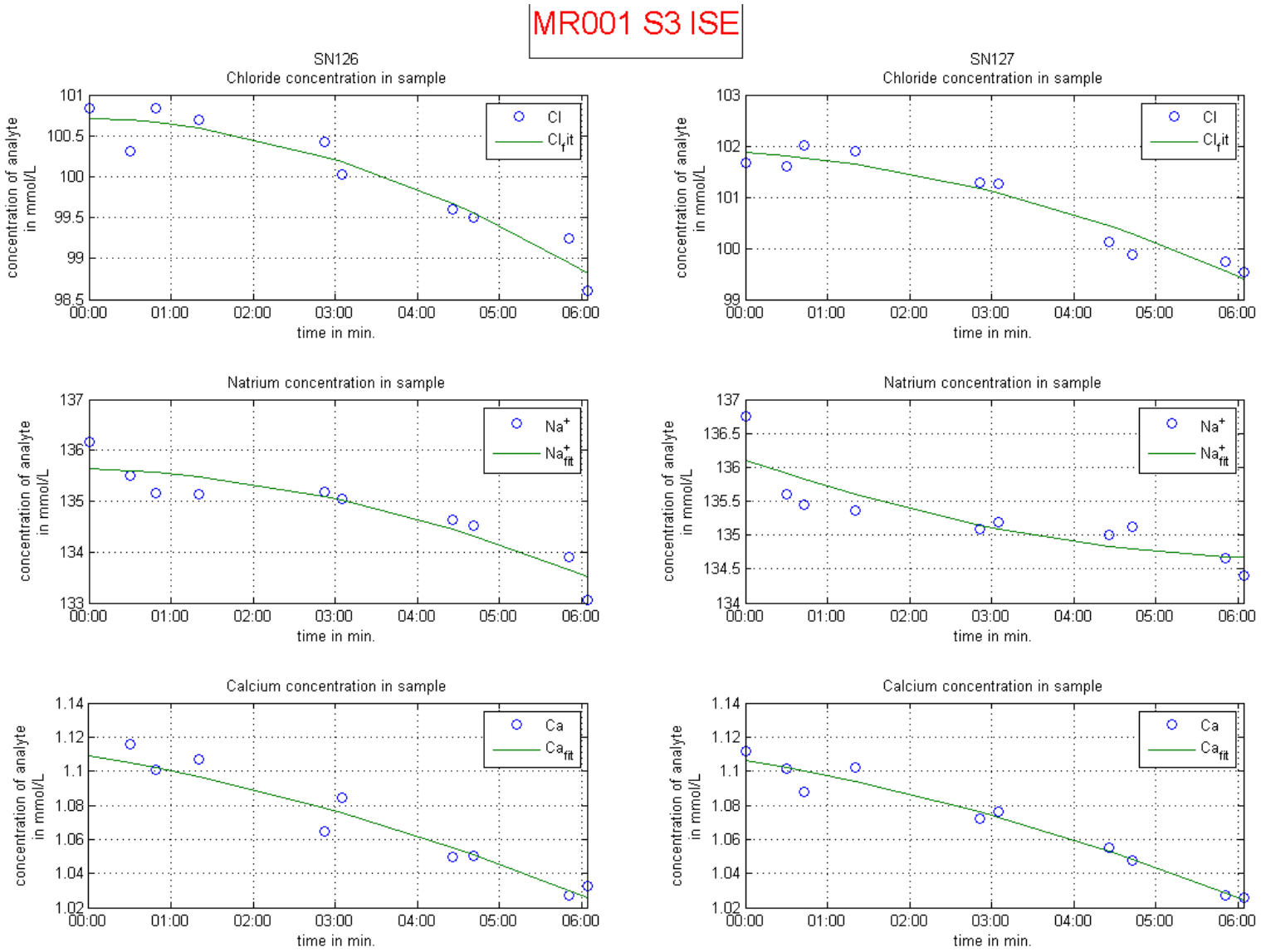

Figure A.6.: **S3 GA**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

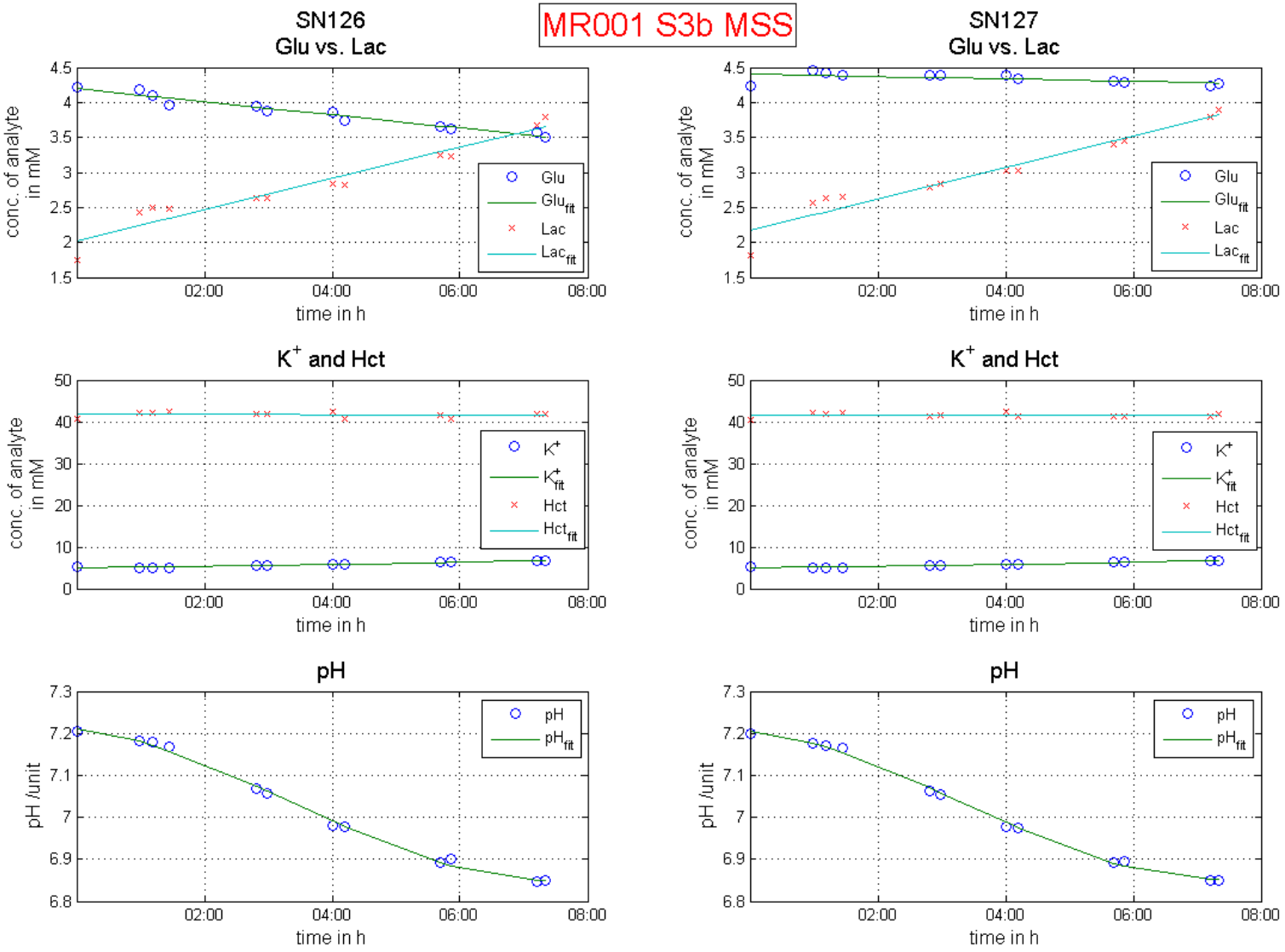

Figure A.7.: **S3b-GA**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

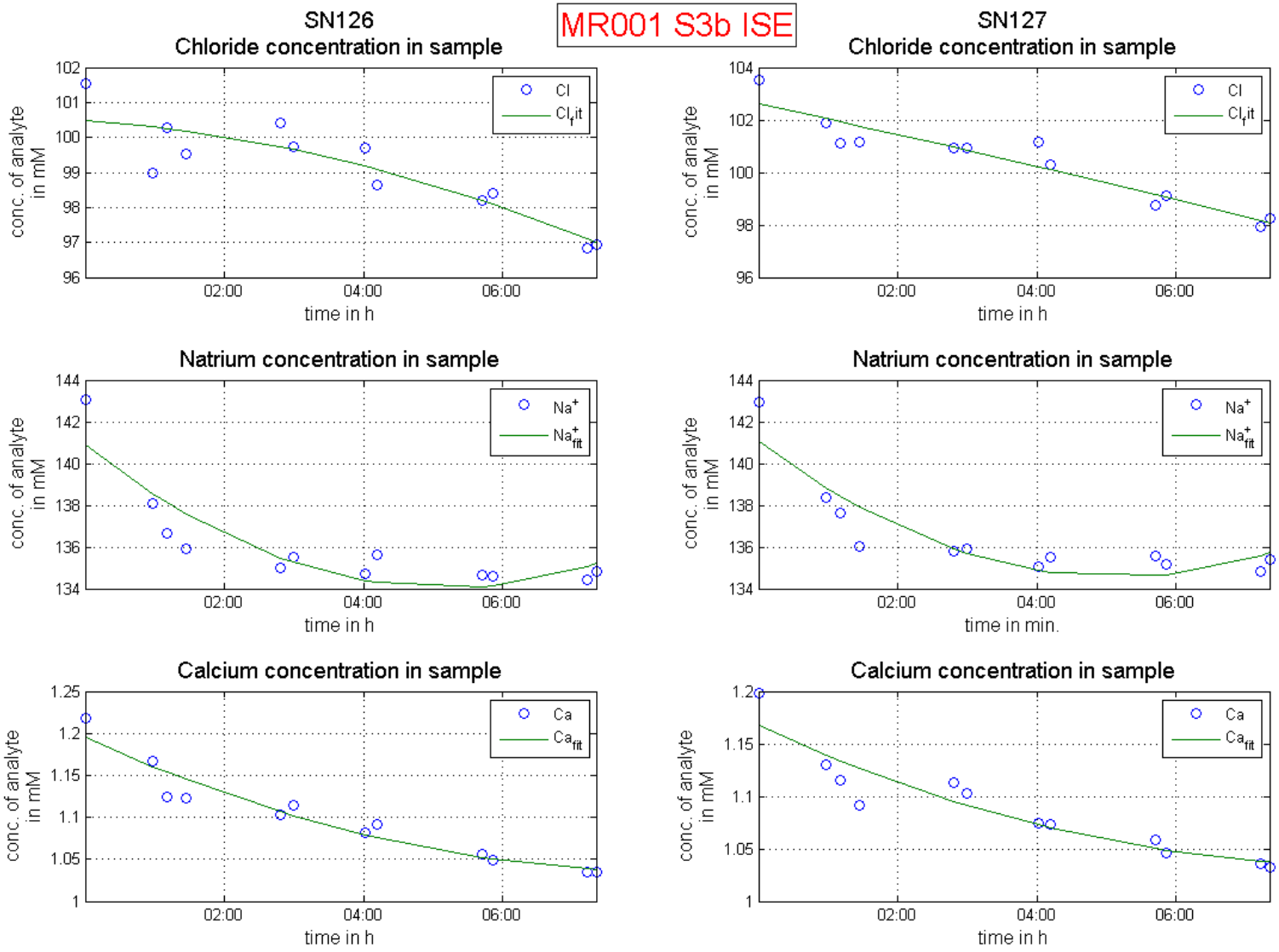

Figure A.8.: **S3b-GA**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

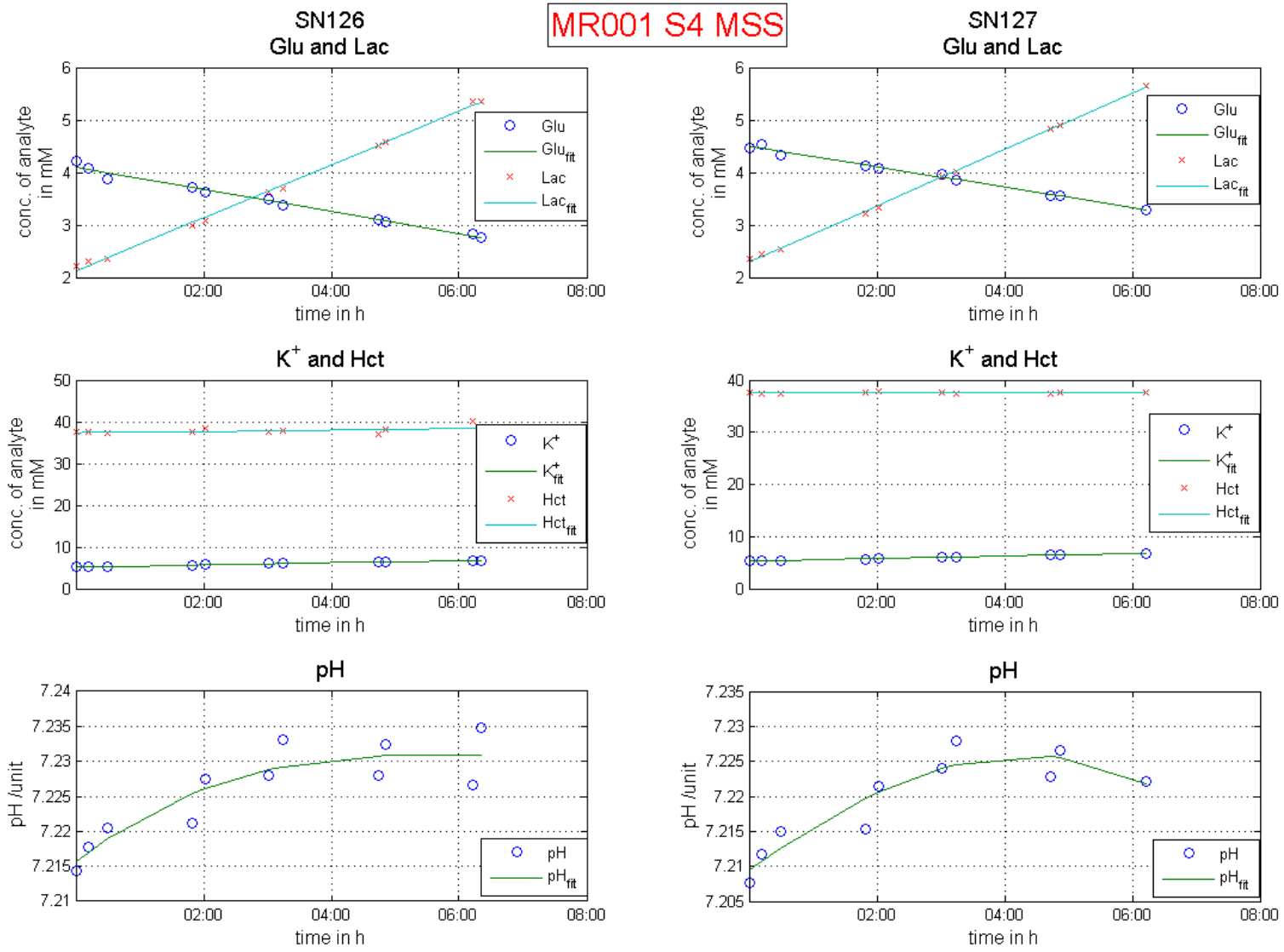

Figure A.9.: **S4 Ouabain**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

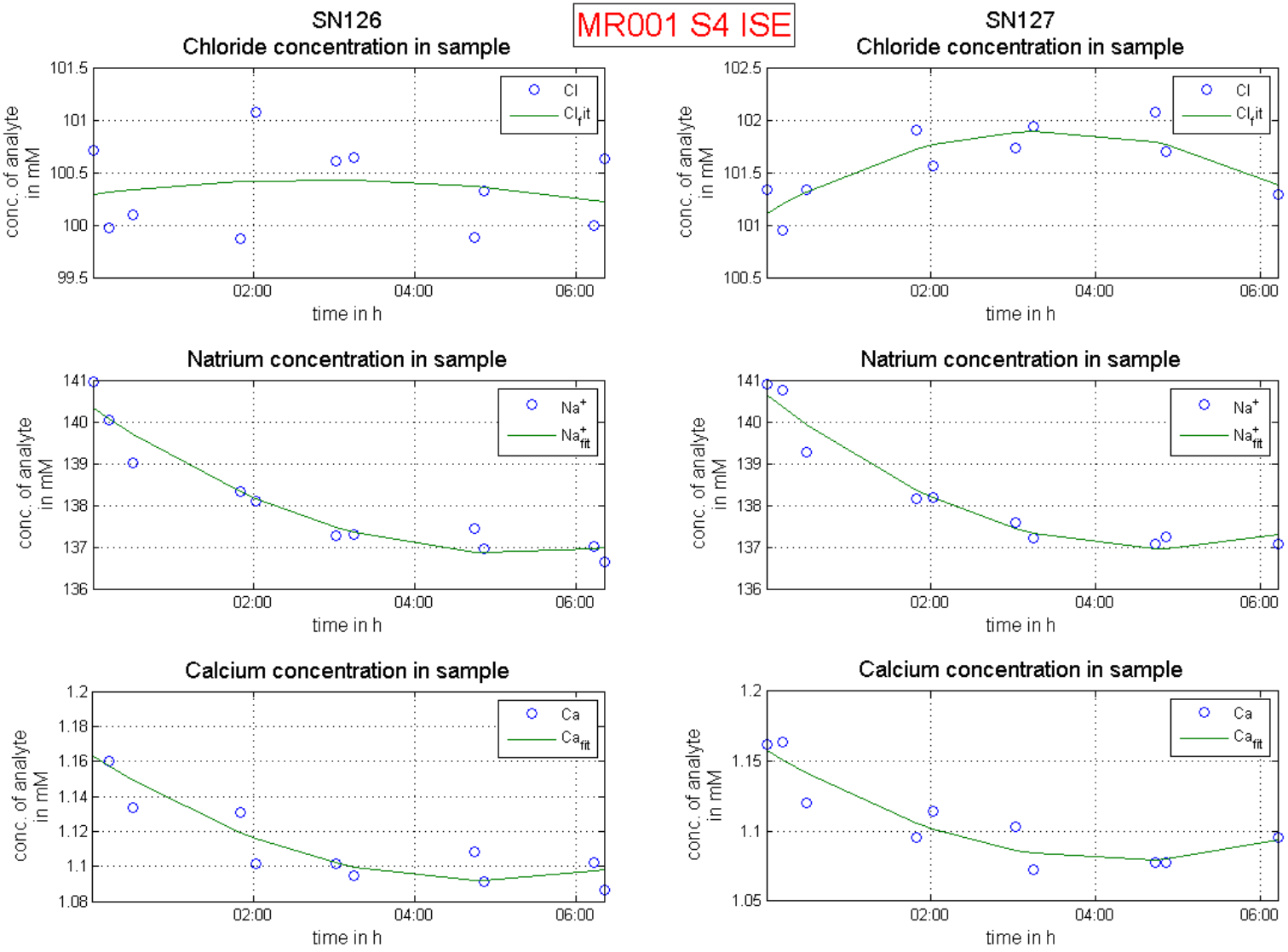

Figure A.10.: **S4 Ouabain**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

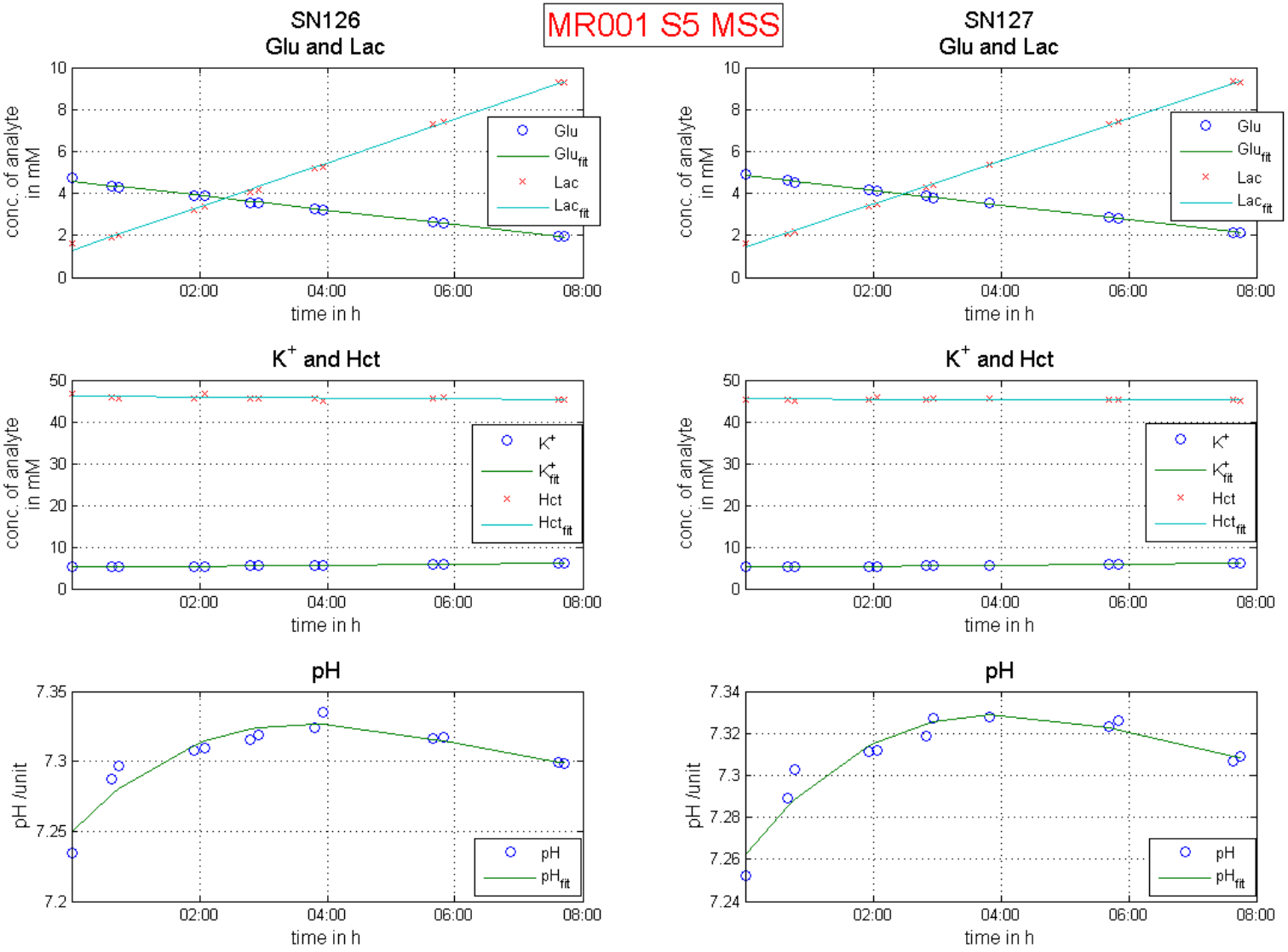

Figure A.11.: **S5 Quercetin**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

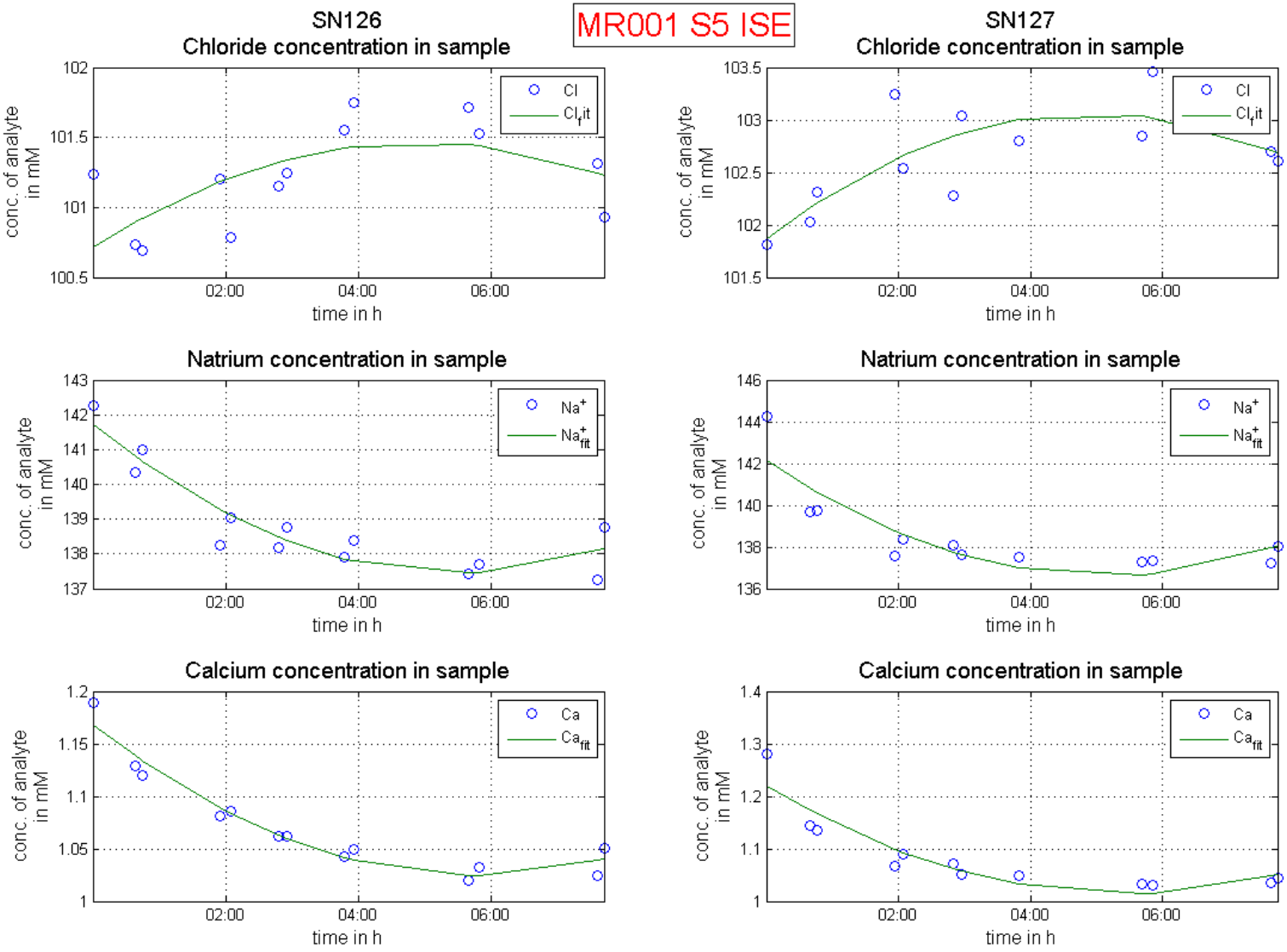

Figure A.12.: **S5 Quercetin**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

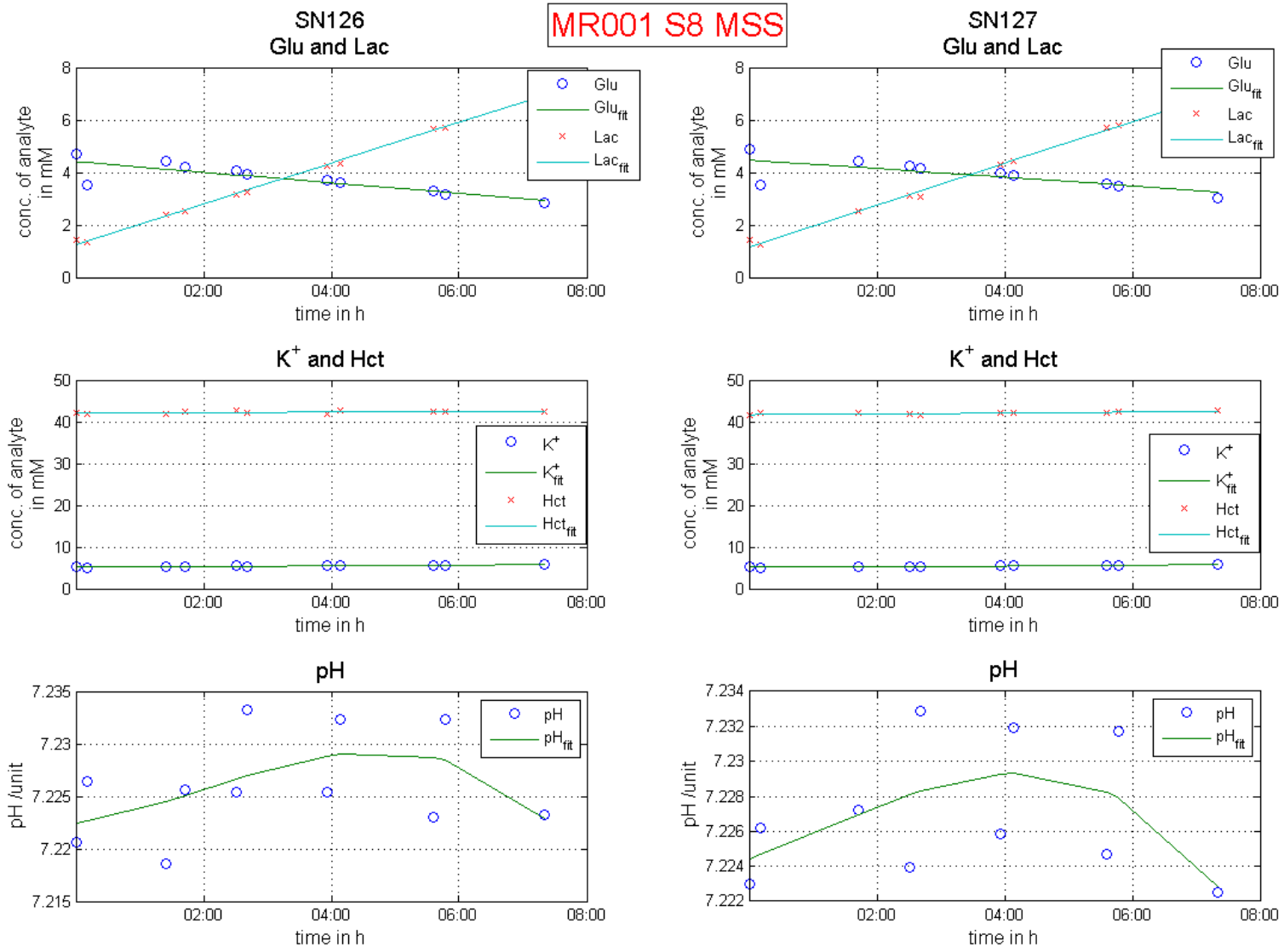

Figure A.13.: **S8 Quercetin**: *MSS* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

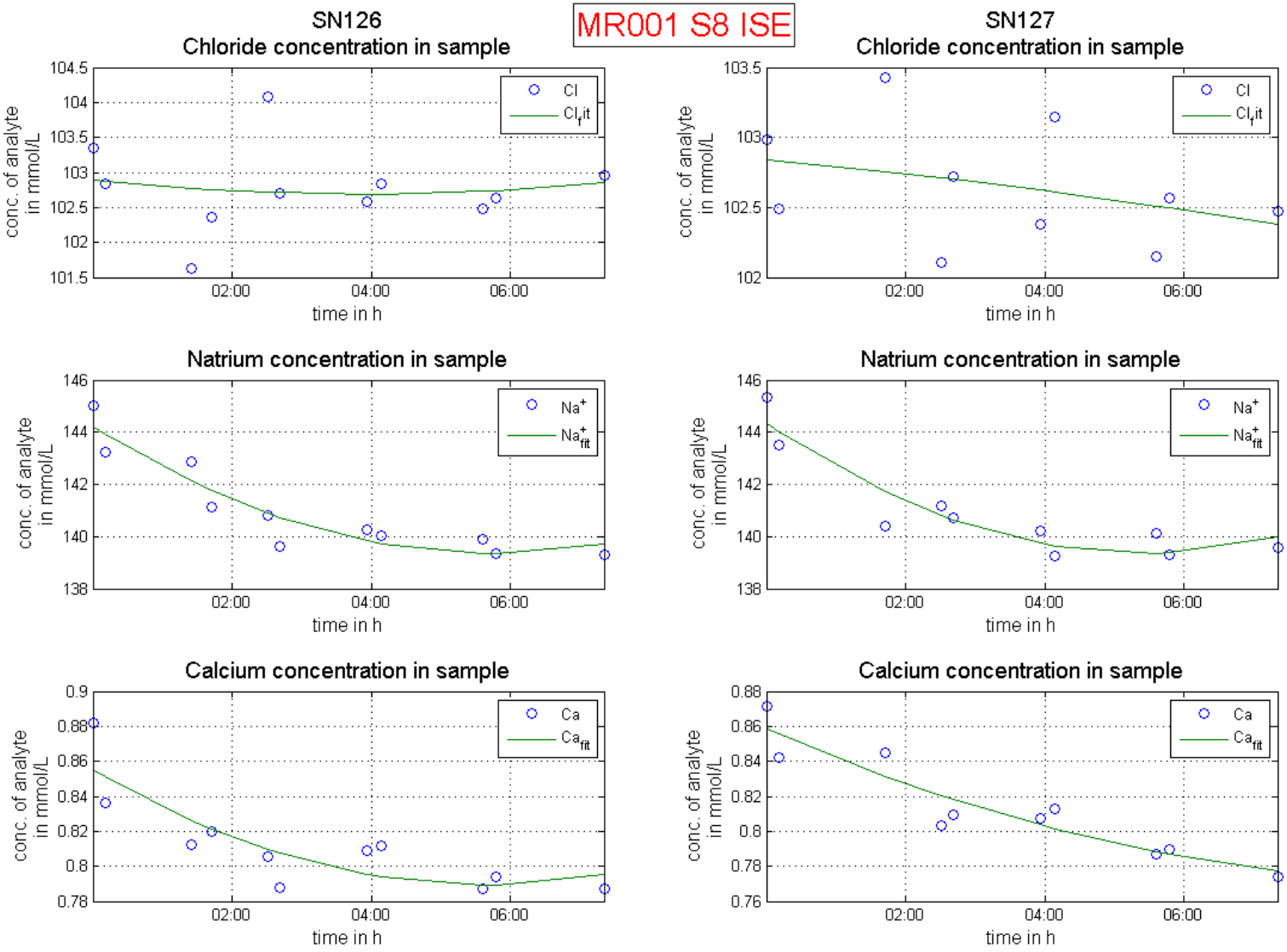

Figure A.14.: **S8 Quercetin**: *ISE* parameters were measured in regular intervals (30 - 60 min) over a total time span of 6 hours. The results were plotted in a concentration vs. time diagram. In order to illustrate the trend of the concentration over time, a polynom of 2^{nd} order was fitted and plotted into the data points.

A.2. Charts and figures for experiment 2 (MR002)

A.2.1. Glyceraldehyde

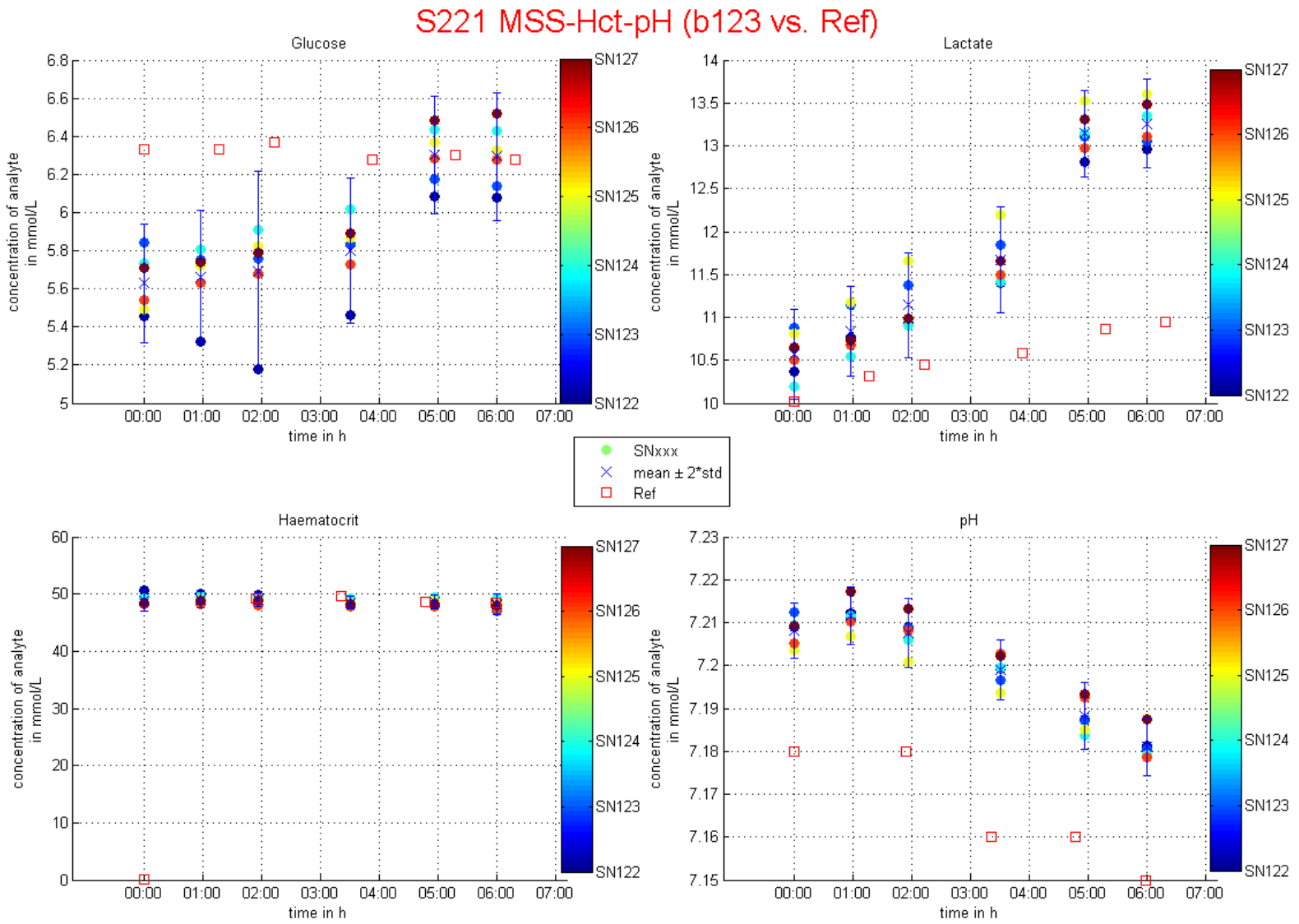

Figure A.15.: Sample s221 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

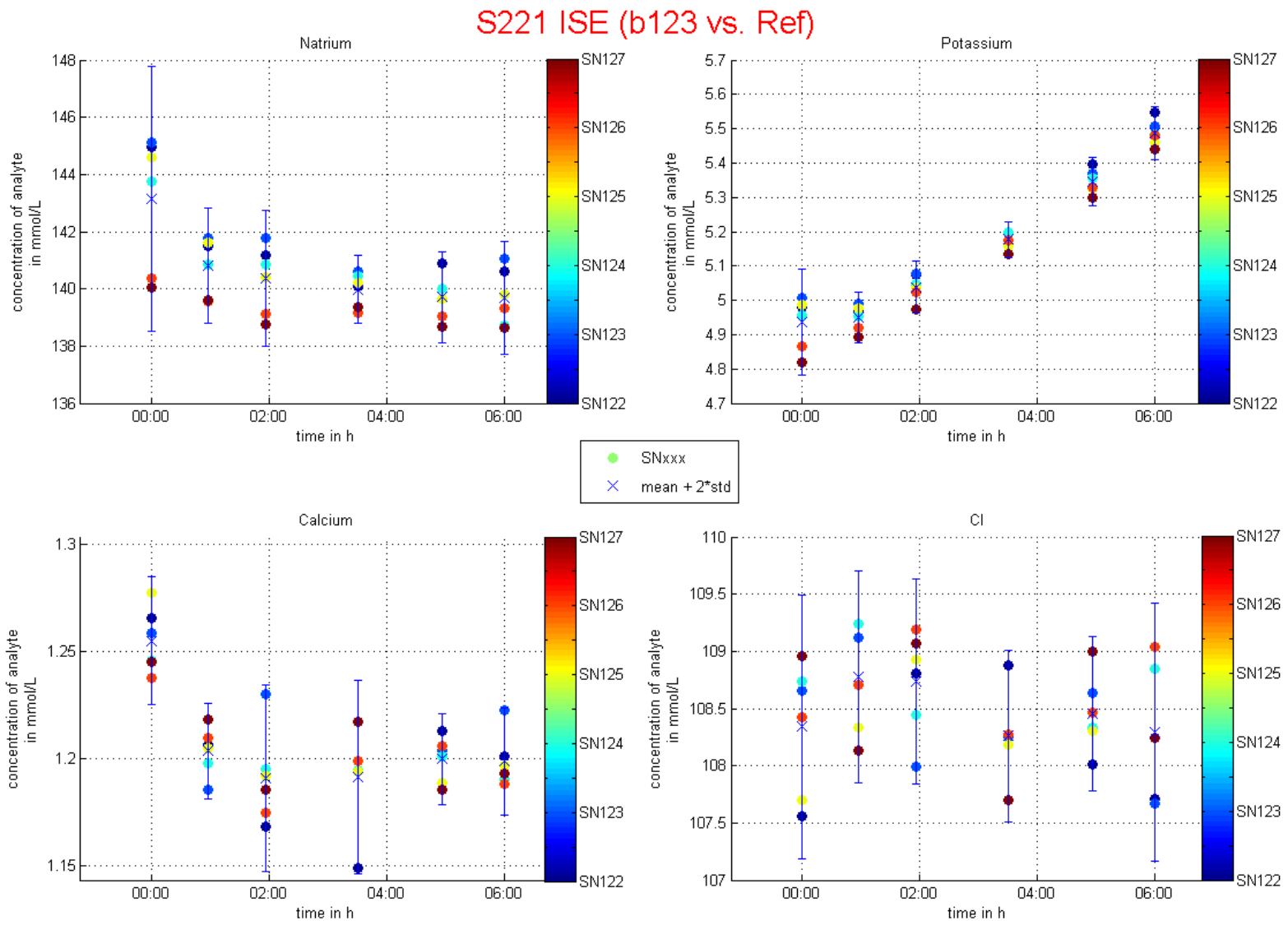

Figure A.16.: Sample S221 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

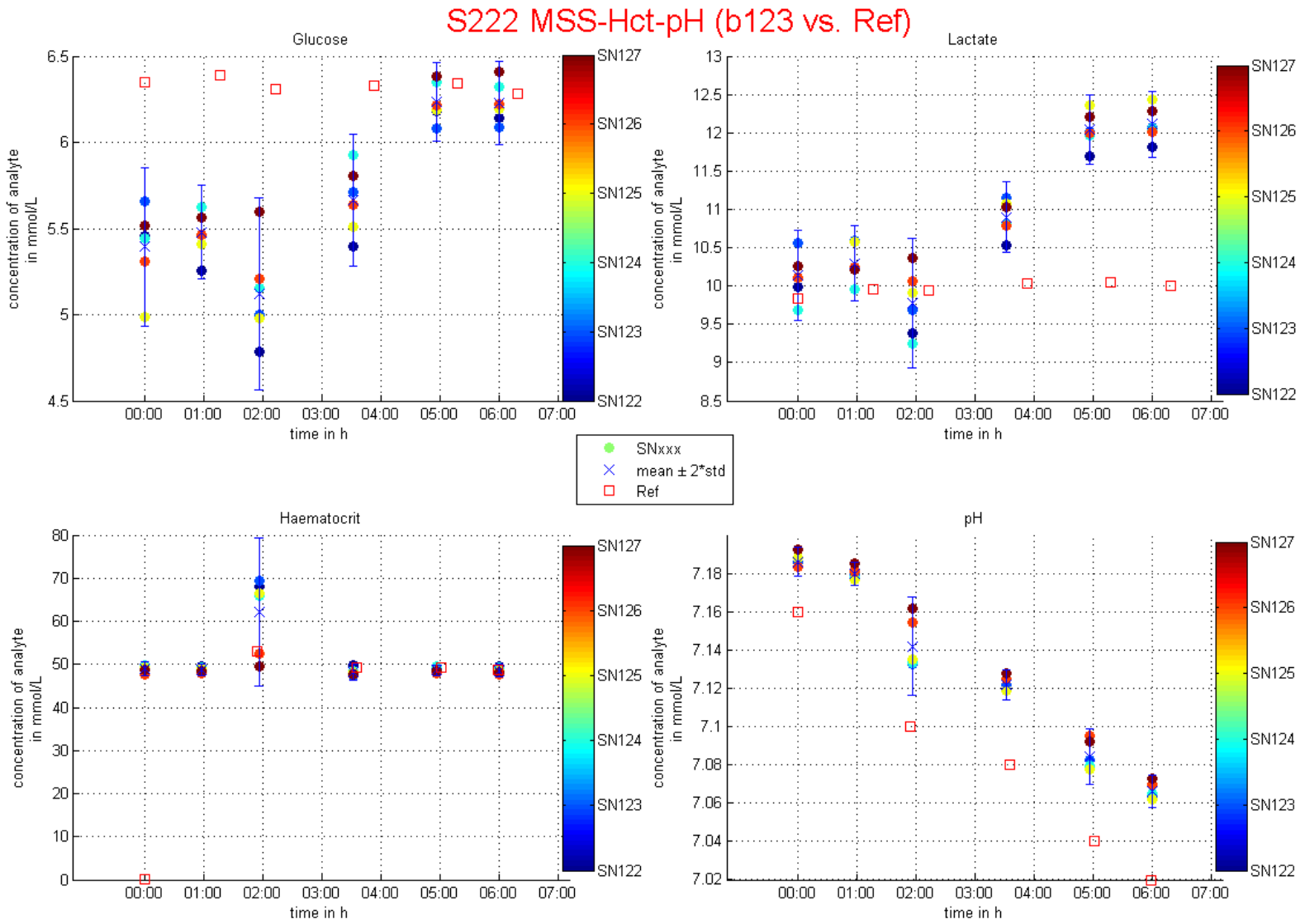

Figure A.17.: Sample S222 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

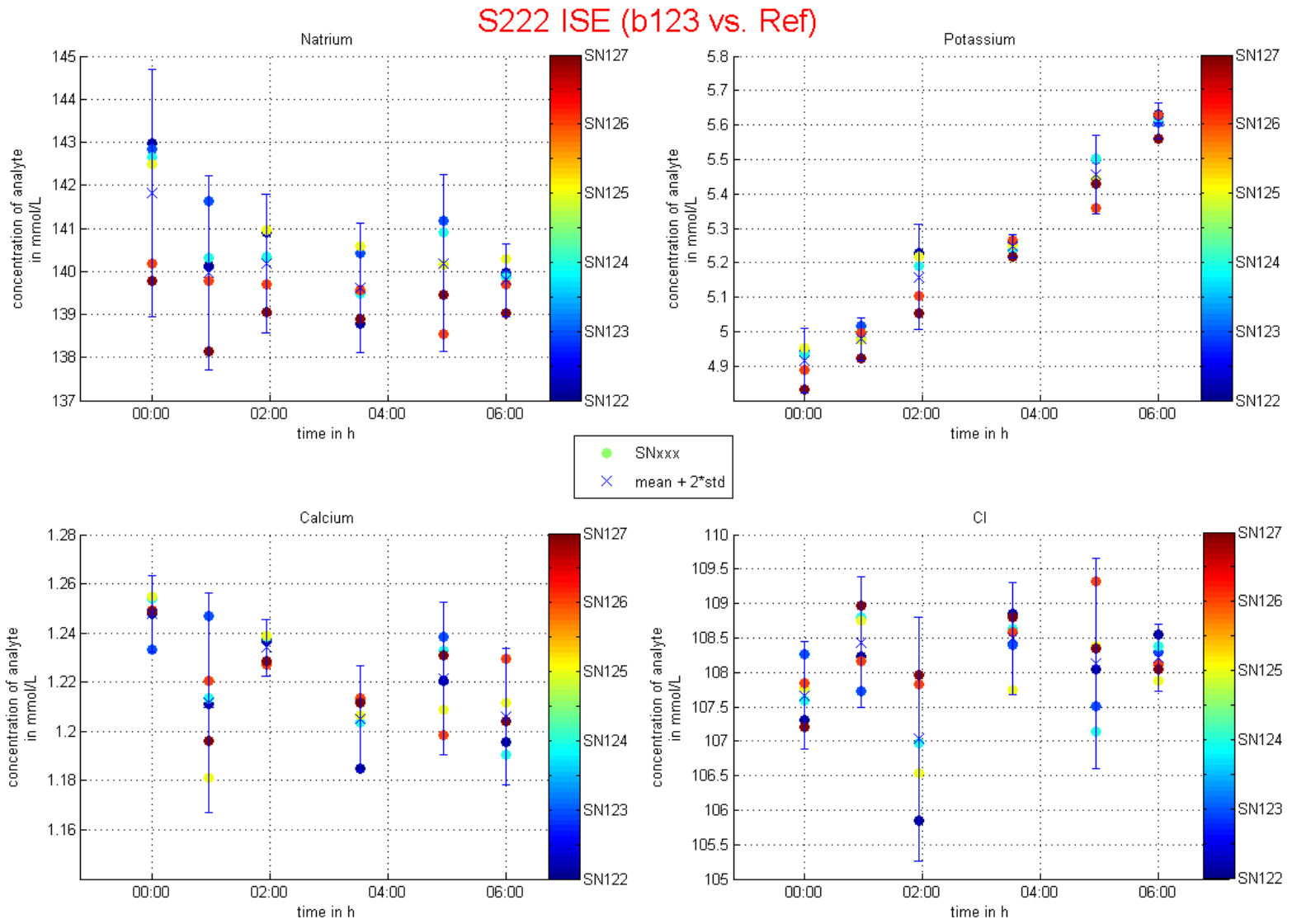

Figure A.18.: Sample S222 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

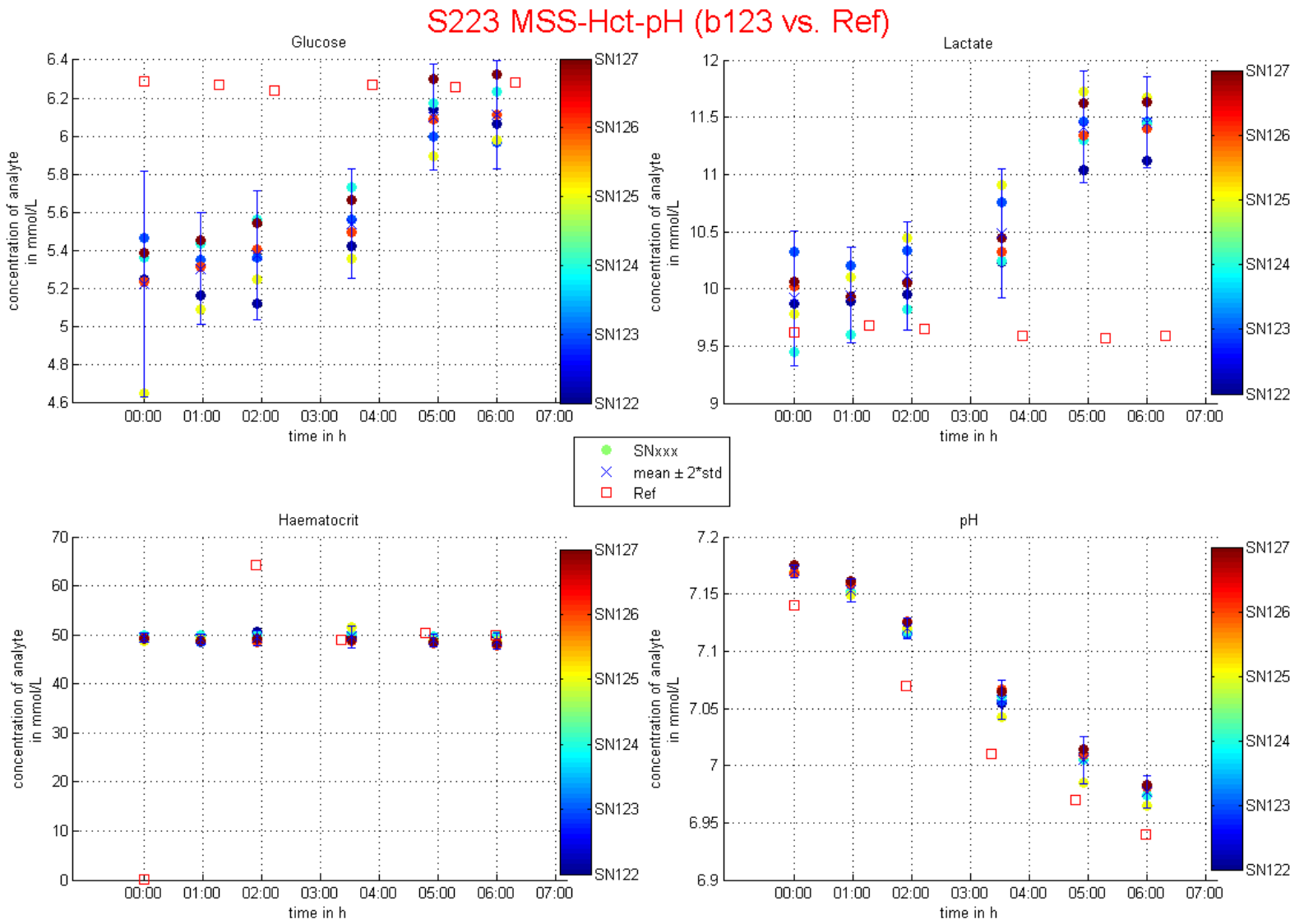

Figure A.19.: Sample S223 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

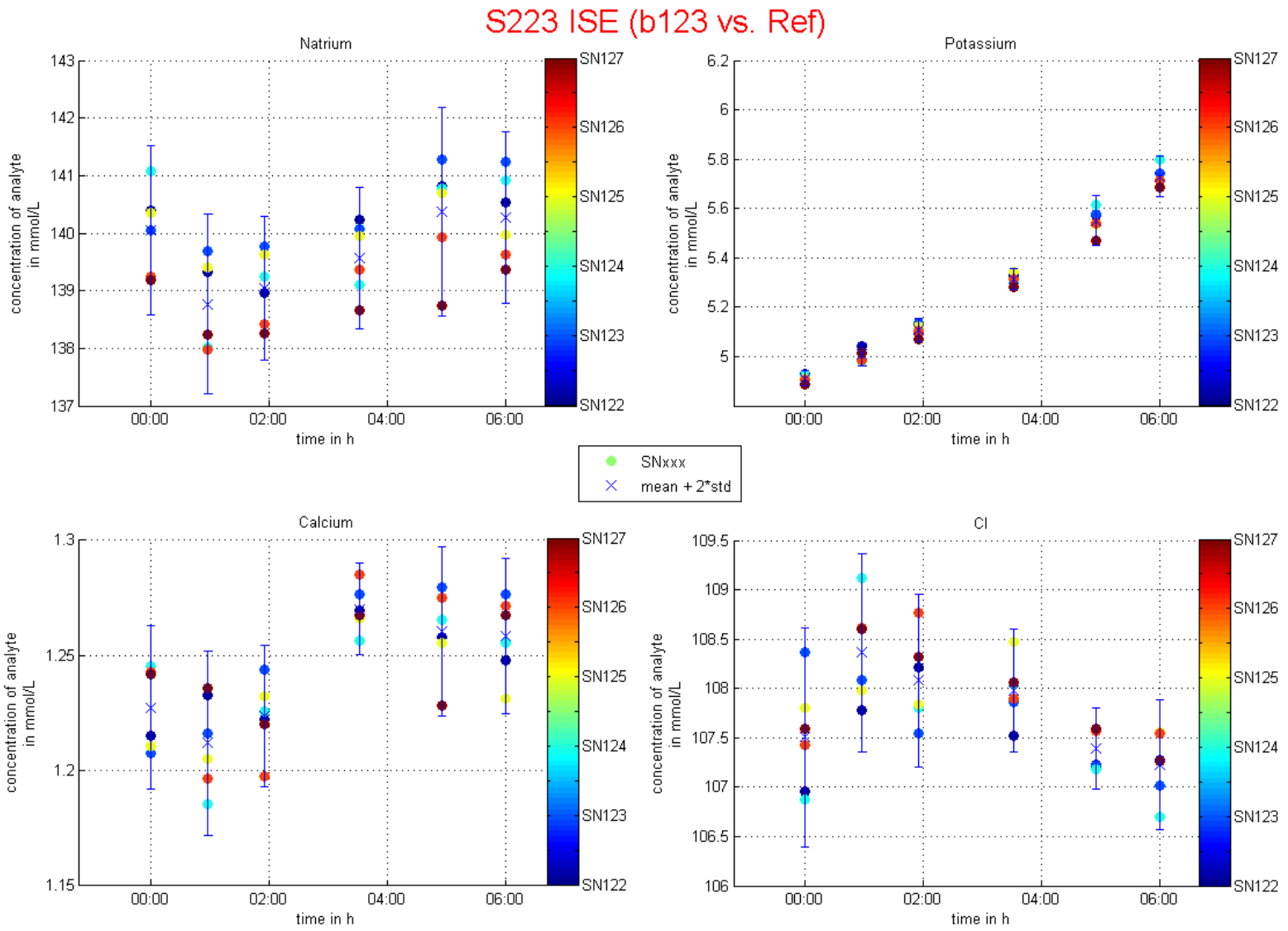

Figure A.20.: Sample S223 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

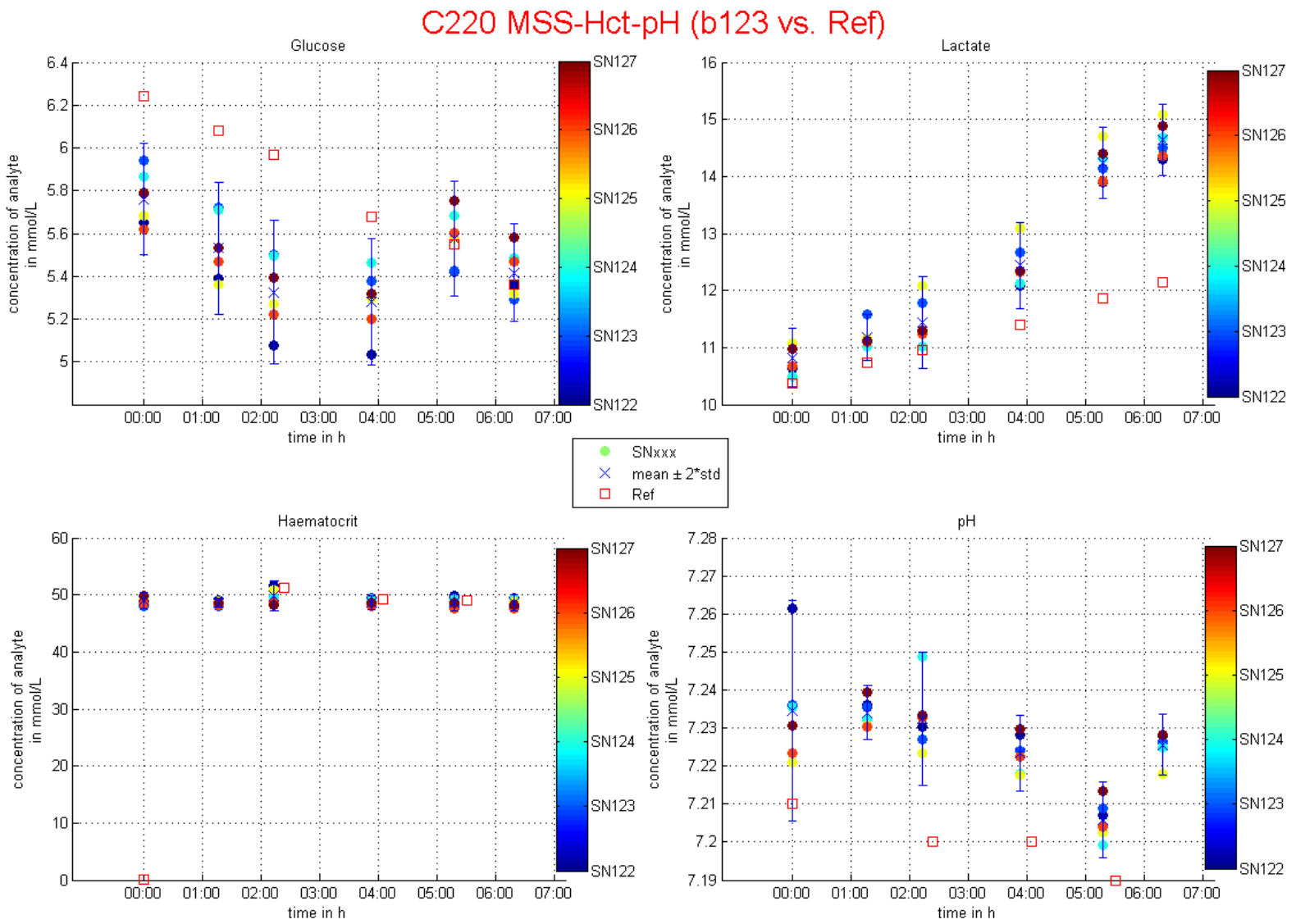

Figure A.21.: Sample C220 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

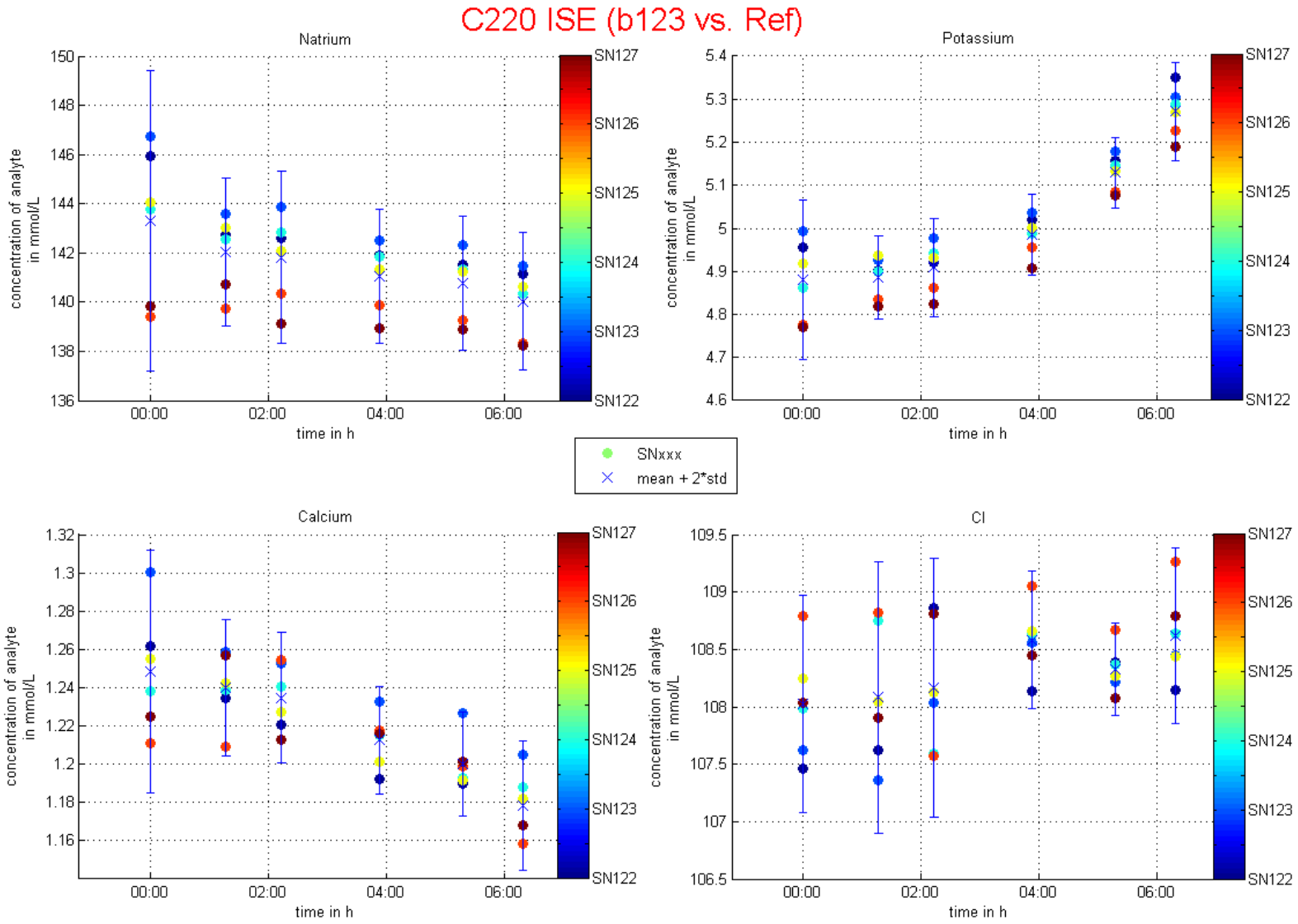

Figure A.22.: Sample C220 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

A.2.2. Bromopyruvic acid

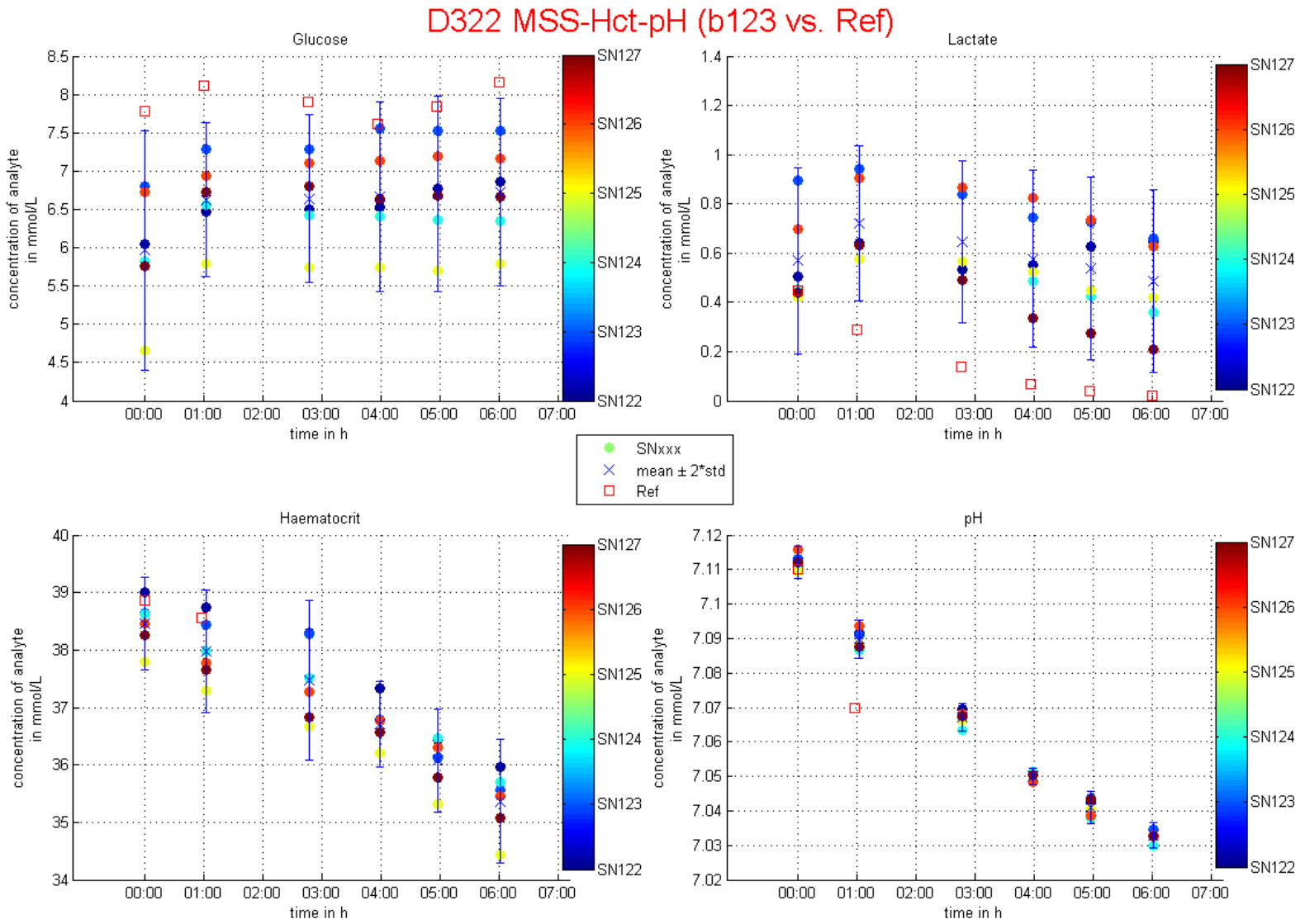

Figure A.23.: Sample D322 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

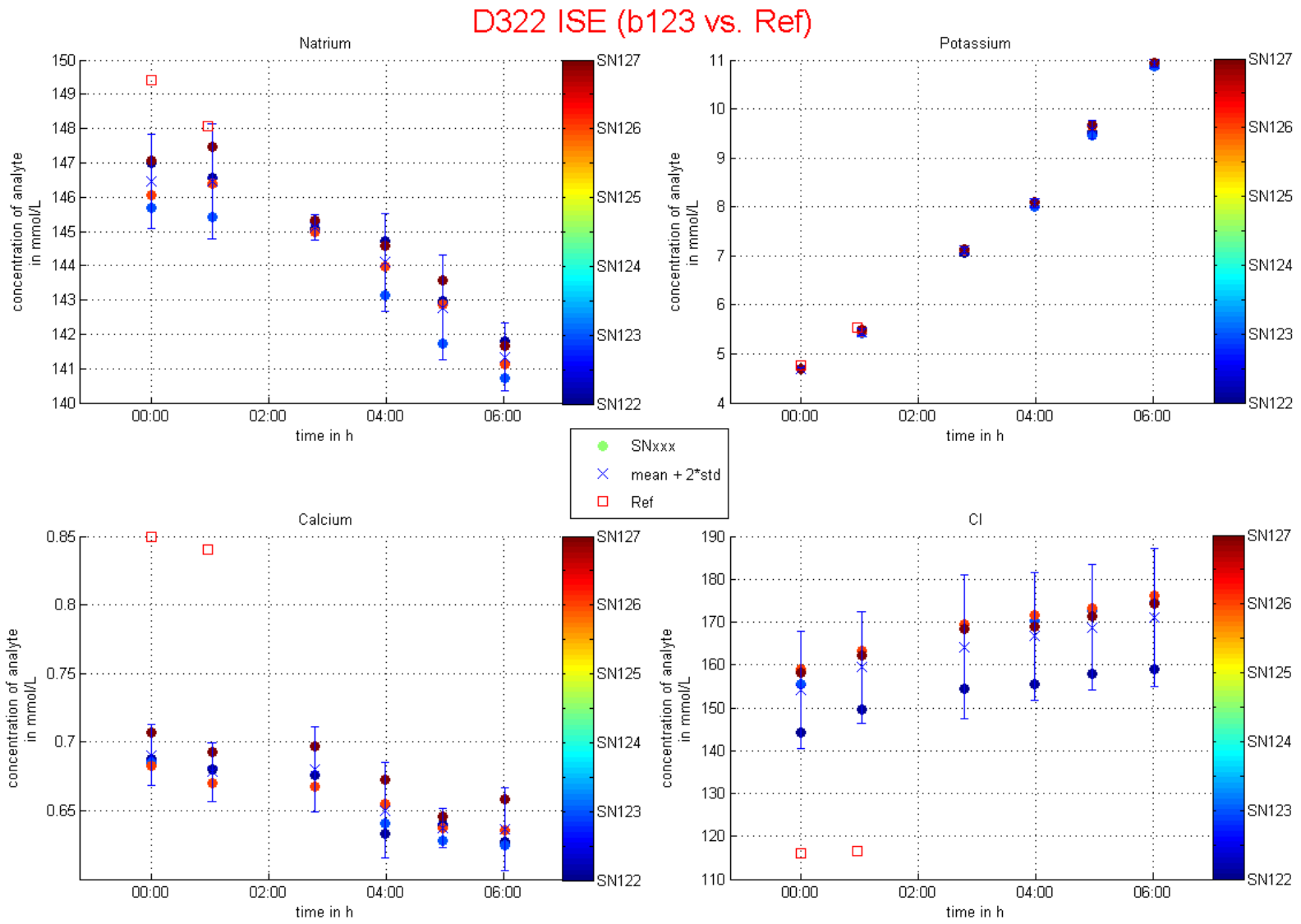

Figure A.24.: Sample D322 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

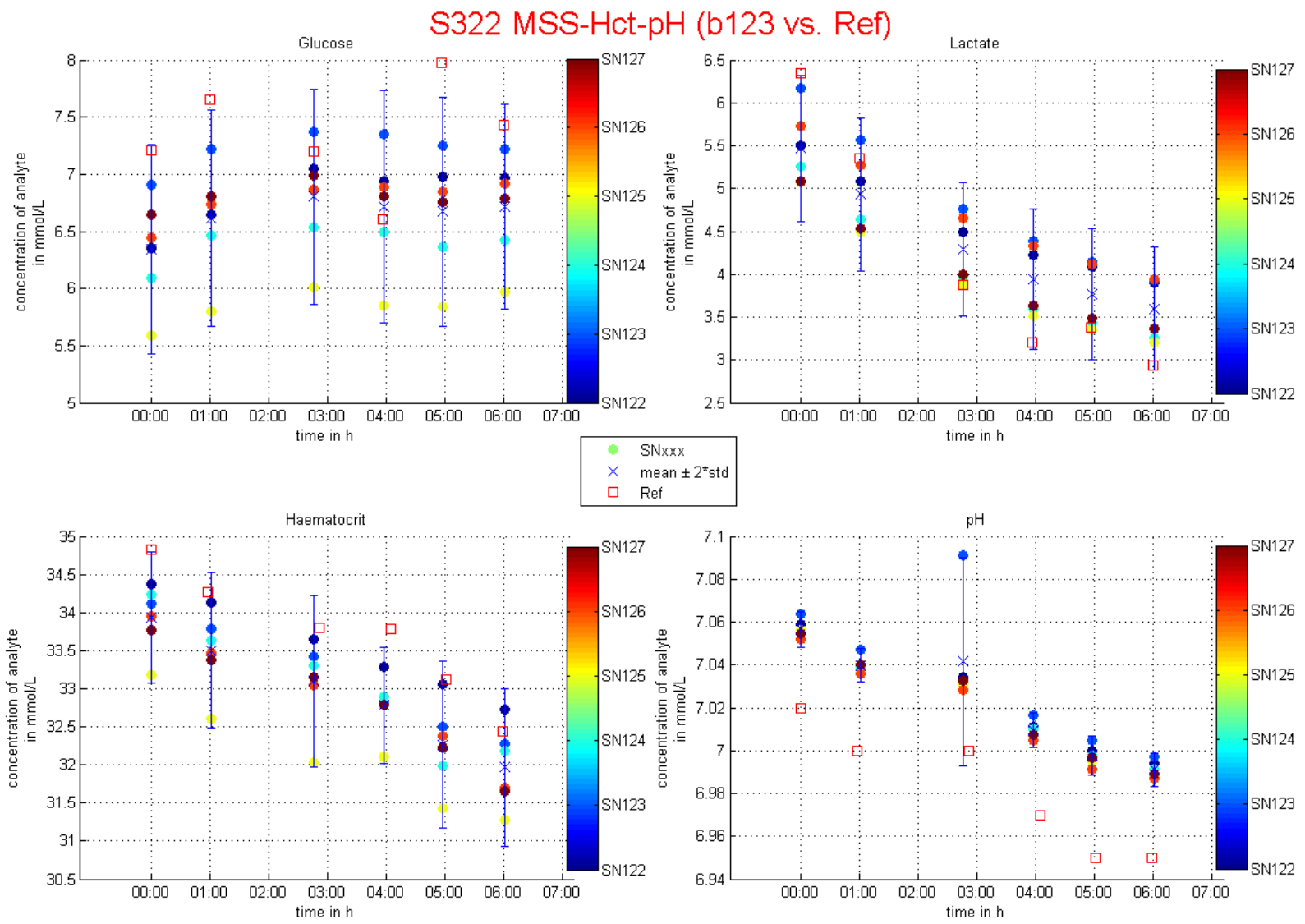

Figure A.25.: Sample S322 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

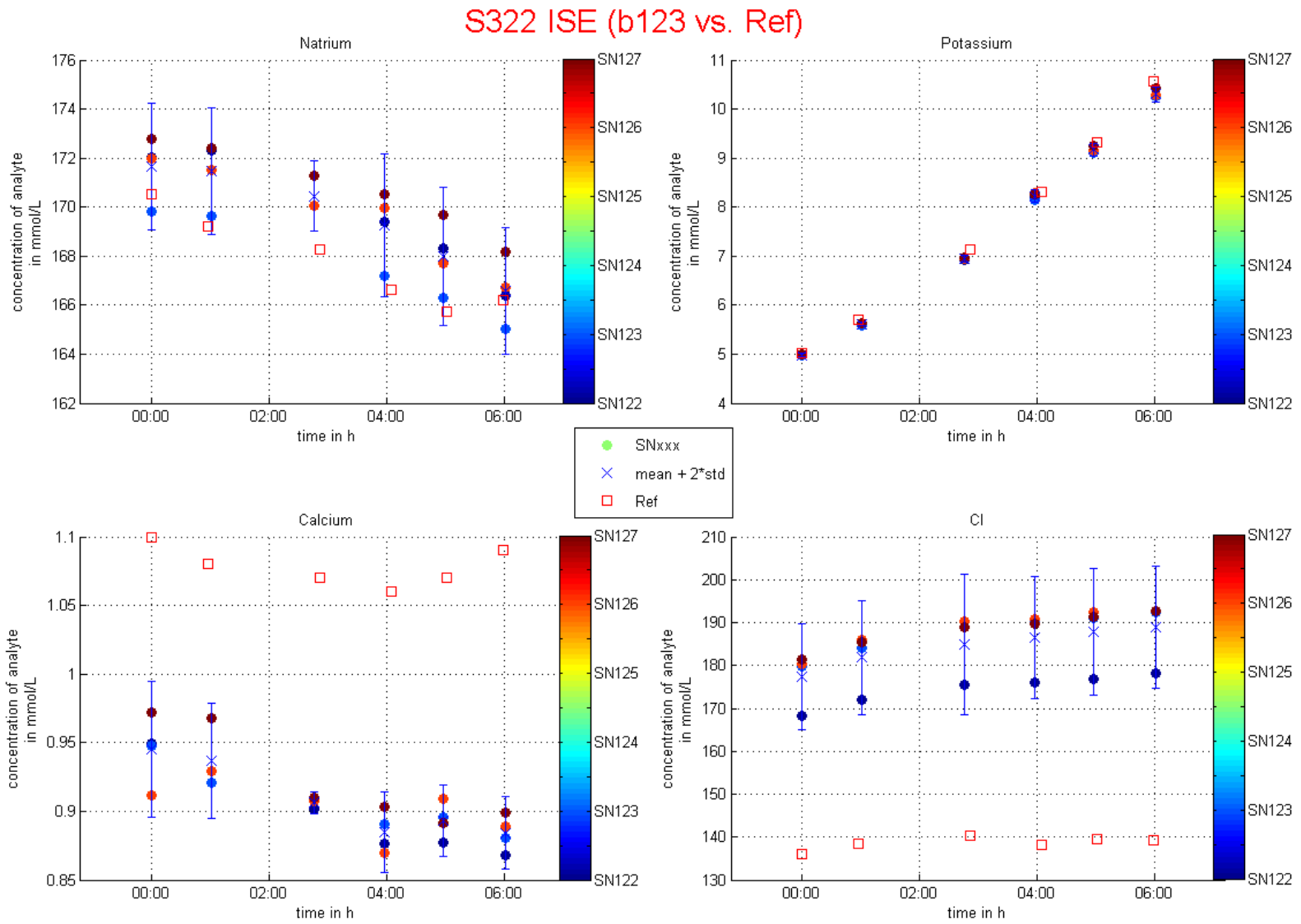

Figure A.26.: Sample S322 glyceraldehyde: comparison of the ISE parameters of the b123 and the reference device (b221).

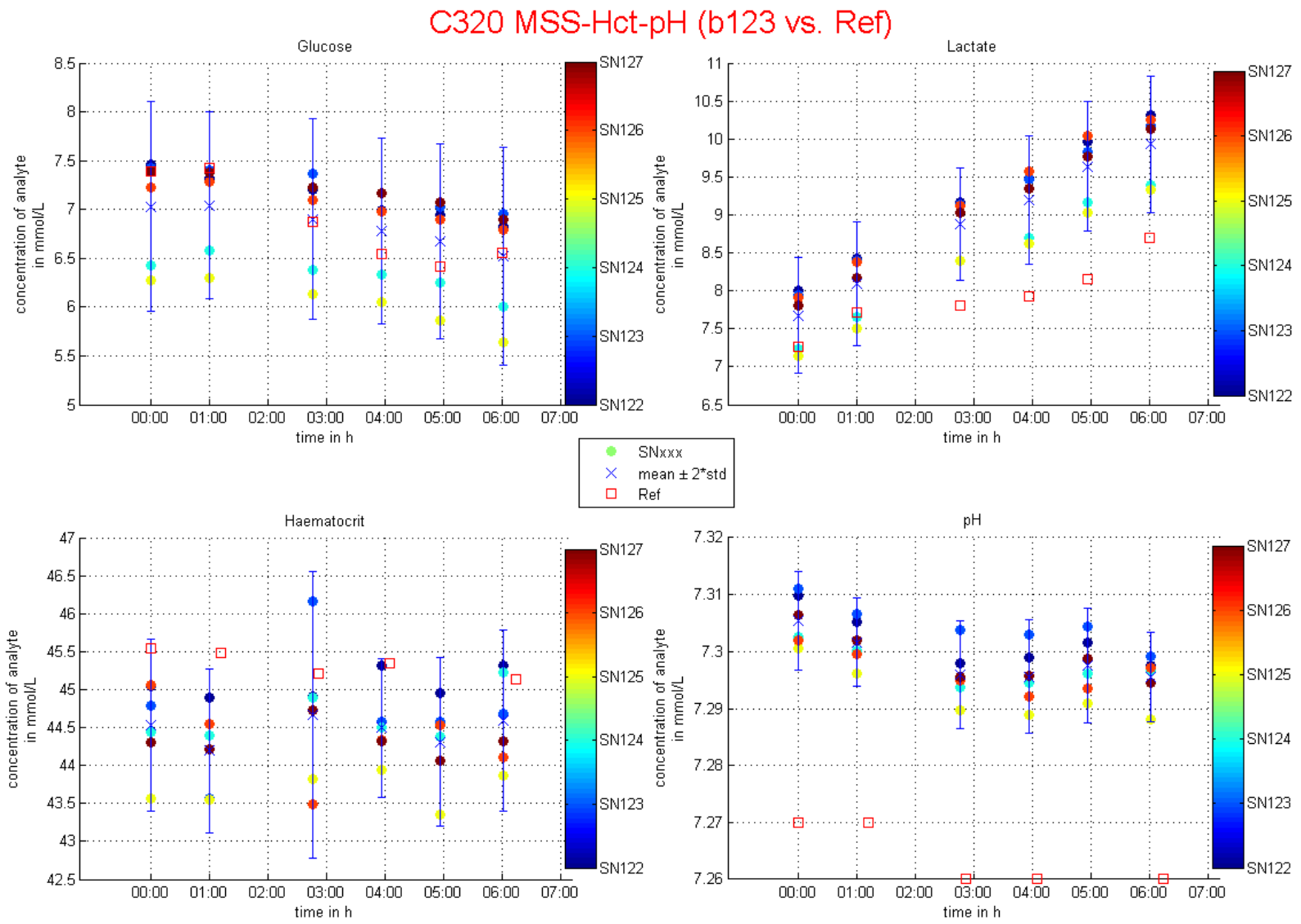

Figure A.27.: Sample C320 glyceraldehyde: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

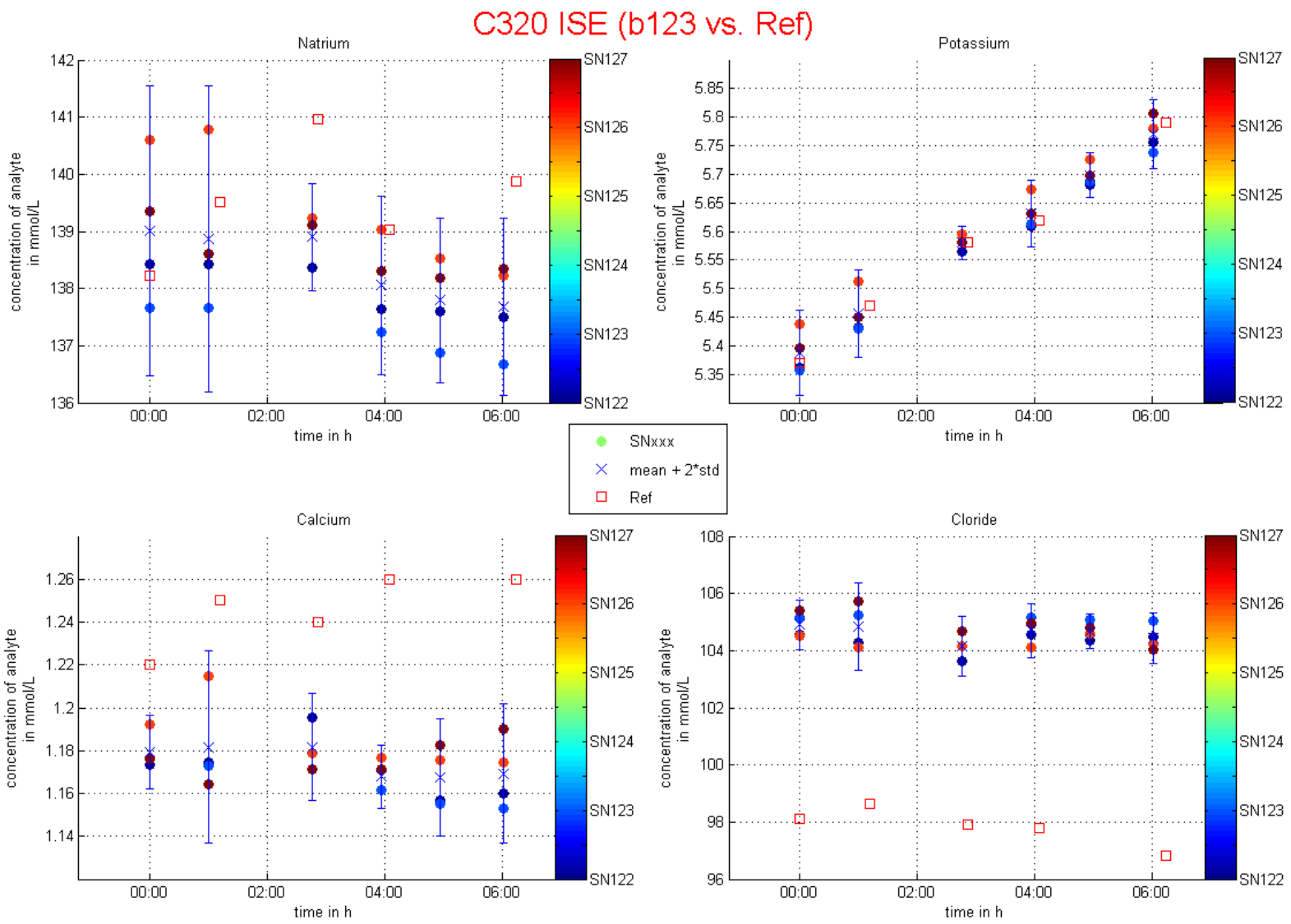

Figure A.28.: Sample C320 glyceraledhyde: comparison of the ISE parameters of the b123 and the reference device (b221).

A.2.3. Sodium fluoride

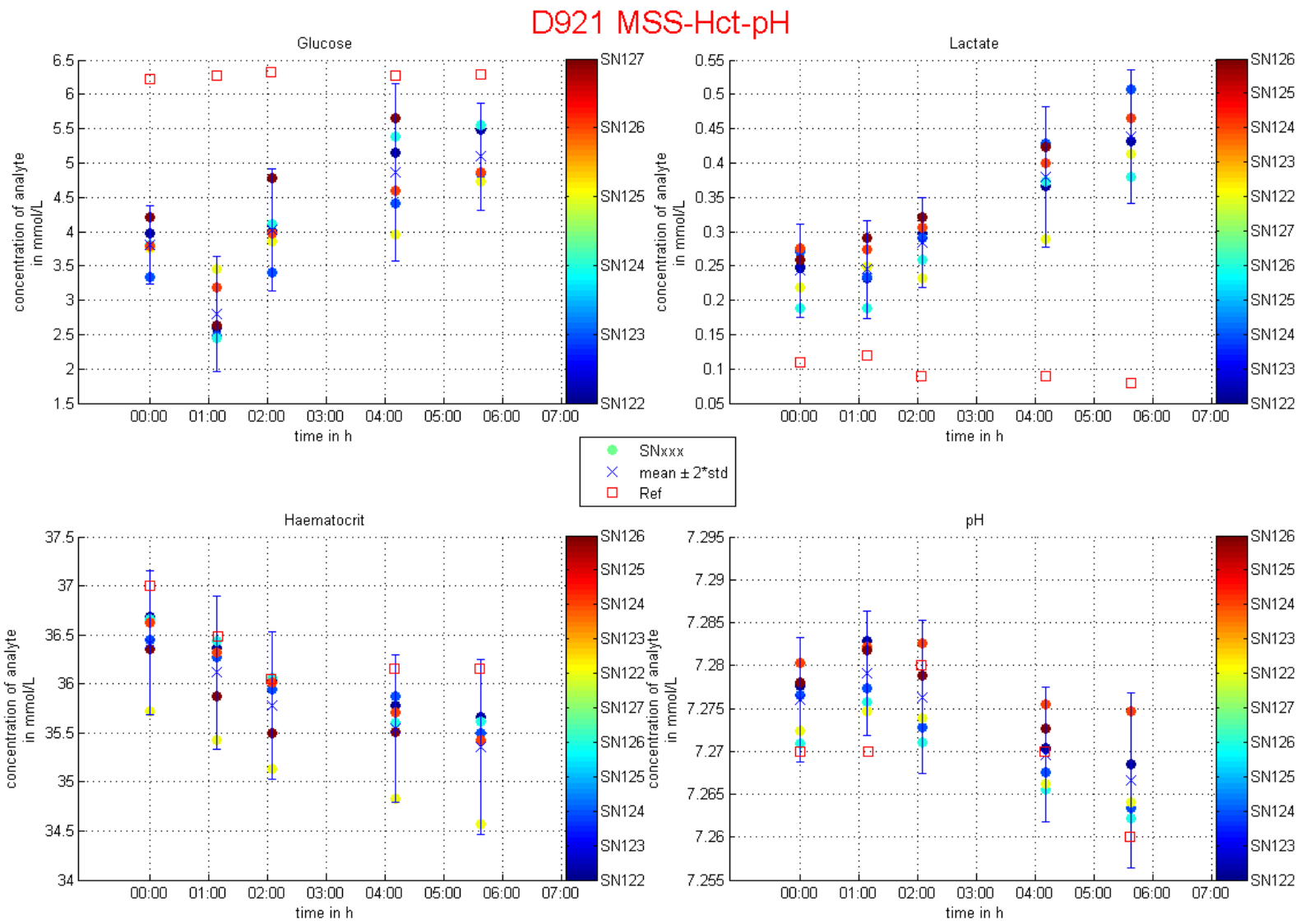

Figure A.29.: Sample D921 sodium fluoride (dialysed): comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

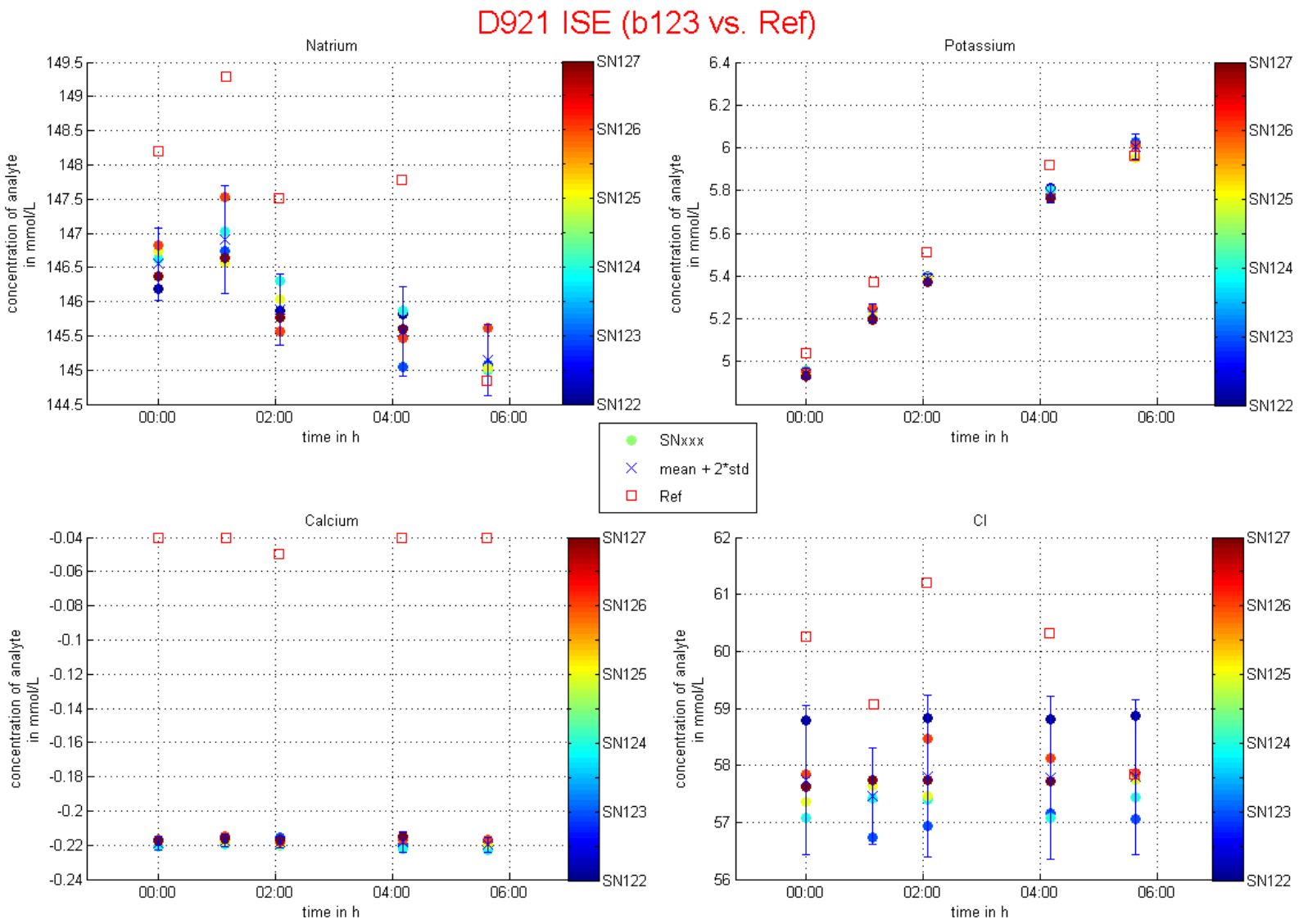

Figure A.30.: Sample D921 sodium fluoride (dialysed): comparison of the ISE parameters of the b123 and the reference device (b221).

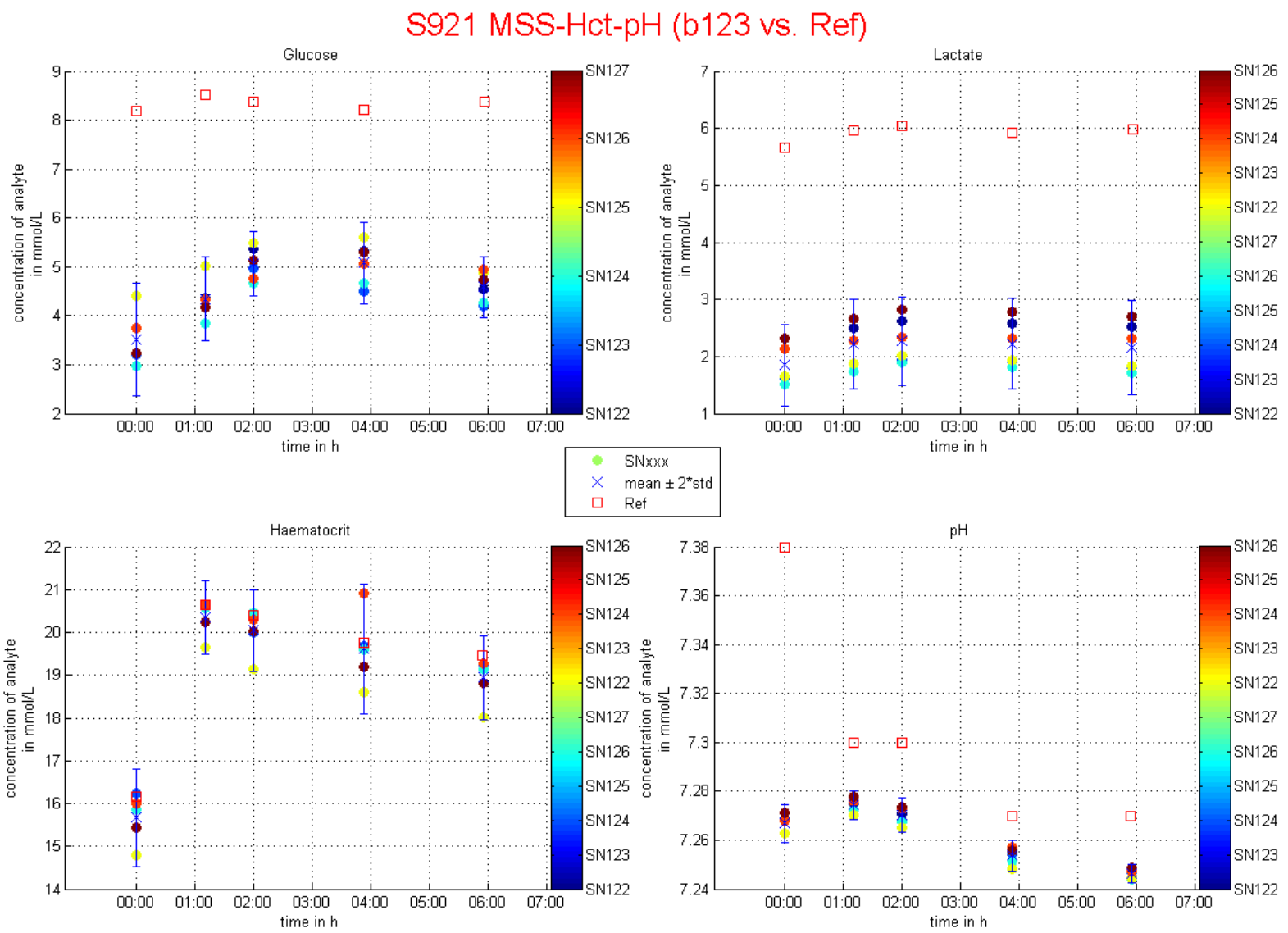

Figure A.31.: Sample S921 sodium fluoride (spiked): comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

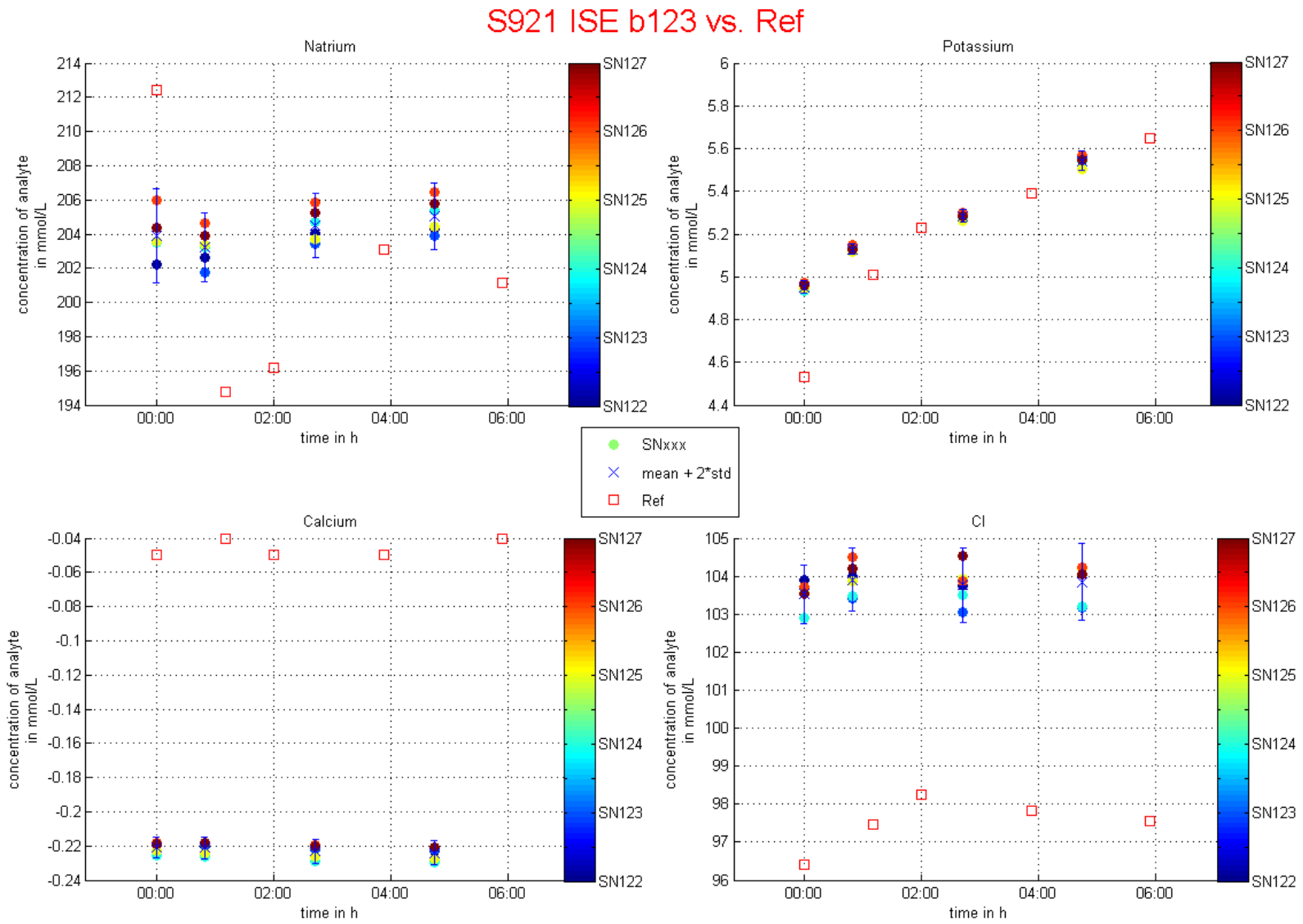

Figure A.32.: Sample S921 sodium fluoride (spiked): comparison of the ISE parameters of the b123 and the reference device (b221).

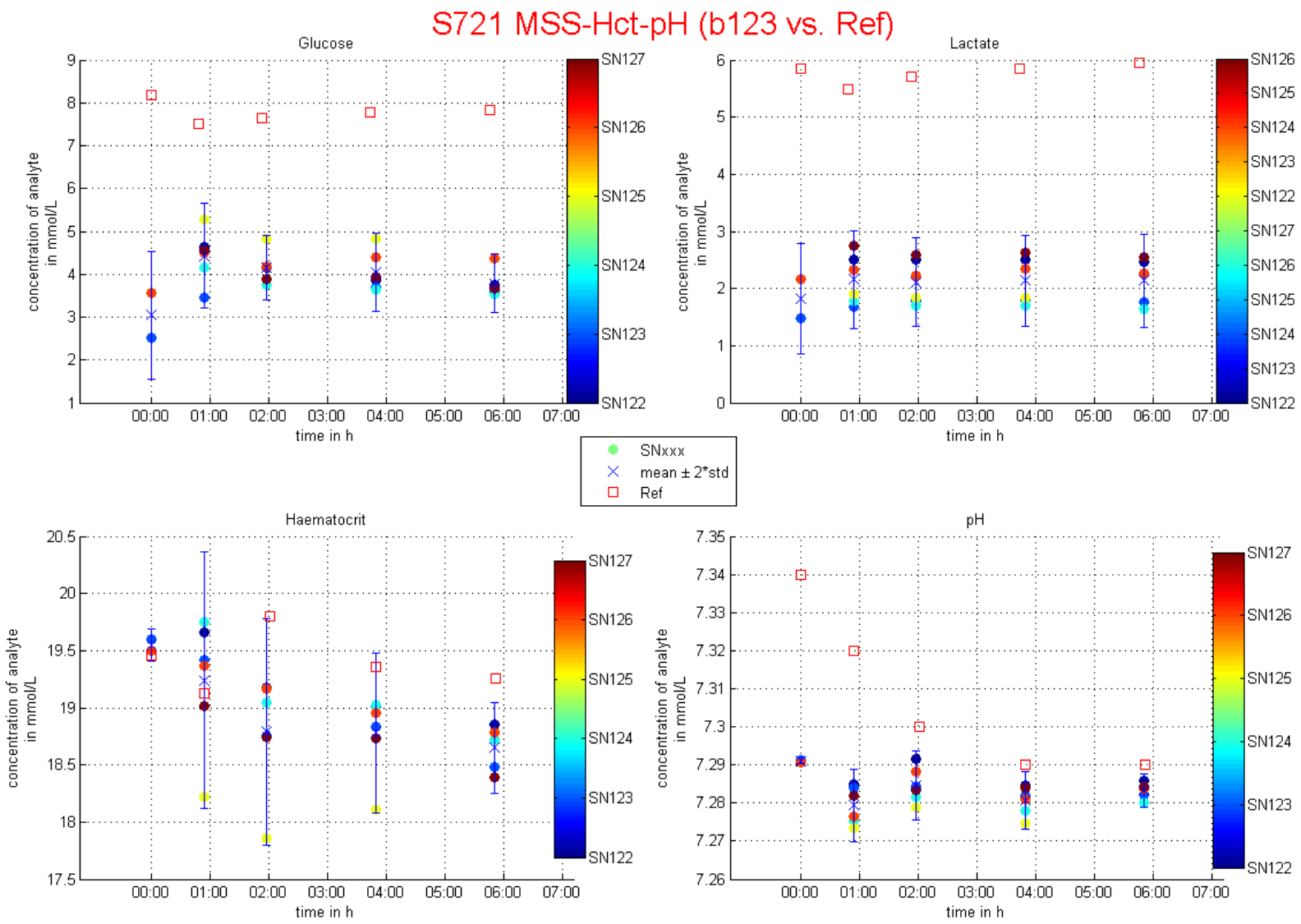

Figure A.33.: Sample S721 sodium fluoride (spiked): comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

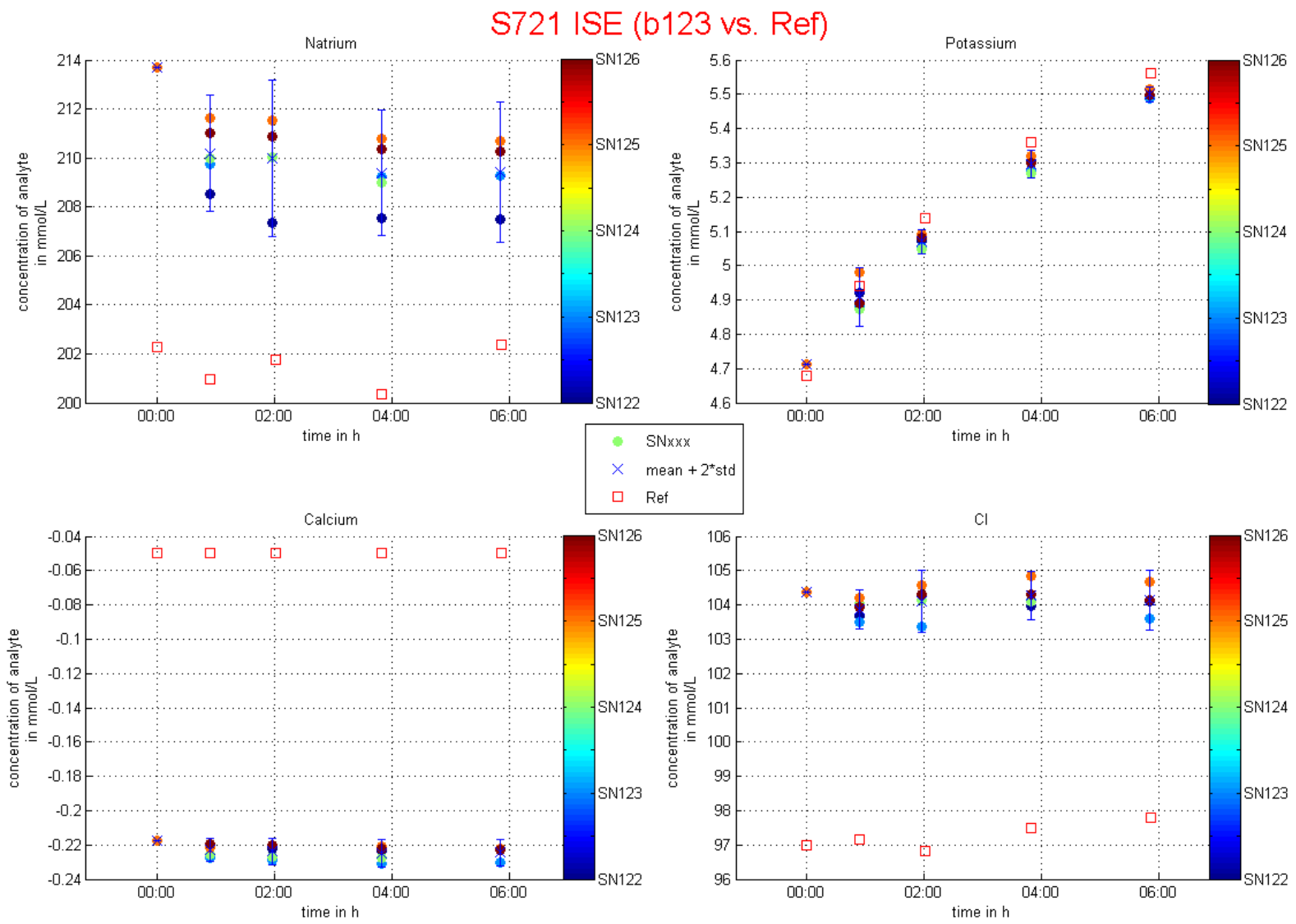

Figure A.34.: Sample S721 sodium fluoride (spiked): comparison of the ISE parameters of the b123 and the reference device (b221).

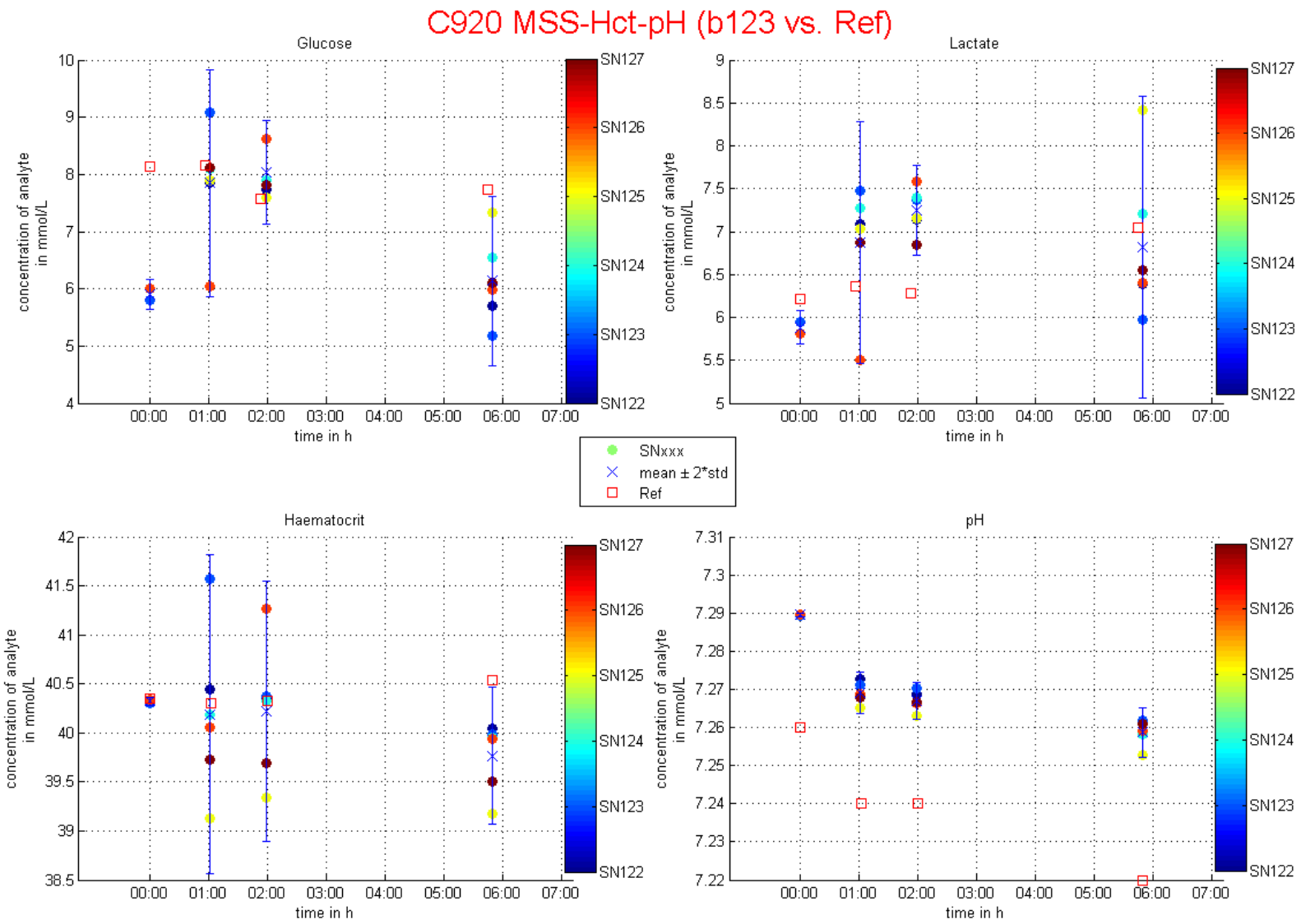

Figure A.35.: Sample C920: comparison of the MSS parameters, haematrocrit and pH-level of the b123 and the reference device (Hitachi 902 for glucose and lactate, b221 for haematocrit and pH).

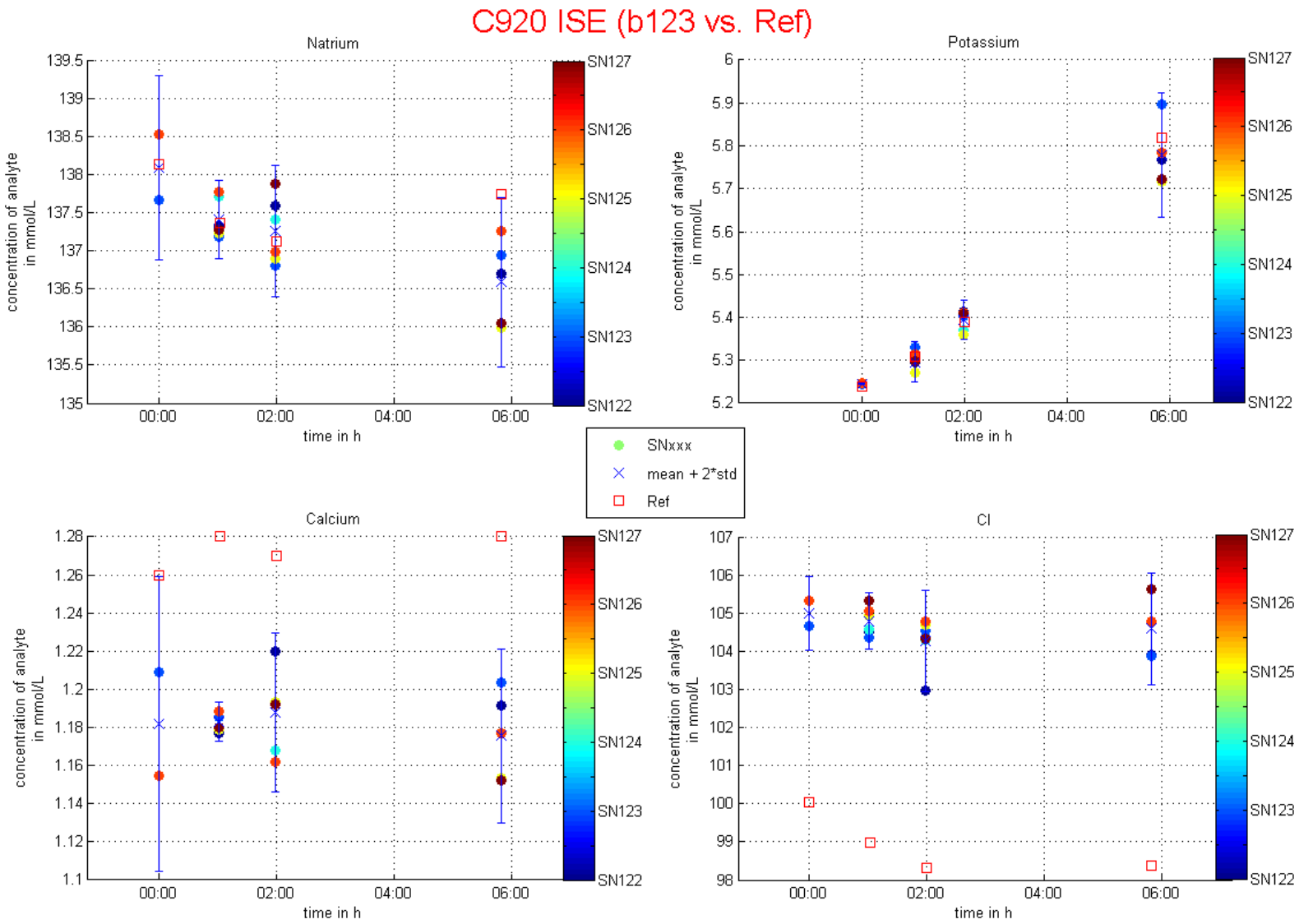

Figure A.36.: Sample C920: comparison of the ISE parameters of the b123 and the reference device (b221).

B. Appendix: calculations

B.1. Influence of BSA correction algorithm

Calculation of the influence of the BSA correction for BSA-levels $c_{BSAs_1} = 1$, $c_{BSAs_2} = 2.0$ and $c_{BSAs_3} = 3.0$:

The corrected signal is calculated according to the equation 2.5, already described in section 2.2.2

The batch parameters were $c_p = 0.3$ and $u_{D2BSAn} = 0.8$ mV/BSA-equiv. Inserting c_{BSAs_1}, c_{BSAs_2} and c_{BSAs_3} into equation B.1. The signal of the glucose sensor corresponding to a glucose concentration of $c_{glu} = 6.00$ mM is $u_{glu_{6mM}} = 46.7$ mV.

$$w_{c_{BSA}} = \begin{cases} 0 & \text{if } c_{BSA_s} \leq z_0 \\ \frac{c_{BSA_s} - z_0}{z_1 - z_0} & \text{if } z_0 < c_{BSA_s} < z_1 \\ 1 & \text{if } c_{BSA_s} \geq z_1 \end{cases} \tag{B.1}$$

$$\begin{aligned} w_{c_{BSA_1}} &= \frac{1-1}{2.5-1} = 0.0 \\ w_{c_{BSA_2}} &= \frac{2-1}{2.5-1} = 0.7 \\ w_{c_{BSA_1}} &= \frac{3-1}{2.5-1} = 1.0 \end{aligned}$$

$$\begin{aligned} u_{glu_{corr}} &= u_{glu} - b_p * w_{c_{BSAs}} * c_{BSAs} * u_{D2BSAn} \\ u1_{glu_{corr}} &= 46.7mV - 0.3 * 0.0 * 1 * 0.8mV = 46.7mV \\ u2_{glu_{corr}} &= 46.7mV - 0.3 * 0.7 * 2 * 0.8mV = 42.7mV \\ u3_{glu_{corr}} &= 46.7mV - 0.3 * 1.0 * 3 * 0.8mV = 39.9mV \end{aligned}$$

The voltage $u_{glu_{corr}}$ is proportional to the glucose concentration by the following relationship

$$c_glu = \frac{u_{glu_{corr}} * \alpha}{(\beta - u_{glu_{corr}})} \tag{B.2}$$

with α and β being calibration parameters that are obtained from calibrating the sensor with specific calibration solutions.

$$u1_{glu_{corr}} = 46.7mV \propto c1_{glu_{corr}} = 6.00mM$$
$$u2_{glu_{corr}} = 42.7mV \propto c2_{glu_{corr}} = 5.61mM$$
$$u3_{glu_{corr}} = 39.9mV \propto c3_{glu_{corr}} = 5.13mM$$

Calculating the error in percent

$$x = \frac{c_{glu_{corr}} - c_{glu}}{c_{glu}} * 100 \qquad \text{x ... error in \%}$$

$$x1 = \frac{6.00 - 6.00}{6.00} * 100 = 0\%$$

$$x2 = \frac{5.61 - 6.00}{6.00} * 100 = -0.39\%$$

$$x3 = \frac{5.13 - 6.00}{6.00} * 100 = -0.87\%$$

B.2. Influence of Cl-correction

Depending on the measured chloride concentration C_{Cl}, the signal for glucose (in mV) is corrected and weighted according to equation B.3:

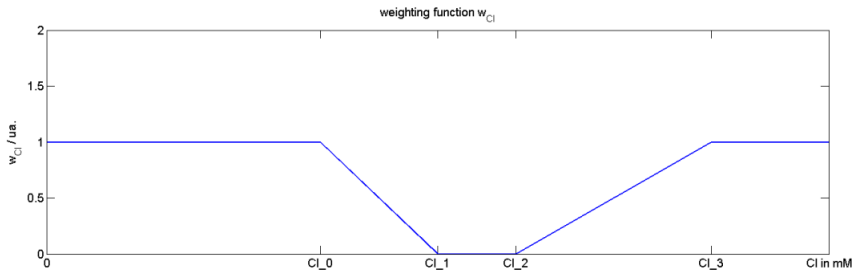

Figure B.1.: The influence of the chloride correction algorithm on the measured glucose concentration is governed by the depicted weighting function w_{Cl}.

$$w_{Cl} = \left\{ 1 - \left\{ \begin{array}{ll} 0 & \text{if } C_{Cl} \leq Cl_0 \\ \frac{C_{Cl}-Cl_0}{Cl_1-Cl_0} & \text{if } Cl_0 < C_{Cl} < Cl_1 \\ \frac{C_{Cl}-Cl_2}{Cl_3-Cl_2} & \text{if } Cl_2 < C_{Cl} < Cl_3 \\ 1 & \text{if } C_{Cl} \geq Cl_3 \end{array} \right. \right. \tag{B.3}$$

with C_{Cl} being the measured Cl-value within the sample, Glu_{Cl_0} being the lower limit, below this value, the correction term is weighted with 100%, between Glu_{Cl_1} and Glu_{Cl_2} the

correction term does not contribute to the signal and Glu_{Cl_3} being the upper limit, above which the correction term is applied to its fullest.

The correction term u_{corrCl}, representing the influence of the chloride concentration in equation 2.4, is then calculated according to equation B.4.

$$u_{corrCl} = w_{Cl} * Glu_{sub_{corrCl}} * (Cl_{Stdby} - C_{Cl}) \tag{B.4}$$

with $Glu_{sub_{corrCl}}$ being a sensor and batch specific parameter and Cl_{Stdby} being the Cl concentration measured in a calibration solution while in standby mode.

For a glucose concentration of 6 mM in the whole blood sample, a mean Cl_{Stdby} value being 103 mM and $Glu_{sub_{corrCl}} = -0.053$ different chloride concentrations may lead to different glucose concentrations

$$\begin{aligned}
&\text{for } C_{Cl} = 60 \text{ mM (NaF sample)} && \rightarrow \quad w_{Cl_{60}} = 1 \\
&\text{for } C_{Cl} = 110 \text{ mM (GA sample)} && \rightarrow \quad w_{Cl_{110}} = 0 \\
&\text{for } C_{Cl} = 180 \text{ mM (3-BrPA sample)} && \rightarrow \quad w_{Cl_{180}} = 1
\end{aligned}$$

leading to a correction term u_{corrCl}

$$\begin{aligned}
u_{corrCl_{60}} &= 1 * -0.053 * (103 - 60) = -2.28mV \\
u_{corrCl_{110}} &= 0 * -0.053 * (103 - 110) = 0.0mV \\
u_{corrCl_{180}} &= 1 * -0.053 * (103 - 180) = +4.08mV
\end{aligned}$$

the voltage signal for glucose concentration = 6 mM is 54.46 mV. The corrected signal (taking only the influence of chloride into account) is then calculated

$$\begin{aligned}
&u_{s_{corr}} = u_s + u_{corrCl} \\
&u_{s_{corr60}} = 54.46mV - 2.28mV = 52.18mV && \rightarrow \text{glucose concentration of} \quad c_{glu} = 5.74mM \\
&u_{s_{corr110}} = 54.46mV + 0.00mV = 54.46mV && \rightarrow \text{glucose concentration of} \quad c_{glu} = 6.00mM \\
&u_{s_{corr180}} = 54.46mV + 4.08mV = 58.54 && \rightarrow \text{glucose concentration of} \quad c_{glu} = 6.47mM
\end{aligned}$$

This shows that the two extreme cases of a chloride concentration of 60 mM, as it was the case for the NaF samples and a chloride concentration of 180 mM, present in the 3-BrPA samples lead to final glucose concentrations with errors of $\approx$ 0.5 mmol at the maximum.

B.3. Why aim for a glycolytic rate of 0.1% per hour?

The precision is influenced by the the sensor cartridge, the system, the reagent pack and by the sample used for the precision test. For the sake of of simplicity it is assumed that each compartment contributes to the imprecision equally with 25% each.

For the medium glucose level (4-25 mM), a *total precision* of 5% is specified for the *cobas b123* resulting in a precision of 0.2 mM at the lower limit and 1.25 mM at the upper limit.

Table B.1.: Components contributing to the imprecision of the device and their contribution in %.

Sensor	0.25
Device(system excl. sensor)	0.25
reagent pack	0.25
sample	0.25

$$prec_{total} = (prec_{sens} + prec_{dev} + prec_{reag} + prec_{sample}) \tag{B.5}$$

leading to

$$prec_{sample} = 0.25 * prec_{total} = 0.25 * 5\% = 1.25\% \tag{B.6}$$

Aiming for a mean duration of approximately 6h this would result in

$$rate_{max} = 1.25\%/6h \approx 0.2\%/h \quad \rightarrow \quad 0.2\%/h \; + \; buffer = 0.1\%/h \tag{B.7}$$

taking some buffer into account, to be absolutely sure, that a glucose or lactate drift does not influence the evaluation of precision, results in a stipulated rate of at least 0.1% per hour.

C. Pictures

Figure C.1.: Dialysis setup: 2L beaker containing dialysis buffer solution, magnetic stirrer, 2 magnetic clips, dialysis membrane.

Figure C.2.: laboratory setup, row of six cobasb123. from left to right: SN122 - SN127

Figure C.3.: laboratory setup, *cobas b221*, used as reference measuring devices for the ISE parameters.