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INTERFERENCE STUDY OF GLUCOSE LEVEL MEASUREMENTS USING GLUCOMETERS BASED ON GLUCOSE OXIDASE**

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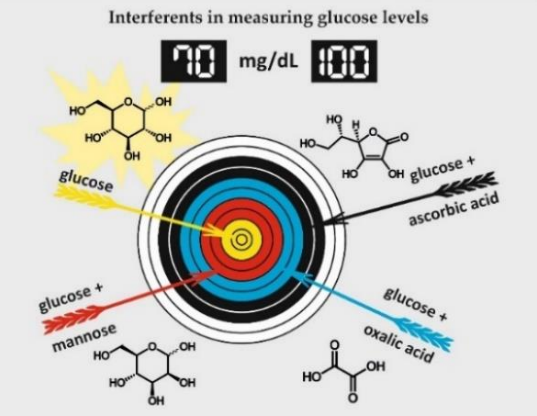
Glucometer readings are not only affected by the intrinsic accuracy of the device, but it has been proven that several substances can give rise to selectivity issues and display inaccurate readings. This study aims to investigate the influence of interfering substances, namely ascorbic acid, oxalic acid, and mannose on three commercially available glucometers in Roumania. The devices were tested on aqueous buffer glucose solutions along with different interferents at physiologically relevant concentrations to establish the influence of these substances on the final glucometer readings. We found that ascorbic acid has a significant interference on the studied devices (which to the best of our knowledge has not been previously assessed) – a greater than seven-fold increase from the reference value on all tested devices. Both oxalic acid and mannose exhibited interference on the devices tested at different concentration ranges and were signaled as new potentially interfering substances.



INTRODUCTION

Commercial glucometers[1] aiming to measure fast and reliable blood glucose levels have had an appreciable impact on the population ever since their first appearance on the market[2] and research directed toward effective and efficient devices to act as point-of-care diagnostic tools[3] is still a topic of high interest.[4] The reliability of the results provided by these devices is affected by numerous factors that must be carefully considered,[5] such as the meter-inherent factors (*i.e* accuracy[6]) environmental factors (*i.e.* temperature,[7] electromagnetic radiation[8]) or interfering chemicals[9] (*i.e.* ascorbic acid,[10] maltose,[11,12] acetaminophen[13]).

Strip-based glucometers available on the market rely on the capacity of enzymes such as glucose-oxidase and glucose-dehydrogenase[14] to generate an electric current which is displayed as the glucose concentration in the sample. The enzymes differ by their redox potential and use of each has advantages and disadvantages, for example the dependence of

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glucose-oxidase for dissolved oxygen.[1] The test strips also contain an excess of a mediator agent, such as ferricyanide ions or ferrocene derivatives that transfer the signal from the enzyme to the working electrode. Currently, the test strips are electrochemically based,[15] but the first generation of glucometers used to be colorimetric, as they used dyes to relay signal intensity. For exemplification, simplified illustration of a glucometer is presented in Fig. 1– the blood sample is drawn by the capillary and the glucose in the blood is oxidized by the immobilized enzyme to gluconic acid in presence of oxygen or the oxidizing mediator; the enzyme, in a temporarily reduced, inactive form, is oxidized by the mediator agent; the mediator is reoxidized at the electrode surface and the measured current is proportional to glucose concentration in the sample.[1]

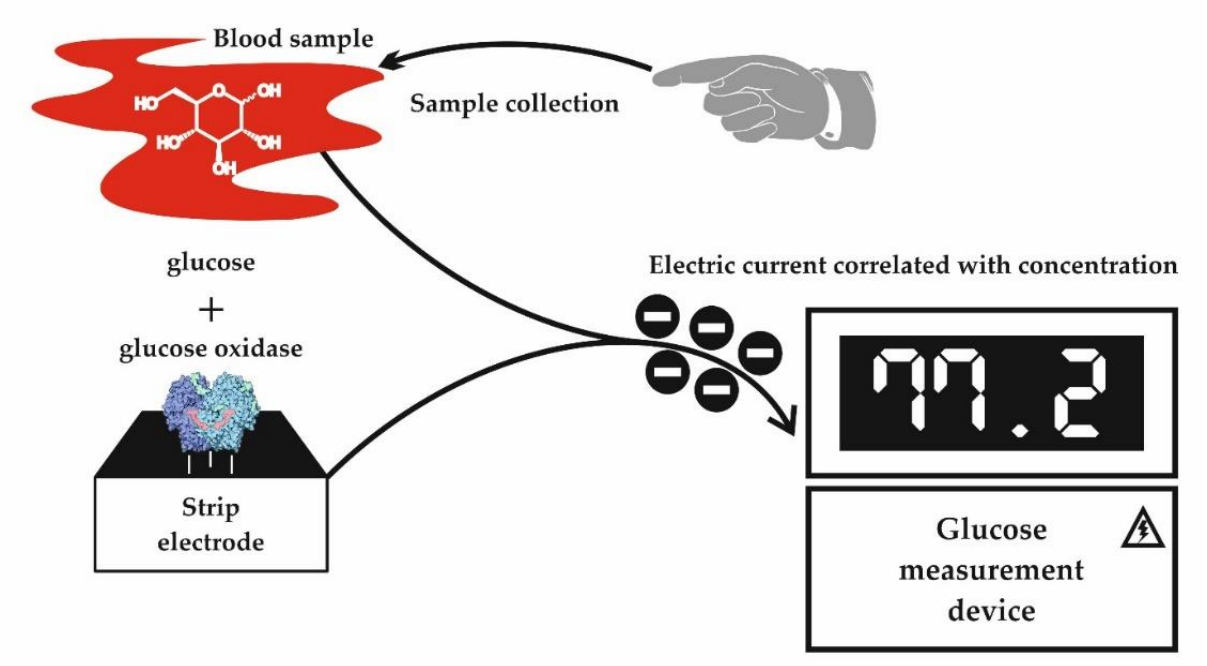


Fig. 1– Simplified working principle of a glucometer.

Even though the enzymes have high specificity, several substances can give rise to signals when processed by the enzymes and thus constitute inaccurate measurements.[16]

Vitamin C (ascorbic acid) supplementation has become widespread due to its immunomodulatory and antioxidant properties and therefore use in prophylaxis and adjunctive treatments.[17] Diabetic individuals are advised to consume low glycemic index foods, and citrus fruits, which are high in ascorbic acid, are good replacements for other high glycemic index meals. It is therefore evident how elevated levels of blood ascorbic acid can be common in the human population, which inquires that commercial glucometers should have a high tolerability threshold for blood ascorbic acid levels so that they do not interfere with the final reading.

Oxalic acid, a naturally occurring substance in human blood, is easily oxidized by a number of different oxidants,[18] and given the oxidative nature of the sensing part of the glucose meters (*e.g.* using glucose oxidase) oxalic acid may alter readings of these devices, as it has been shown to be the case for ascorbic acid,[19] both being similar reducing agents.

Mannose, an epimer of glucose, can theoretically give rise to specificity issues of the glucometers. As a dietary supplement, mannose is sometimes used for prevention of urinary tract infections along traditional treatment plans,[20] which can alter glucose readings.

Although this topic of research has considerable potential for improving lives of many people across the globe, it has been scarcely explored in Roumania. For example, previous research reported fabrication of a novel glucose biosensor and to demonstrate its robustness, several possible interferents such as uric acid, dopamine, ascorbic acid, oxalic acid, and mannose[21] were assayed and compared to commercially available devices. However, to the best of our knowledge, screening of commercial devices available on the Roumanian market and their response in glucose readings in presence of known interferents has not been reported.

Thus, this study aimed to test three potential interferents on three commercial glucometers available for purchase online on the Roumanian market, in order to establish their relevance in the context of devices accessible to the general population and evaluate their impact on glucose concentration readings. The interferents we have chosen to test were ascorbic acid, oxalic acid, and mannose, each to be tested at physiologically relevant concentrations. We used synthetic solutions of glucose and interferents. We hereby present the results we obtained and how the interferents affected readings of the glucose levels.

RESULTS AND DISCUSSION

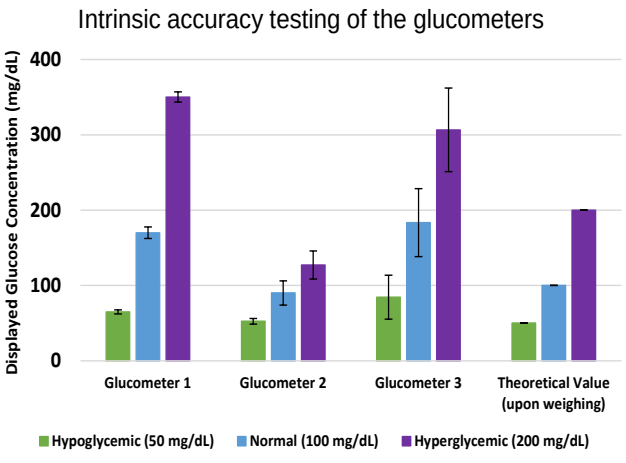
The experiments were designed so as to perform various glucose concentrations covering the physiologically relevant glucose concentration ranges – hypoglycemia (below 70 mg/dL), normal (70–99 mg/dL fasting (before breakfast), under 140 mg/dL post-prandial (after meal) and hyperglycemia (over 126 mg/dL fasting (before breakfast), over 200 mg/dL post-prandial (after meal).[22,23]

Each glucometer was initially tested on aqueous/buffer glucose solutions to establish reference values for the other measurements. The phosphate buffer used in all experiments was prepared following a previously described procedure. Red dye was also employed for final testing solutions because not all glucose meters were able to display readings for the colorless buffered solutions.

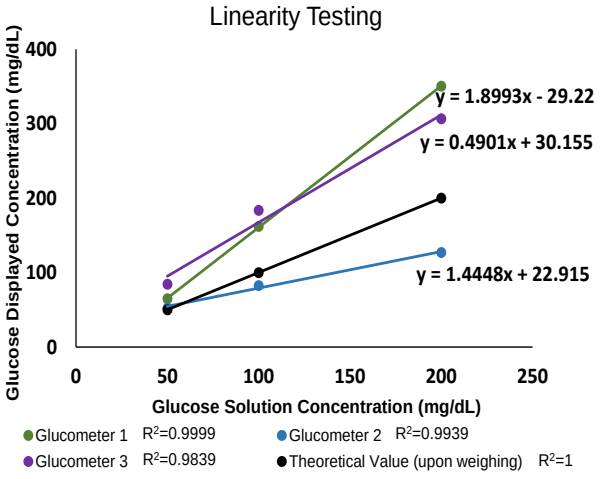
The concentrations of the interferents were chosen based on values of the physiologically relevant concentrations. These values, corresponding to the average concentration of ascorbic acid, oxalic acid and mannose in human blood plasma, were estimated to be approximately 80 mg/dL,[24] 0.26 mg/dL,[25] and 0.73 mg/dL,[26] respectively. Values outside this range were tested as well to account for patients receiving supraphysiological doses or that have nutrient deficiencies and these are specified in the experimental section.

Intrinsic accuracy testing

For determination of the intrinsic accuracy of the devices, preliminary tests were conducted in order to establish the response of the devices and their mean error from the theoretical glucose concentrations of the tested solutions obtained by initial accurate weighing (Fig. 2). The theoretical value data set has a maximum coefficient of determination, representing the ideal values, as weighed before preparation of the solutions. Measurements were performed for each glucometer at least in triplicates and the values are reported as average (see Table S1 in Supporting Information for raw data). Measurements were performed both on aqueous glucose solutions and red-colored buffer solutions of glucose.



a)



b)

Fig. 2 – a) Displayed glucose solution concentrations for each glucometer for all three concentration ranges, compared to theoretical value of glucose concentration, as weighed;.b) coefficients of determination and equations are presented for each linear dependence, as displayed glucose concentration *vs* theoretical glucose solution concentration.

While there is an appreciable linearity between the displayed and the theoretical glucose concentration for each device, with glucometer 1 achieving almost perfect linearity from the data collected during the preliminary testing, their accuracy is only moderate.

Table 1

Average displayed glucose concentration values with the respective mean deviations ± extended uncertainty, 95% confidence interval from the theoretical value, as weighed

Device	Average Displayed Glucose Concentration (mg/dL)			Mean Deviation from theoretical value (mg/dL)		
	Hypoglycemic (50 mg/dL)	**Normal (100 mg/dL)**	**Hyperglycemic (200 mg/dL)**	**Hypoglycemic (50 mg/dL)**	**Normal (100 mg/dL)**	**Hyperglycemic (200 mg/dL)**
1	65 ± 2	170 ± 5	350 ± 5	15	70	150
2	52 ± 2	90 ± 9	127 ± 9	2	–10	–73
3	84 ± 15	183 ± 23	307 ± 28	34	83	107

As displayed in Table 1, the highest recorded absolute mean error was 150 mg/dL glucose above the theoretical value for glucometer **1** in the simulated hyperglycemic range and the lowest recorded absolute mean error was 2 mg/dL glucose above the theoretical value for glucometer **2** in the simulated hypoglycemic range. The device with the lowest absolute mean errors was glucometer **2** for every concentration range, while glucometer **1** had the highest absolute mean error for the hyperglycemic ranges and glucometer **3** had the highest absolute mean errors for the hypoglycemic and normal ranges. In general, the devices appear to be skewed towards lower glucose concentrations, as those were more accurately predicted than the higher glucose concentration ranges, as shown by the larger systematic error for the hyperglycemic range. Glucometer **1** possibly exhibits a chromatic requirement for the analyzed sample, as it responded to measurements only when the solution of glucose contained both buffer and the azorubine red dye. Glucose concentrations acquired through accuracy testing for each glucometer are further used as reference values, such that the observed deviations are reported from experimental concentrations, not theoretical values.

Interference Testing

Interference testing was conducted on buffer solutions containing glucose and each interferent at varying concentrations. The displayed values were recorded, and their averages were compared to the interference free glucose measurement value (Fig. 1).

Any value exhibiting an absolute deviation of more than 10% from the reference value was considered a significant interference. All values that met these criteria for interference are written in bold (Tables 2, 3, and 4).

Table 2

Interference testing data for ascorbic acid. Deviations from the reference value readings are displayed as percentages. Absolute deviations >10% are considered significant interference and are bolded

Glucose concentration range	Device	Ascorbic Acid (mg/dL)				
		5	25	50	100	200
Hypoglycemic (% deviation from reference value)	**1**	4	**98**	**213**	**419**	**724**
	2	9	**102**	**178**	**362**	**485**
	3	**29**	**122**	**204**	**334**	**482**
Normal (% deviation from reference value)	**1**	**33**	**80**	**131**	**217**	**error high**
	2	**32**	**74**	**107**	**214**	**357**
	3	8	**42**	**71**	**115**	**164**
Hyperglycemic (% deviation from reference value)	**1**	**–13**	**–11**	**–13**	**–13**	–6
	2	**error high**	**error high**	**error high**	**error high**	**error high**
	3	–4	5	4	2	–*

*Readings were not displayed despite numerous attempted measurements.

Ascorbic acid was found to significantly interfere at the hypoglycemic and normal glucose simulated concentration levels on all devices. The highest deviation from the reference value was observed in the hypoglycemic range at the highest ascorbic acid concentration on glucometer 1, represented by a 7.2-fold increase from the reference. These results are comparable to previously reported results, for example a study indicated a 4.5-fold increase from the actual glucose concentration measured using a laboratory-based instrument[10] as a result of ascorbic acid spiking of blood plasma samples. At the hyperglycemic range, glucometer 2 displayed a high glucose error message for each ascorbic acid concentration, while there was no interference recorded for glucometer 3 and a negative interference (approximately –13% from reference) recorded for glucometer 1.

Table 3

Interference testing data for oxalic acid. Deviations from the reference values readings are displayed as percentages. Absolute deviations >10% are considered significant interference and are bolded

Glucose concentration range	**Device**	**Oxalic Acid (mg/dL)**				
		0.05	0.1	0.2	0.3	0.4
Hypoglycemic (% deviation from reference value)	**1**	–*	–*	**–11**	–*	–*
	2	9	0	–1	–1	1
	3	**21**	**23**	**25**	**23**	**21**
Normal (% deviation from reference value)	**1**	**10**	3	**29**	**26**	**24**
	2	**33**	**12**	**22**	**22**	**20**
	3	6	4	5	4	3
Hyperglycemic (% deviation from reference value)	**1**	7	**13**	**15**	6	**15**
	2	3	5	4	3	**10**
	3	9	**11**	**12**	9	**10**

*Readings were not displayed despite numerous attempted measurements

Oxalic acid was also found to interfere with glucose at all concentration levels. For the hypoglycemic range, glucometer 3 displayed positive interference at all concentrations tested, with deviations ranging from approximately 21% to 25.5% above the reference value. For the medium glucose range, both glucometer 1 and glucometer 2 displayed positive interference, up to a 29% deviation above reference value for glucometer 1 and up to 33% above the reference value for glucometer 2. Concerning the hyperglycemic range, glucometer 1 and glucometer 3 displayed interference at most of the oxalic acid concentrations – up to 15% for glucometer 1 and up to a 12% deviation for glucometer 3, while glucometer 2 only exhibited notable interference at the highest concentration of oxalic acid, reaching the 10% deviation from reference value threshold.

Table 4

Interference testing data for mannose. Deviations from reference value readings are displayed as percentages. Absolute deviations >10% are considered significant interference and are bolded

Glucose concentration range	**Device**	**Mannose (mg/dL)**				
		0.5	1	1.5	2	2.5
Hypoglycemic (% deviation from reference value)	**1**	**–28**	**–33**	–*	**–21**	**–19**
	2	**–11**	–5	–2	–2	–1
	3	**25**	**19**	**22**	**26**	**24**
Normal (% deviation from reference value)	**1**	**10**	**11**	7	**10**	**13**
	2	**20**	**26**	**27**	**31**	**23**
	3	4	7	1	0	5
Hyperglycemic (% deviation from reference value)	**1**	0	**–19**	**–19**	**–17**	**–18**
	2	–3	–2	**–20**	8	–1
	3	8	0	–5	-2	3

*Readings were not displayed despite numerous attempted measurements

Mannose displayed negative interference for glucometer 1 at both the hypoglycemic and hyperglycemic levels – up to a deviation of approximately –33% for the hypoglycemic range and –19% for the hyperglycemic range. Mannose was found to interfere positively with all glucometers at select concentration levels as follows: both glucometer 1 and glucometer 2 registered positive interference for mannose at the normal concentration range, up to 13% and 31% from the reference value, respectively, and glucometer 3 displayed positive interference for mannose at the hypoglycemic range, up to a deviation of approximately 26%.

The results obtained for solutions of glucose with interferents indicate that the glucometers are skewed towards lower glucose concentrations, as the normal glucose range recorded the most values of positive interference for glucometer 1 and glucometer 2, while glucometer 3 displayed the highest number of recorded positive interferences at the hypoglycemic range.

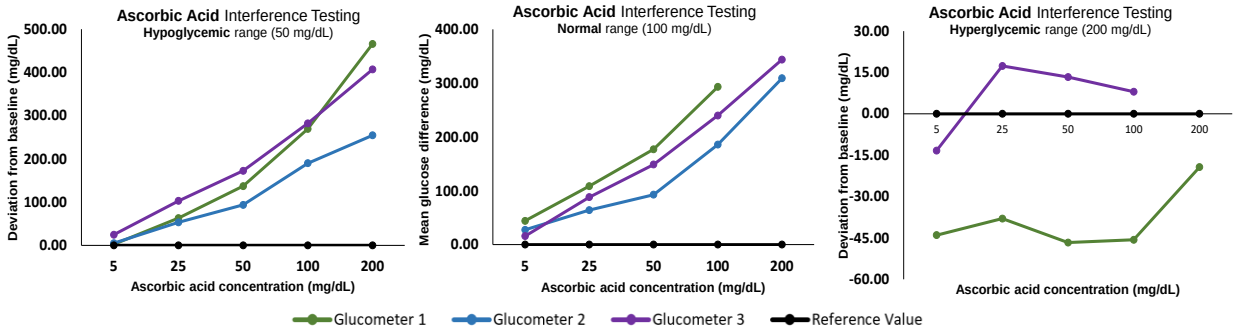


Fig. 3 – Ascorbic acid interference testing graphs
(ascorbic acid concentration in mg/dL *vs* glucose concentration deviation from baseline in mg/dL).

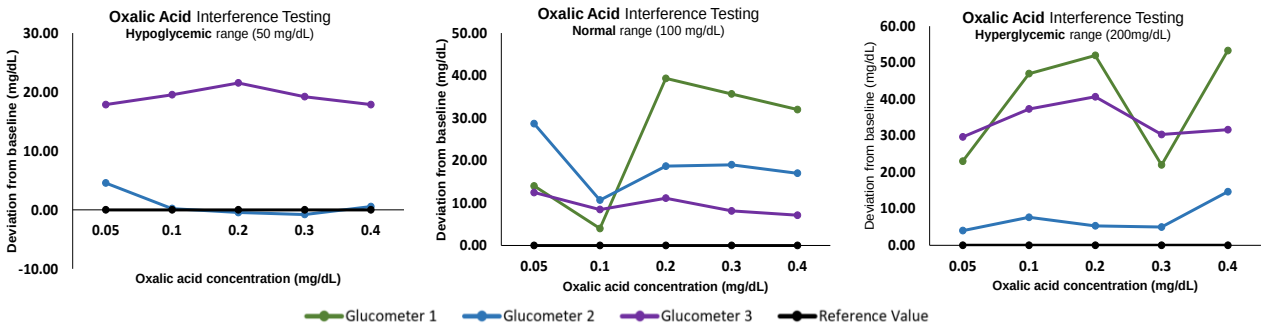


Fig. 4 – Oxalic acid interference testing graphs
(oxalic acid concentration in mg/dL *vs* glucose concentration deviation from baseline in mg/dL).

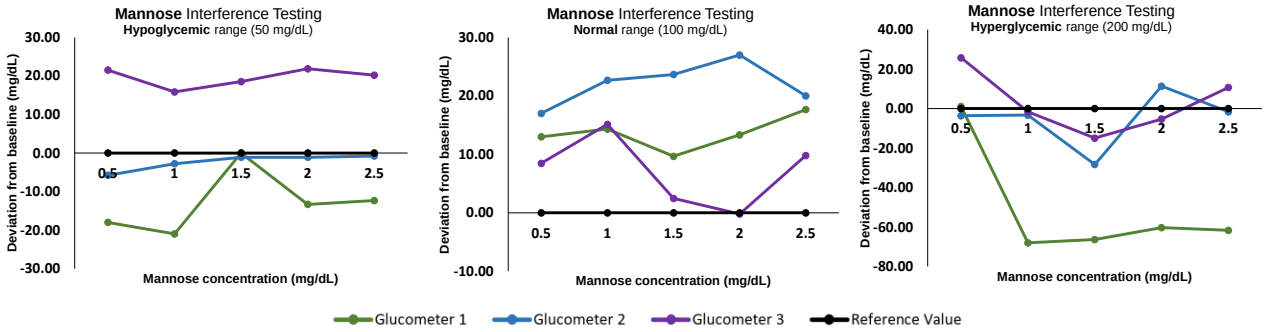


Fig. 5 – Mannose interference testing graphs
(mannose concentration in mg/dL *vs* glucose concentration deviation from baseline in mg/dL).

In the case of ascorbic acid, the results obtained suggest that the interferences are, to a certain extent, proportional with concentrations, while oxalic acid and mannose did not display such an intense correlation with increasing concentration; this could indicate that the mechanism through which they interfere with the measurements might be more complex or that after a benchmark concentration is reached, increasing concentration leads to diminishing returns. It should be noted that the error readings can be considered significant positive interference as the message is displayed in case the device detects very high concentrations of glucose, which are above the reference concentrations recorded by more than 10%, as per the minimum criteria for interference.

EXPERIMENTAL

Materials and methods

All reagents and solvents were analytical grade commercially available substances and therefore, they were used as purchased, without any further purification. Distilled water was employed to perform testing solutions in volumetric flasks of 25 or 50 mL. Stock solutions of glucose, ascorbic acid, oxalic acid, and mannose were prepared and sequentially mixed and diluted to the target concentrations.

The masses of glucose and interfering species, respectively, necessary for preparation of the stock solutions, were accurately weighed using an analytical balance (Mettler Toledo ME104T/M00) with 4 decimal places.

The concentrations of the glucose solutions were as follows: 50 mg/dL (low glucose level), 100 mg/dL (normal glucose level), and 200 mg/dL (high glucose level).

The buffer used for the solutions preparation was adapted from a patent for glucose control solutions.[27] It was obtained by mixing 2.777 g $Na_2HPO_4 \cdot 2H_2O$, 0,7332 g $NaH_2PO_4 \cdot 2H_2O$, 3 g polyethylene glycol and distilled water to 1L fixed volume.

Red dye was added (azorubine, 300 µL) when testing solutions were performed by dilutions because not all glucometers were able to display readings for the colorless buffered solutions.

The concentrations employed for the interferents were for ascorbic acid 5, 25, 50, 100, 200 mg/dL, for oxalic acid 0.05, 0.1, 0.2, 0.3, 0.4 mg/dL and for mannose 0.5, 1, 1.5, 2, 2.5 mg/dL. The working solutions were prepared from stock solutions, by proper dilutions for each target glucose and interferents concentrations.

Principle of enzyme-based sensors in this study

The working devices were three commonly used strip-based glucometers in Roumania. The glucometers and test strips used in the study all function on glucose oxidase (GOx) based systems, with potassium ferricyanide as the oxidizing mediator.

Statistical Analysis

A minimum of three measurements was conducted on three replicates for each glucose concentration and glucose/ interferent concentration combinations respectively (Table S1). First, the reference value was established by conducting measurements on glucose buffer solutions, without addition of interferent. The average of all the measurements recorded for each glucose/interferent concentration combination was calculated and compared to the reference measurements in form of percentages. The resulted values were reported in tables as percentage deviations from the reference: measurements representing less than 100% of the reference value are reported as negative values, and measurements representing more than 100% of the reference are reported as positive values.

Limitations of the study

Considering the samples prepared for analysis were made in the laboratory and not procured from human volunteers, a potential caveat of this study would be that results obtained from tests done on human blood plasma might differ, since blood is a more complex matrix than the buffer used to prepare the solutions. Furthermore, the possibility of errors arising due to lack of calibrating solutions of the instrument provided by the manufacturer should be considered.

CONCLUSIONS

In summary, we prepared and tested synthetic glucose and glucose/interferent buffer solutions, using three commercially available glucometers in Roumania, aiming to analyze the intrinsic accuracy of these devices and subsequent interference tests to evaluate the effect of other substances on glucometer readings. While the linearity for each device was found appropriate, the accuracy of the performed measurements was indicated as moderate, considering that the devices failed to provide glucose concentrations close to the expected theoretical values in the higher concentration ranges. Generally, we observed a bias towards lower glucose concentrations, as suggested by the significant systematic error for the hyperglycemic range. The interference of ascorbic acid was confirmed on all three commercial glucometers and oxalic acid and mannose were signaled as potentially interfering substances.

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