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Development of urine glucose meter based on micro-planer amperometric biosensor and its clinical application for self-monitoring of urine glucose

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ABSTRACT

The highly sensitive urine glucose meter based on amperometric glucose sensor was developed and commercialized. It shows remarkable performances of wide measurement range in 0–2000 mg dl^{-1}, rapid response time as 6 s and robustness against influence by interferents like ascorbic acid or acetaminophen. Correlation between the developed urine glucose meter and commercialized clinical-use urine glucose analyzer showed excellent linear relationship. The monitoring of postmeal blood glucose levels by assess of urine glucose of actual subjects was performed with the developed urine glucose meter. The experimental results suggest the urine glucose level 120 min following the meal should be the appropriate index for diabetes or impaired glucose tolerance to control blood glucose level. The new portable meter was developed, and is expected for flexible use at places other than home or office.

1. Introduction

According to the International Diabetes Federation (IDF) report in 2007 autumn, 246 million people suffer from diabetes in worldwide, and it is predicted to become 380 million by 2025. The report points out serious increase of diabetes in developing and newly industrialized nations.

The predominant focus of therapy has been given on decrease of fasting plasma glucose for long. However, the monitoring of the fasting plasma glucose levels is demonstrated insufficient to optimal postmeal glucose control. Recent studies suggest that reduction in postmeal plasma glucose is quite important to achieve lower hemoglobin A1c (HbA1c) which reflects diabetes development (Monnier et al., 2003, 2007; Woerle et al., 2007). The importance of self-monitoring of blood glucose (SMBG) has widely been recognized as an effective method to control diabetic blood glucose level, and a lot of efforts have been done for developing blood glucose sensors (Cass, 1990; Uenoyama and Nankai, 1994; Mizutani and Yabuki, 1992; Jobst et al., 1996; Sharlkhammer et al., 1994; Yang et al., 1998; Gao et al., 2005; Cui et al., 2000). However, a momentary glucose level obtained by SMBG with a single measurement does not always provide the highest glucose level after the meal precisely, which may result in the monitoring of inaccurate postmeal hyper glucose. Much more frequent SMBG are less recommended due to the disadvantages associated with SMBG; pains by lancets, possible infection risks driven from injured skin.

Meanwhile, urine glucose monitoring is expected as an alternative tool for self-monitoring of the postmeal blood hyper glucose.

In 1996, the first personal use urine glucose meter was introduced to the Japan market (Nakajima et al., 1996) which semi-quantitatively monitored urine glucose levels in the range of 0–500 mg dl^{-1}. In 1999, another urine glucose monitor system developed by TOTO Corporation was launched to the domestic market that was built-in the stool seat of toilet. Urine samples collected from a urine receptacle and diluted was monitored by the built-in meter.

Authors developed urine glucose sensor that permits the measurements of glucose levels in wider range, with high accuracy and rapid response time, applying semiconductor planer technology to fabricate multi-layered immobilized enzyme membrane (Matsumoto et al., 1998, 2001; Ohashi et al., 2003; Ito et al., 2003). In 2004, the urine glucose meter containing the glucose sensor was released to the Japanese market, which can measure the range of 0–2000 mg dl^{-1}. The product was followed by the recent development of a portable urine glucose meter. This report describes the technology employed to the urine glucose meter based on the urine glucose sensor, the performance of the meter and its availability of the monitoring of postmeal glucose levels with clinical application examples to actual diabetic patients.

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2. Development of highly sensitive micro-planer urine glucose sensor for urine glucose meter

The urine glucose meter contains a biosensor that amperometrically detects glucose by an immobilized glucose oxidase (GOX) membrane on planer peroxide electrode. The electrode is fabricated by photolithography technology. Glucose is enzymatically converted to hydrogen peroxide (H_2O_2) by GOX, and the yielded H_2O_2 is electrochemically detected by the electrodes. The enzymatic reaction (1) and the electrode reactions (2) are as follows:

(1) Enzymatic reaction

$$\text{GOX}: \quad \text{Glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2$$

(2) Electrochemical reactions at electrodes

$$\text{Workingelectrode}: \quad H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

$$\text{Counterelectrode}: \quad 2H^+ + 1/2\,O_2 + 2e^- \rightarrow H_2O$$

$$\text{Entireelectrodesystem}: \quad H_2O_2 \rightarrow H_2O + 1/2\,O_2$$

Fig. 1a shows the layout of the electrodes of the H_2O_2 sensor. Three electrodes are placed on the glass plate. Two platinum electrodes act as working and counter electrodes and Ag/AgCl electrode works as the reference electrode. The three electrodes occupy a 3-mm diameter circle, so that all electrodes fit within the cartridge which has 4 mm diameter opening for the sensor. The circle-shaped working electrode has a 1.35-mm diameter, and the surface area is 1.43 mm^2. The Ag/AgCl reference electrode employed here shows approximately 85 mV stable potential vs. Ag/AgCl (saturated NaCl) (Matsumoto et al., 2002). In the beginning of the development, authors used 550 mV (vs. Ag/AgCl) as the operation voltage, which was corresponding with 635 mV vs. Ag/AgCl (saturated NaCl). After some experimental works, until 2000 authors decided to employ 450 mV as the operational voltage which corresponded with 535 mV vs. Ag/AgCl (saturated NaCl) to reduce the excess oxidation current by acetaminophen. Authors are still using 450 mV as the operational voltage vs. Ag/AgCl electrode to prevent unpredicted excess current, although the cation layer has been so improved that it is less affected by acetaminophen.

Urine samples may contain interferents such as ascorbic acid or acetaminophen, or adherents such as urea compounds. These factors make urine glucose measurement problematic. Fig. 1b shows a cross-sectional schematic of the sensor. This sensor consists of four different thin layers on H_2O_2 electrode; restricted permeable layer, enzyme immobilized layer, cation-exchanging layer and adhesive layer. The restrictively permeable layer at the top controls the permeation of glucose into the enzyme-immobilized layer which results in the expansion of the measurement of glucose concentration spectrum. The layer made of special fluorine simultaneously blocks adherents in urine samples. The second enzymatic layer converts glucose to hydrogen peroxide, which is monitored by the H_2O_2 electrode. The sensor's response without the restricted permeable membrane has narrow linear measurement range as 0–20 mg/dl and has typically 500 nA at 20 mg/dl glucose in proper products. After restricted membrane was formed, the output has wide linear range as 0–2000 mg/dl, and its typical output at 20 mg/dl glucose is around 1 nA, and output 100 nA at 2000 mg/dl glucose is obtained. Then, 500 times dilution for glucose seems to occur in the restricted permeable membrane, so authors can cover the very wide measurement range.

The third cation-exchange membrane has two functions to prevent the penetration of negatively charged interferents such as ascorbic acid or acetaminophen as well as transmit hydrogen peroxide. The bottom adhesive layer strengthens the adhesiveness between above three-layered organic membranes and glass substrate/electrodes.

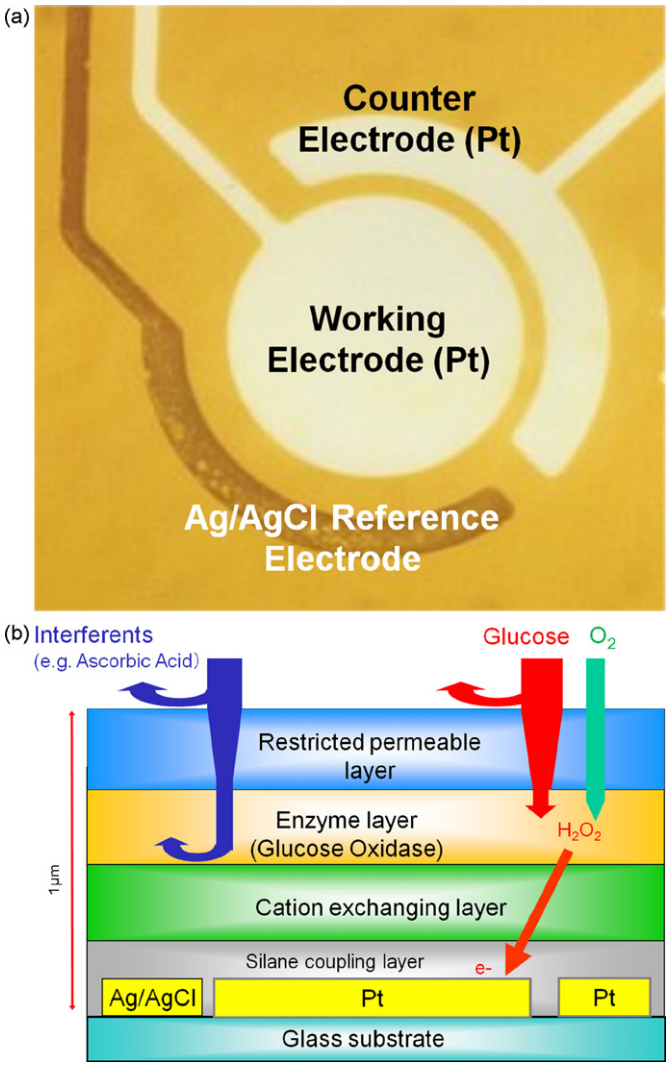

Fig. 1. Glucose sensor construction. (a) Electrodes layout in H_2O_2 sensor. (b) Cross-sectional schematic of sensor.

3. Developing of urine glucose meter

3.1. Feature of the urine glucose meter

A urine glucose meter using a micro-planer amperometric biosensor UG-101 was developed and commercialized in June 2004. In February 2007, the latest model UG-103 was released after improvements of the original version. The supplementary figure S1a and b shows the exterior view of UG-103. It consists of the main body of urine glucose meter and the holder to store the urine glucose meter. Inside the holder which houses the meter of the standby mode, there is buffer solution tank to dip the top-end of the sensor to prevent it from drying.

Supplementary Figure S1b shows the main body of urine meter with sensor cartridge. The electric circuit board, LCD and dry batteries are built in the main body. The equipment is 200 mm length and can be operated with one hand.

The top of the sensor cartridge has urine glucose sensor to monitor urine glucose levels by dropping the urine directly, and the thermistor is integrated around the sensor to compensate the temperature. The sensor cartridge can be discarded from the main body by pushing the release buttons after the use over 200 times or 60 days. The main body has only a single button-switch for managing calibration mode and calling the memorized data. Two coin lithium butteries (CR2032) are used for power supply.

Supplementary Figure S1c describes handling procedures of the meter for urine glucose monitoring. The meter automatically becomes active from the standby mode when it is pulling out from the holder. The relay switch in the meter keeps "on" while it is detached from the magnet incorporated by the holder. The magnet makes simple to regulate active/standby modes of the meter. It is controlled by the detachment/attachment of the meter to the holder. The measurement starts, when the urine is dropped on the sensor. Six seconds later, the meter exhibits the output of urine glucose level on LCD. After washing off the sensor with clean water, the meter returns to the standby mode in the holder.

3.2. Performance

3.2.1. Repeatability

The glucose standard solution was prepared by dissolving glucose in 100 mM TES (*N*-Tris(hydroxymethyl-methyl-2-aminoethanesulfonic acid, pH 7.0) with 150 mM NaCl. The standard solution was applied directly to the sensor of the glucose meter at room temperature. Supplementary Table S1 shows the results obtained at low, middle, and high concentrations. Coefficient of variation (CV) was 3.6% for low concentration, 3.4% for middle and 3.6% for high concentration area, respectively.

3.2.2. Effects of interferential substances

To determine the influences of interferents on the glucose meter, 100 mg dl^{-1} glucose standard solution with various levels of ascorbic acid or acetaminophen were prepared and measured by the urine glucose meter. Fig. 2a and b shows the effects of ascorbic acid and acetaminophen on the measured glucose levels, respectively. These results clearly demonstrate that less than 20 mg dl^{-1} ascorbic acid or less than 15 mg dl^{-1} acetaminophen gives very little influence on the measurements of glucose meter.

3.2.3. The long-term stability of the sensor

The stability of the urine glucose sensor was studied where eight urine glucose meters were employed to continuously measure 500 mg dl^{-1} standard glucose solution for 62 days. The calibrations were carried out at the frequency of once a week. As shown in Fig. 2c, all the eight meters demonstrated quite a uniform and stable output data for the 62-day period.

3.2.4. Comparison with clinical glucose analyzer

A comparison was made between the urine glucose meter and a commercialized clinical-use urine glucose analyzer (A&T

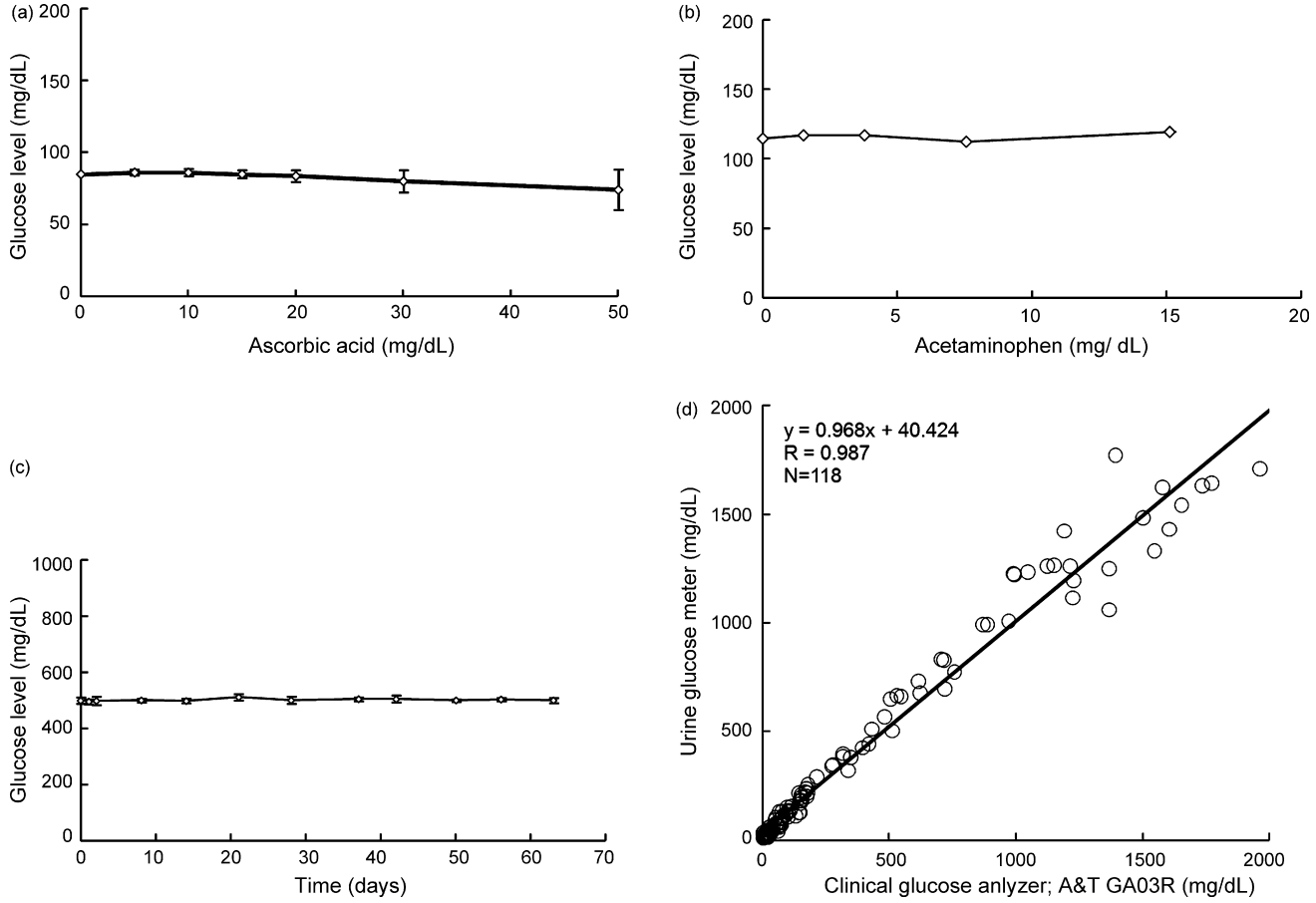

Fig. 2. Property of the urine glucose meter with RSD for each 10 times. The entire CV value is 2.0%. (a) Influence of ascorbate on glucose levels with 10 meters (different sensors). Up to 20 mg/dl of acerbate, there is no influence by ascorbate to glucose level. At 30 mg/dl, ascorbate decreases the signal of glucose by 4%. And at 50 mg/dl of acerbate, it becomes 13%. (b) Influence of acetaminophen on glucose levels. (c) The long-term stability of urine glucose meter (eight machines are used). Outputs from eight sensors are shown. (d) Comparison with clinical glucose analyzer.

GA03R). The excellent liner relationship between the urine glucose meter and the clinical-use analyzer was obtained, as demonstrated through the least squares method of $y = 0.968x + 40.4$, $R = 0.987$ ($N = 118$). Especially, there is almost no discrepancy at low concentration area, which has been considered most critical to clinical laboratory-use (Fig. 2d).

4. Developing of portable urine glucose meter

Authors have developed a portable glucose meter that allows users to monitor at any convenient time and/or places remaining its inherent high performance (Fig. S2). The features specific to the portable type are as follows. This model (UG-201) is available in Japanese market as of the end of June 2008.

(1) The mechanism to carry sensors in buffer solution has been introduced. It permits to shorten the preparing time to less than 30 min. In the former models, the sensor was shipped under dried conditions, so that users have to spend much time of 5 h to restore the sensor to wet condition. Newly introduced mechanism to carry sensors in buffer solution has enabled to shorten the preparation time to less than 30 min.
(2) The length of the meter is 20 cm on-use, and sized down to 12 cm when carrying by introducing the hinge mechanism incorporated.
(3) The water resistance construction was introduced to cover many kinds of usage including outdoor use.
(4) To maximize usability, the power on/off is controlled by the open/close of the hinge. Only a single button switch is located near the hinge for the calibration or reading of the stored data.

This equipment provides easy management of urine glucose level for diabetes and impaired glucose tolerance (IGT) to the people who need to determine urine glucose level out of the home or office. Frequent measurement will contribute to decline the number of diabetes and IGT.

5. Examples of clinical application of urine glucose meter

Two experiments by using UG-103 are described below.

5.1. Availability of determination of postmeal high glucose level

Authors have examined the availability of the urine glucose meter for postmeal glucose monitoring.

The meal load examination of 580 kcal (elemental diet) was executed to 10 subjects with IGT (average HbA1c: $6.0 \pm 0.7\%$). These subjects have no clinical diabetes treatment including insulin or any other medicines. The blood glucose levels were measured every 15 min for 2 h after eating and every 30 min for another 1 h. Concurrently, the urine glucose levels were measured every 1 h for the same period.

The highest postmeal blood glucose level was observed in the period from 45 to 105 min after the meal (Fig. 3a), and its increase from premeal levels of respective subjects was in the range from 72 to 144 mg dl^{-1}. The blood glucose levels 15 min respectively before and after the peak level fluctuated remarkably among subjects; 17 mg dl^{-1} in average and 41 mg dl^{-1} at maximum.

In case of one subject, the urine glucose level 120 min following the meal reached to 658 mg dl^{-1} (Fig. 3b), while postmeal blood glucose level 120 min following the ingestion of diets restored

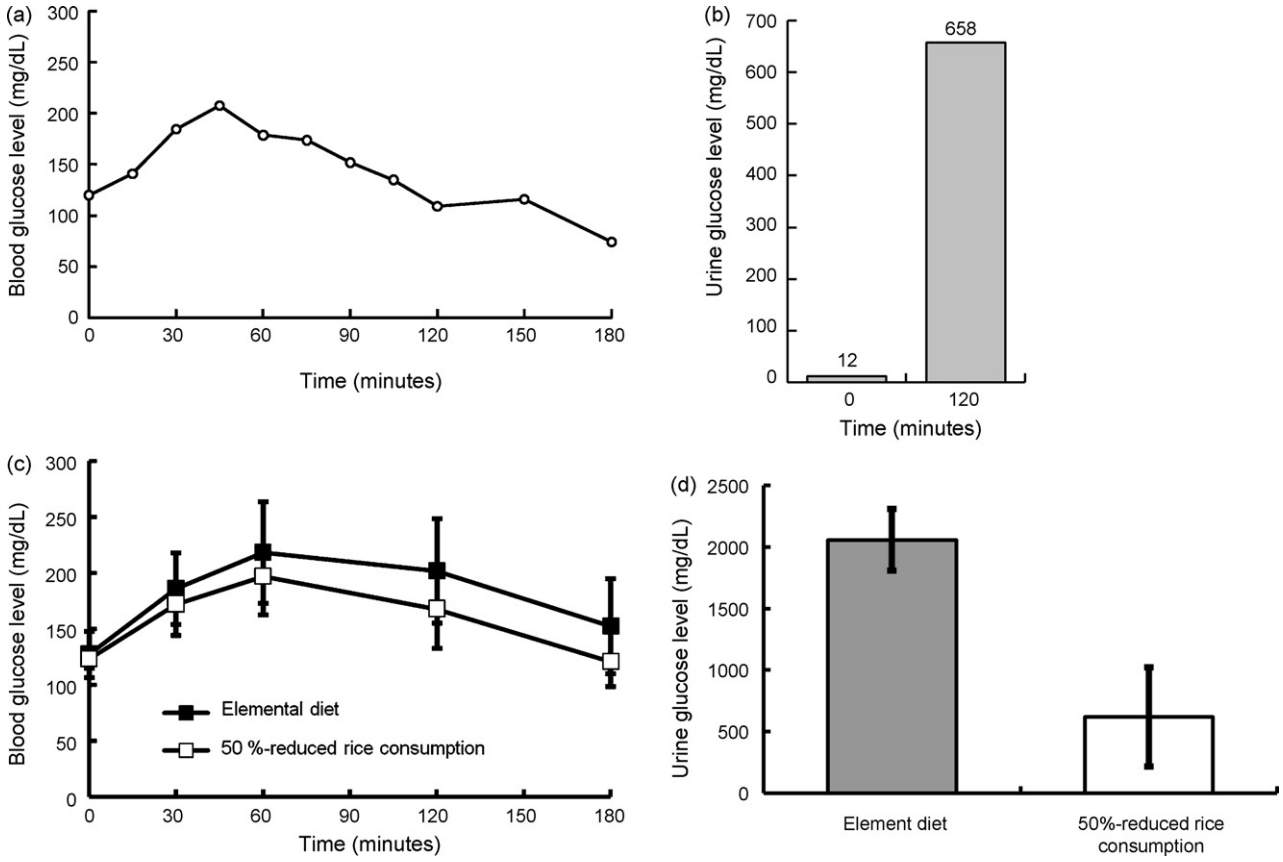

Fig. 3. Comparison between blood glucose level and urine glucose level in postmeal testing and reduced rice testing. (a) Blood glucose level after meal in postmeal testing. (b) Urine glucose level before and 2 h after meal in postmeal testing. (c) Blood glucose level ($n = 4$) in reduced rice testing. (d) Urine glucose level 120 min after the meal ($n = 4$) in reduced rice testing.

to the normal level (109 mg dl^{-1}). The further detailed observation demonstrated the blood glucose reached to the peak level of 208 mg dl^{-1} was obtained at 45 min after the meal. Several measurements of urine glucose levels can detect the transient high blood glucose level that may not be captured by a single blood glucose measurement.

5.2. Availability to confirm the effects by meal

As the next step, the low-level meal load examination (50% reduced rice consumption) was performed on the selected four subjects (average HbA1c: 5.7 ± 0.5%) to measure blood and urine glucose levels.

When rice volume was half, the decrease in the blood glucose level 60 min following the meal was in the range from 7 to 43 mg dl^{-1} and that of the urine glucose level postmeal 120 min was in the range from 486 to 1439 mg dl^{-1}, compared with the levels in case of the ingestion of the elemental diet (Fig. 3c and d). The above results suggest the urine glucose monitoring by the urine glucose meter is effective for postmeal blood glucose management in some cases. It, we understand, should be confirmed by further studies with more subjects.

Authors would like to emphasize the importance of postmeal blood glucose monitoring by postmeal urine glucose level monitoring. Sometimes urine glucose level after meal reflects transient increasing of blood glucose level, even though blood glucose level will be restored to the normal level after 2 h following the ingestion of diets.

6. Results and discussion

The developed urine glucose meter has exhibited the following performances through practical assessments of urine samples: (1) wide measurement range of 0–2000 mg dl^{-1} was obtained; (2) rapid response in 6 s was achieved; (3) the excellent repeatability and little influence by some interferent included in urine like ascorbic acid and acetaminophen was observed; (4) the long-term stability of sensor for 60 days was shown. Furthermore, the accuracy of urine glucose meter was proven by the remarkable linear relationship with clinical-use glucose analyzer. These high performances were achieved by applying semiconductor photolithography technology to the mass-fabrication of the multi-layered thin enzyme membrane for the sensor.

The portable quantitative urine glucose meter has developed by receiving the strong demand from the customers who require monitoring postmeal urine glucose levels out of the home or office. By carrying the urine glucose meter, users can simply and easily monitor urine glucose levels following the food ingestion even outside.

Recently, IDF emphasized the importance of postmeal blood glucose management to control diabetes. In the report, fasting blood glucose should not exceed 110 mg dl^{-1} and 2-h postmeal plasma glucose should not exceed 140 mg dl^{-1} to avoid hyperglycaemia. The efficacy of management of postmeal blood glucose by postmeal urine glucose monitoring after fasting urine excretion is shown in Section 5.2.

Furthermore, the effectiveness reduced calorie in carbohydrate can be confirmed by self monitoring of urine glucose (SMUG) as there is clear correlation between urine glucose level and blood glucose level.

To improve postmeal hyper glucose, self-management of the diet or therapeutic exercise is considered as key factors. Additionally, simple and easy monitoring system of postmeal glucose levels provide subjects with high motivation to improve their blood glucose level. Also, it is effective for improve metabolic syndrome with impaired glucose tolerance.

7. Conclusion

This study demonstrates that the urine glucose meter developed by the authors allows the measurements of wide-range urine glucose levels by rapid response time without influence by various interferents. It has the stability of more than 60 days in one sensor, and showed excellent linear relationship with the existing clinical-use glucose analyzers on the market. Authors examined the availability and effectiveness to employ 120 min postmeal urine glucose monitoring to postmeal blood glucose monitoring. Through the results we have recognized the SMUG enables diabetic patients to manage their blood glucose level simply. Furthermore, it suggested that the frequent measurements of urine glucose levels should detect the transient high blood sugar that may not be captured by a single blood glucose measurement. Addition to the present urine glucose meter, authors have developed the portable urine glucose meter by which many diabetic patients can monitor urine glucose level at places other than home or office.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.07.072.

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