

Article

α -Glucosidase-Mediated Glucometer Readout for Portable Monitoring of Acarbose and Migliol

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Abstract: The α -glucosidase inhibitor is regarded as one of the most important drugs for the treatment of diabetes, which can control postprandial blood glucose levels via prolonging the carbohydrate digestion time and retarding the carbohydrates' absorption. The present work aims to establish a facile bioanalytical method, based on α -glucosidase catalyzing the hydrolysis of 2-O- α -D-Glucopyranosyl-L-ascorbic acid (AA-2G), for the quantification of acarbose and migliol using a personal glucose meter (PGM). The hydrolysis products (ascorbic acid and glucose) can trigger the reduction of $K_3[Fe(CN)_6]$ to $K_4[Fe(CN)_6]$ in the glucose test strips, which results in the formation of the electron, which can be measured by PGM. Thus, ascorbic acid and glucose can be simultaneously measured by a simplified and miniaturized PGM method. However, the products produced by the hydrolysis of AA-2G will be decreased after the addition of acarbose or migliol to inhibit the activity of α -glucosidase, thereby resulting in a decreased PGM readout. After being incubated with α -glucosidase for 3.0 min and enzymatic reaction for 5.0 min, the quantitative detection of acarbose and migliol can be achieved within the ranges of 1.0–30.0 μ M with the limit of detection of 0.33 μ M and 3.0–33.3 μ M with the limit of detection of 1.0 μ M, respectively. IC_{50} values for acarbose and migliol are calculated to be 10.0 μ M and 16.0 μ M, respectively. The recoveries of the acarbose and migliol spiked with three different concentrations (final concentrations of 10.0, 20.0, and 30.0 μ M) in human serum sample are in the ranges of 89.6–114.5% and 93.9–106.5%, respectively. These results demonstrate that the developed PGM method may be useful in future studies on therapeutic monitoring of acarbose and migliol.

Keywords: α -glucosidase; personal glucose meter; acarbose; migliol; therapeutic monitoring



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1. Introduction

Diabetes mellitus, a serious and common chronic disease with high incidence, has become a global health problem [1–3]. Type I, type II, and gestational diabetes are the main types of diabetes mellitus. Among them, type II diabetes accounts for more than 90% of diabetes mellitus [4]. Moreover, diabetes mellitus is commonly accompanied with many complications such as cardiovascular diseases, nephropathy, foot ulcers, blindness, and so on [5–7]. After years of research, α -glucosidase (α -Glu) inhibitor has been regarded as one of the most important drugs for the treatment of diabetes, which can control postprandial blood glucose level via prolonging the carbohydrate digestion time and retarding the carbohydrates absorption in the intestine. To date, all α -Glu inhibitor drugs have demonstrated efficacy and safety in randomized controlled clinical trials. In addition to effectiveness, the definition of optimal therapeutic effect must be based on safety. However, studies have shown that several α -Glu inhibitors used in the clinic still have gastrointestinal adverse effects, including flatulence, abdominal discomfort, and gastrointestinal spasm pain [8,9]. Therefore, to ensure the treatment effect and minimize adverse effects, the dosage of drug is

often adjusted according to the needs of the patient. In this case, it is significant to develop simple and facile methods with low-cost for performing therapeutic monitoring.

Spectrophotometry is the most commonly used method for screening inhibitors of α -Glu using p-nitrophenol glucopyranoside as the enzyme substrate [10]. However, this method is susceptible to interference from the absorption peak at 400 nm of the sample, which hindered its application. Currently, there are many other approaches developed for the screening of α -Glu inhibitors, including capillary electrophoresis [11], high performance liquid chromatography-mass spectrometry [12], electrochemical [13], colorimetric [14], and fluorescent methods [15]. Most of these methods are based on nanomaterials that require long-term synthesis, complex manipulation and purification steps, and bulky and expensive equipment, which increase the cost of testing and are not available to the public all over the world.

In recent years, considerable efforts have been devoted to developing portable, simple, low-cost, and user-friendly detection methods that allow people to quickly take care of themselves without advanced instrument, especially in developing countries and resource-limited areas. The personal glucose meter (PGM), one of the most commercially successful sensors, has improved the quality of life of people with diabetes worldwide [16]. As compared to other devices, the advantages of PGM are their portability, low cost, environmental friendliness, and ease of operation [17,18]. Most importantly, PGM can be widely used and will bridge the gaps between people living in urban areas and remote rural areas regarding access to medical diagnosis [19]. PGM was initially used to measure the blood glucose of diabetic patients. Recently, PGM-based measurement of other non-glucose analytes has attracted wide attention, including heavy metal ions, aflatoxin, DNA, enzymes, dopamine, and protein [17,20–24]. Most of the published methods utilize mediators that are closely related to the glucose concentration (such as invertase) or another mediator that can bind the mediator (such as invertase) to non-glucose targets to achieve PGM measurement. For example, Chen et al. [25] fabricated a portable antibody-free sandwich assay for the quantitative detection of chloramphenicol based on PGM. In the study, invertase was linked to the β -cyclodextrin to form β -cyclodextrin/invertase polymer bioconjugate, and a magnetic molecularly imprinted probe was synthesized by using 2,2-dichloroacetamide as the template. Finally, the limit of detection for chloramphenicol is about 0.16 ng/mL. Fang et al. [26] developed a sensitive and portable biosensor for the quantitative detection of DNA using nanogold-functionalized poly(amidoamine) (PAMAM) dendrimer composites and PGM. In the study, invertase was linked to the DNA nanogold-functionalized PAMAM, and the signal DNA undergoes a strand displacement reaction with the target DNA. Finally, the limit of detection for the target DNA sequence is 0.26 pM. Another way of expanding the application of PGM is to establish a direct redox reaction relationship with the mediator in the glucose test strip. For example, a PGM method was developed for the direct determination of hydrogen peroxide and hypochlorite by us [27]. Acetylcholinesterase catalyzes the hydrolysis of acetylthiocholine iodide (ATCI) to generate thiocholine iodide, which triggers the reduction of $K_3[Fe(CN)_6]$ to $K_4[Fe(CN)_6]$ in the glucose test strips and generates a PGM detectable signal. The PGM readout can be reduced after the hydrogen peroxide or hypochlorite was pre-incubated with the ATCI. The limits of quantitation are 1.7 mM and 0.9 mM for hydrogen peroxide and hypochlorite, respectively. Enzyme cascades have sparked tremendous attention in bioanalysis. The coupled biocatalysis can generate extremely amplified signals, enabling quantitative detection of trace targets. For example, Zhang et al. [28] developed versatile enzyme cascade-based colorimetric bioassays for the ultrasensitive detection of diabetes-related glucose. Zhou et al. [29] developed a hybrid nanozyme cascade system with high reaction selectivity, which can transform the substrate into the targeted product by more than 2000 times.

Ascorbic acid (AA) has a strong reducing property, which can reduce $K_3[Fe(CN)_6]$ to $K_4[Fe(CN)_6]$. The 2-O- α -D-Glucopyranosyl-L-ascorbic acid (AA-2G) has been extensively studied as a substrate of α -Glu [30,31]. In this study, a simple and facile bioanalytical method for the acarbose and miglitol determination was developed based on α -Glu cat-

alyzing the hydrolysis of AA-2G in PGM. As shown in Figure 1, α -Glu catalyzes the hydrolysis of AA-2G to produce glucose and AA, and the glucose can be catalyzed by FAD-dependent glucose dehydrogenase in glucose test strips. Meanwhile, AA can trigger the reduction of $K_3[Fe(CN)_6]$ to $K_4[Fe(CN)_6]$ through redox reaction. Afterwards, $K_4[Fe(CN)_6]$ is re-oxidized in the glucose test strips to generate PGM detectable signals. When the anti-diabetic drugs (such as acarbose and miglitol) are added in the reaction mixtures, the activity of α -Glu will be inhibited, resulting in the decrease in products and a lower PGM readout. Thus, acarbose and miglitol can be detected without any additional modifications of the PGM, α -Glu, and substrate, as well as the complex manipulation and purification steps, bulky, and expensive equipment. Finally, the developed PGM method was used to detect acarbose and miglitol in normal human serum for validating its applicability in real sample analysis.

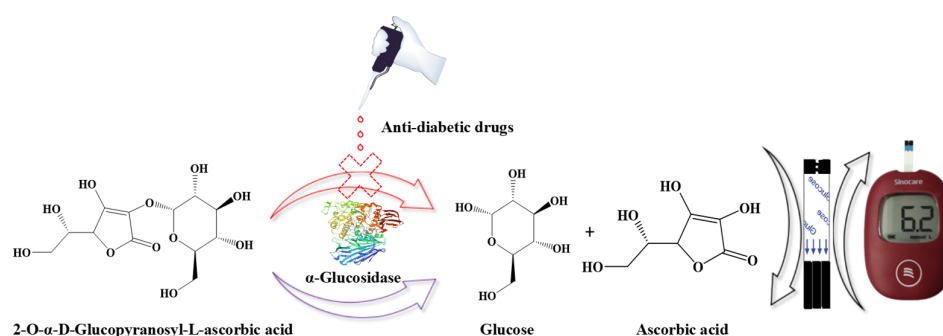


Figure 1. Schematic illustration of the principle of the PGM method based on α -glucosidase-mediated reaction.

2. Materials and Methods

2.1. Materials and Reagents

The α -Glu (754,360 U/mL, liquid), L-arginine hydrochloride, L-lysine hydrochloride, L-hisidine monohydrochloride, L-serine hydrochloride, and L-glutamic acid monosodium salt were purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). AA-2G was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Normal human serum was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Miglitol and acarbose were purchased from Shanghai Bidepharm Technology Co., Ltd. (Shanghai, China). Sodium acetate trihydrate was purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). AA and glacial acetic acid ($\geq 99.5\%$) were purchased from Chengdu Chron Chemicals Co., Ltd. (Chengdu, China). Glutathione was purchased from Sigma-Aldrich (Shanghai, China) Trading Co., Ltd. (Shanghai, China).

2.2. Instrumentation

The PGM of Sannuo + (glucose detection ranges: 1.1–33.3 mM) and glucose test strips (the sample detection area of the glucose test strip contains potassium ferricyanide and FAD-dependent glucose dehydrogenase) were purchased from Sinocare Inc. (Changsha, China). The pH of solution was measured by a FE28-standard pH meter (Mettler-Toledo Instruments, Shanghai, China). The UC-2H ultrasonic cleaner was purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China).

2.3. Preparation of Solutions and Samples

The buffer was prepared by dissolving 10.0 mM sodium acetate trihydrate in ultrapure water and the required pH was adjusted by glacial acetic acid. The α -Glu was diluted 800 times by sodium acetate buffer (10.0 mM, pH = 5.0). AA-2G (42.0 mM) solution was prepared by dissolving 7.1 mg of AA-2G in 0.5 mL sodium acetate buffer (10.0 mM, pH = 5.0) and protected from light. Acarbose and miglitol were prepared by

dissolving them in sodium acetate buffer (10.0 mM, pH = 5.0) with the final concentration of 1.0 mM, respectively.

2.4. Detection of the Anti-Diabetic Drugs Using PGM Method

First, 1.0 μL of $\alpha\text{-Glu}$ was pre-incubated at 40 $^{\circ}\text{C}$ for 3.0 min with the anti-diabetic drugs before the enzymatic reaction. Then, 1.0 μL of AA-2G (42.0 mM) solution was added to initiate the enzymatic reaction and incubated for 5.0 min at 40 $^{\circ}\text{C}$; the concentration of product was measured by the PGM within 5 s. With different amounts of anti-diabetic drugs, different PGM readout values were obtained. Then, the interferences from the sample matrixes were eliminated by subtracting the background of the PGM readout. Finally, comparisons were made with the PGM readout obtained without the addition of antidiabetic drugs. All tests in this study were repeated at least three times, and the data are shown as mean $\pm$ standard deviation. The percentage of inhibition $I(\%)$ can be calculated through Equation (1).

$$I(\%) = \left(1 - \frac{I_t - I_b}{I_0}\right) \times 100\% \quad (1)$$

where I_t and I_0 are the PGM readout with and without the anti-diabetic drug, respectively. I_b represents the PGM readout of the background sample.

The Z' factor is an index to evaluate the performance of the developed PGM method for portable monitoring of anti-diabetic drugs, which is calculated by Equation (2).

$$Z' = 1 - \frac{3\sigma_s + 3\sigma_c}{|\mu_s - \mu_c|} \quad (2)$$

where μ_s and μ_c represent the PGM signal of standard (s) (without inhibition) and the negative (c) group (100% inhibition by the anti-diabetic drug), respectively. The σ_s and σ_c represent the standard deviations of the PGM signal of the standard and the negative (c) group, where 100% inhibition indicates that $\mu_c = 0$; Equation (2) is simplified to Equation (3). The developed PGM method is accurate and reliable when the value of the Z' factor is higher than 0.5.

$$Z' = 1 - \frac{3\sigma_s}{\mu_s} \quad (3)$$

2.5. Determination of the Anti-Diabetic Drugs in Human Serum

Before analysis, the human serum was diluted two times with sodium acetate buffer without any sample cleaning-up step. Spiked samples were prepared by diluting high concentrations of the anti-diabetic drugs with the desired amount of human serum sample. The interferences from the sample matrixes were eliminated by measuring and subtracting the background PGM readout. The solution without $\alpha\text{-Glu}$ served as the background solution and was measured by the PGM. In brief, 1.0 μL of spiked sample was measured by PGM. Then, 1.0 μL of spiked samples was mixed with 1.0 μL of sodium acetate buffer (10.0 mM, pH = 5.0) and 1.0 μL of AA-2G solution, which was detected by the PGM.

3. Results and Discussion

3.1. The Principle of the Anti-Diabetic Drugs Detection

As shown in Figure 1, $\alpha\text{-Glu}$ catalyzes the hydrolysis of AA-2G to produce glucose and AA, and the glucose is oxidized by glucose dehydrogenase in glucose test strips. Meanwhile, AA can trigger the reduction of $\text{K}_3[\text{Fe}(\text{CN})_6]$ to $\text{K}_4[\text{Fe}(\text{CN})_6]$ through a redox reaction. Both of the above pathways can form electrons to generate the PGM-detectable signal. When the anti-diabetic drugs (such as acarbose and miglitol) are added in the reaction mixtures, the activity of $\alpha\text{-Glu}$ can be inhibited, which results in the decrease in products and a lower PGM readout. Thus, the determination of acarbose and miglitol can be achieved without any additional modification to the PGM, $\alpha\text{-Glu}$, and substrate.

To verify the feasibility of the principle using PGM for the monitoring of anti-diabetic drugs based on the AA-2G-mediated reaction, seven solutions were measured using PGM. As shown in Figure 2, the PGM readout is (6.5 ± 0.3) mM after AA-2G is added to the α -Glu solution and incubated for 15.0 min (Figure 2C). After α -Glu is pre-incubated with the anti-diabetic drugs at 40 °C for 3.0 min, 1.0 μ L of AA-2G solution was added to the enzymatic reaction solution and incubated for 5.0 min at 40 °C; then, the PGM readout was decreased to (4.7 ± 0.1) mM (Figure 2D). The PGM readout of the AA-2G or α -Glu + acarbose solutions (Figure 2F or Figure 2G) are all L0 (L0 value means PGM readout < 1.1 mM). On the other hand, the PGM readouts of AA and glucose at the same molar concentration were determined as (9.3 ± 0.6) mM and (3.4 ± 0.5) mM (Figure 2B,E), respectively. Thus, AA contributes more to the PGM readout. Moreover, the PGM readout indicated that the co-existence of AA and glucose (molar ratio of 1:1) was (11.8 ± 0.3) mM (Figure 2A). Therefore, the co-existence of AA and glucose had little effect on the PGM readout. These results indicate that it is feasible to detect an anti-diabetic drug using the PGM method based on an AA-2G-mediated reaction.

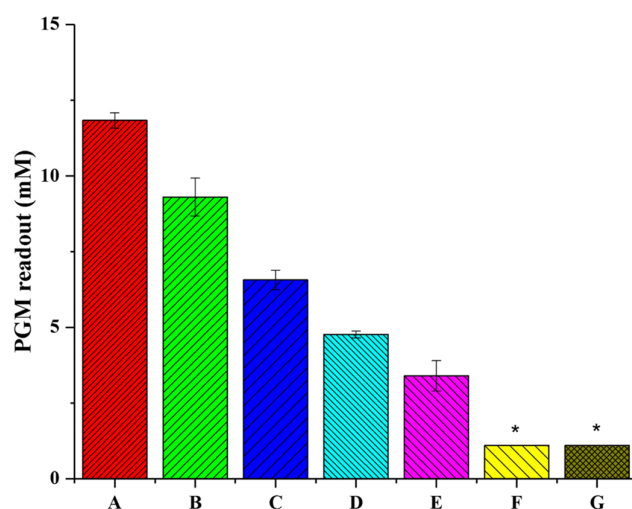


Figure 2. The PGM readout of seven solutions. (A) Ascorbic acid solution + glucose solution (molar ratio: 1:1); (B) Ascorbic acid solution; (C) α -Glucosidase + AA-2G solution; (D) α -Glucosidase + AA-2G + acarbose solution; (E) Glucose solution; (F) AA-2G solution; (G) α -Glucosidase + acarbose solutions. * PGM readout < 1.1 mM.

3.2. Optimization of the Experimental Conditions

The main experimental factors were investigated and optimized using acarbose as an example, including the effect of AA-2G concentration (6.0–22.0 mM) on the PGM readout, the relationships between percentage of inhibition and the enzymatic reaction time (5.0–15.0 min), as well as AA-2G concentration (10.0–14.0 mM) and inhibition time (0.0–7.0 min), to acquire an effective PGM-based method for monitoring the anti-diabetic drugs.

AA-2G concentration is important in the PGM method for monitoring the anti-diabetic drugs. With AA-2G in the α -Glu solution, AA and glucose will be produced, which will cause the increase in PGM readout. As shown in Figure 3, it was found that the PGM readout increased with the increase in the AA-2G concentration. Moreover, the PGM readout shows a good linear relationship with the concentration of AA-2G in a range from 6.0 to 14.0 mM and the regression equation of the PGM readout = $1.2729 \times C_{AA-2G}$ (mM) – 6.1057 ($R^2 = 0.9993$). The results demonstrate that the developed PGM method is feasible to measure the products of glucose and AA. Therefore, an AA-2G concentration of 6.0 to 14.0 mM was chosen for the following experiments.

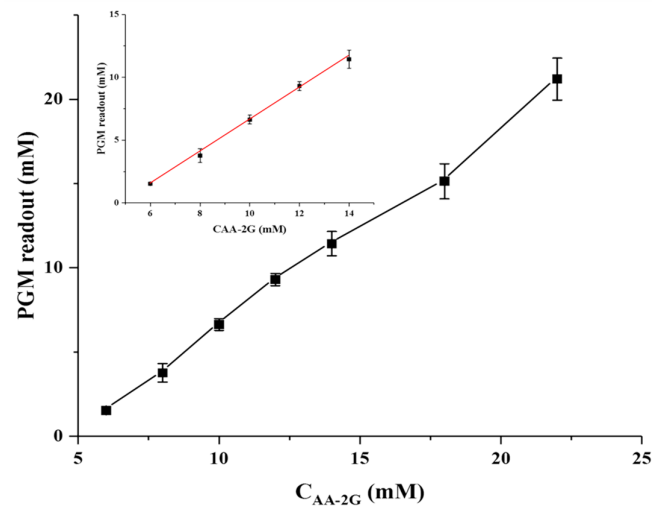


Figure 3. Effect of the concentration of AA-2G on the PGM readout. The inset is the linear relationship between the concentration of AA-2G and the PGM readout. Conditions: α -Glucosidase, 314.3 U/mL; incubation time, 15.0 min; AA-2G, 6.0–22.0 mM.

As shown in Figure 4, the enzymatic reaction can generate enough products for a PGM readout in the concentrations of AA-2G from 10.0 to 14.0 mM after incubation for 5.0 min. The percentage of inhibition decreases with the increase in the enzymatic reaction time and AA-2G concentration. Therefore, the enzymatic reaction time of 5.0 min was selected for the next study. On the other hand, the PGM readout in 10.0 mM and 12.0 mM of AA-2G are 2.2 mM and 3.0 mM after incubation for 5.0 min, respectively, and the PGM readout in 14.0 mM of AA-2G is 4.8 mM. Therefore, considering the analytical performance, the concentration of AA-2G at about 14.0 mM was used for the further study.

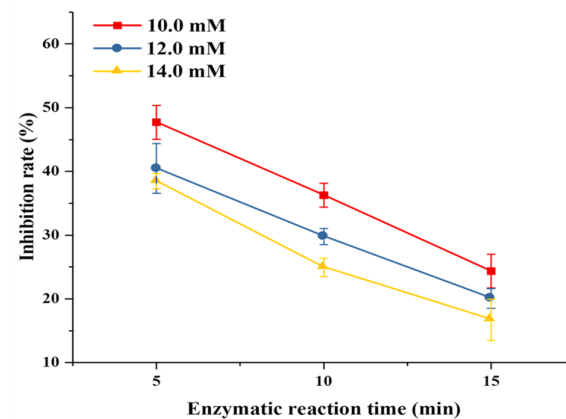


Figure 4. Effect of the concentrations of AA-2G and enzymatic reaction time on the percentage of inhibition. Conditions: α -glucosidase, 314.3 U/mL; incubation time, 5.0–15.0 min; AA-2G, 10.0–14.0 mM; inhibition time, 3.0 min; acarbose, 2.2 μ M.

As shown in Figure 5, the PGM readout decreases with the increase in inhibition time from 0 to 3.0 min, while the PGM readout almost remains constant after inhibition for 3.0 min. Therefore, the inhibition time of 3.0 min was selected for the next study.

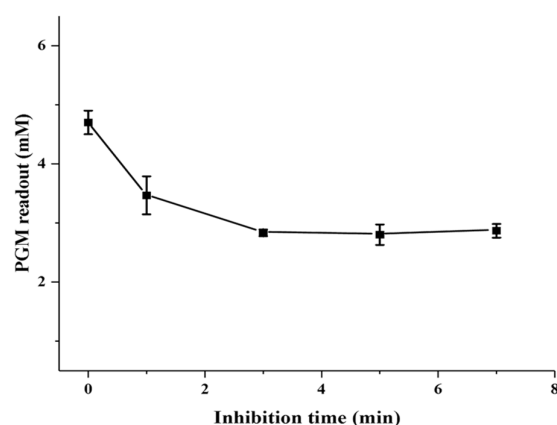


Figure 5. Effect of the inhibition time on the PGM readout. Conditions: α -glucosidase, 314.3 U/mL; incubation time, 5.0 min; AA-2G, 14.0 mM; inhibition time, 1.0–9.0 min; acarbose, 2.2 μ M.

3.3. Determination of Anti-Diabetic Drugs by the PGM Method

The repeatability of the PGM method for the portable monitoring of anti-diabetic drugs was investigated based on the relative standard deviation (RSD) of the PGM readout. The run-to-run repeatability using a batch ($n = 5$) of glucose test strips was calculated to be 2.8%. The repeatability using different batches ($n = 5$) of glucose test strips was calculated to be 4.5%. The Z' factor was determined to be 0.84 ($n = 8$) according to Equation (3), which indicates that the developed method is precise and reliable. The anti-diabetic drugs can be detected based on the PGM method, as they can inhibit the activity of α -Glu through reducing the production of AA and glucose. With less anti-diabetic drugs, more AA and glucose will be produced, and the inhibition rate (%) of anti-diabetic drugs can be easily calculated through the variation of PGM readout. Under the optimized conditions (314.3 U/mL of α -Glu, 5.0 min of incubation time, 14.0 mM of AA-2G, 3.0 min of inhibition time), the anti-diabetic drugs with different concentrations were pre-incubated with α -Glu before the enzymatic reaction. Followed by the AA-2G catalytic reaction, the variations of PGM readout were recorded. Based on the relationship between inhibition rate (%) and anti-diabetic drugs concentration, the calibration curve can be plotted. As shown in Figure 6A, the inhibition rate (%) of acarbose on α -Glu increases gradually with the increase in the concentration of acarbose. The inhibition rate (%) vs. Log[acarbose] presents excellent linear dependence in the range of 1.0–30.0 μ M with the regression equation of $Y = 31.679 \times \text{Log}[\text{acarbose}](\mu\text{M}) + 18.570$ ($R^2 = 0.9868$). The limit of detection of acarbose is 0.33 μ M (inhibition rate (%) is 13.1 ± 3.4 , $n = 3$). The IC_{50} value is calculated to be 10.0 μ M for acarbose, which is comparable to the reported literature with IC_{50} values (Table 1). In addition, using the developed PGM assay, the calibration plot for migliol was constructed (Figure 6B). The inhibition rate (%) vs. Log[migliol] presents good linear correlation in the range of 3.0–33.3 μ M with the regression equation of $Y = 61.201 \times \text{Log}[\text{migliol}](\mu\text{M}) - 22.844$ ($R^2 = 0.9920$). The limit of detection of migliol is 1.0 μ M (inhibition rate (%) is 4.9 ± 1.2 , $n = 3$), and the IC_{50} value is calculated to be 16.0 μ M. Compared to the other anti-diabetic drugs assays (Table 1), this method does not require expensive instrument, skilled operators, or a complicated synthesis process, but it offers a satisfactory linear range and limit of detection. To investigate the selectivity of the PGM determination method, the interferences with the concentration of 0.1 mg/mL were tested, and the relative values are shown in Figure 7. Because of the specificity of α -Glu, no obvious changes in the relative PGM readout were observed under the same conditions. This result confirms the satisfactory selectivity of the PGM method.

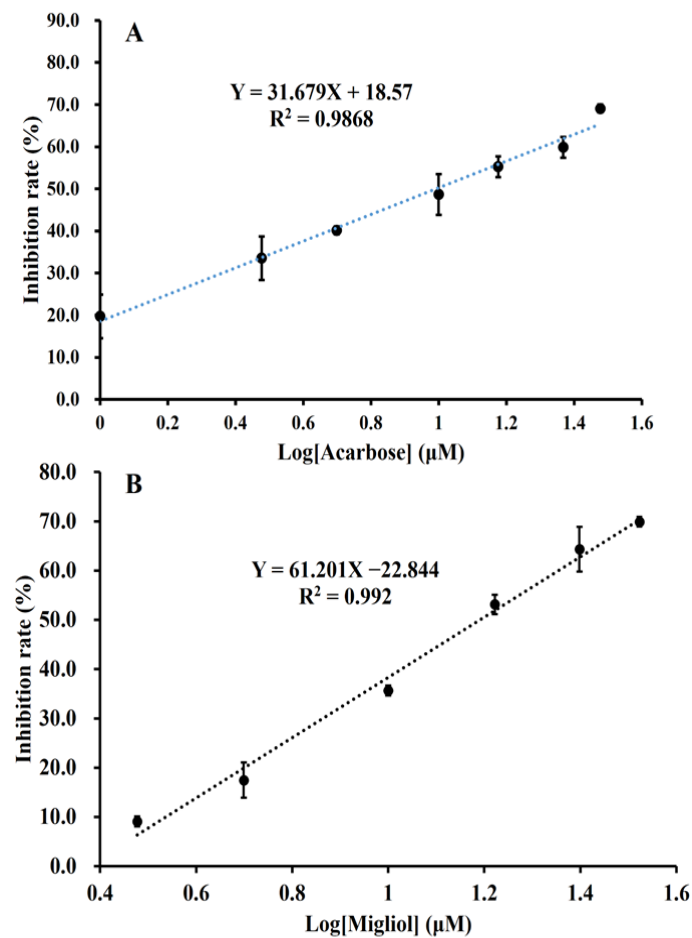


Figure 6. The plots of percentage of inhibition versus the logarithmic concentration of acarbose (A) and migliol (B) Conditions: α -glucosidase, 314.3 U/mL; incubation time, 5.0 min; AA-2G, 14.0 mM; inhibition time, 3.0 min.

Table 1. Comparison of different methods for the anti-diabetic drugs detection in terms of substrates, linear range, limit of detection, and IC_{50} .

Methods	Substrates	Linear Range (μM)	Limit of Detection (μM)	IC_{50} (μM)	Ref.
Liquid crystal-based assay	Dodecyl α -D-glucopyranoside	Acarbose: 1.0–10.0; Migliol: 1.0–20.0	Acarbose: 0.57; Migliol: 1.00	-	[32]
Personal glucose meter	Maltose	Acarbose: 1600–46,500	-	Acarbose: 16,800	[33]
Fluorescence	4-Nitrophenyl- α -D-glucopyranoside	Acarbose: 0.1–1000	Acarbose: 0.01	Acarbose: 58.68	[34]
Colorimetry-naked-eye detection	L-ascorbic acid-2-O- α -D-glucopyranosyl	Acarbose: 0–16	Acarbose: 1.0	-	[31]
Colorimetry	L-ascorbic acid-2-O- α -D-glucopyranosyl	Acarbose: 25–4500	-	Acarbose: 370	[30]
Fluorescence	4-Nitrophenyl- α -D-glucopyranoside	Acarbose: 50–300	-	Acarbose: 203.5	[35]
Personal glucose meter	L-ascorbic acid-2-O- α -D-glucopyranosyl	Acarbose: 1.0–30.0; Migliol: 3.0–33.3	Acarbose: 0.33; Migliol: 1.00	Acarbose: 10.0; Migliol: 16.0	This study

Note: “-” not mentioned.

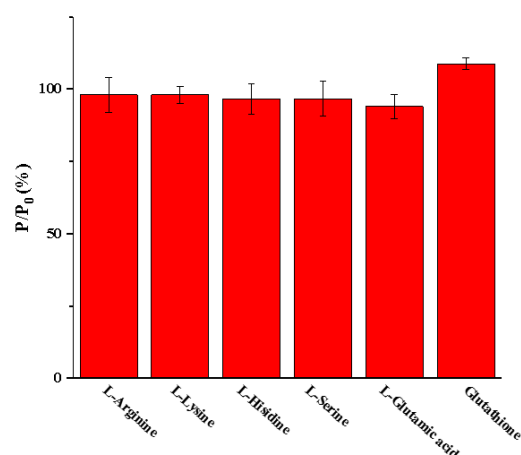


Figure 7. Selectivity of the developed PGM-based method. P/P_0 = the ratio of PGM readout measured with/without interfering substances.

3.4. Detection of the Anti-Diabetic Drugs in Human Serum

The PGM method based on the AA-2G-mediated reaction was further tested by sample spiked recovery in human serum without a sample cleaning-up step to investigate its practical application. The recoveries (Table 2) of the acarbose in the human serum sample spiked with three different concentrations (final concentrations of 10.0, 20.0, and 30.0 μM) are in the range of 89.6–114.5%. On the other hand, the recoveries (Table 2) of the miglitol in the human serum sample spiked with three different concentrations (final concentrations of 10.0, 20.0 and 30.0 μM) are in the range of 93.9–106.5%. The results indicate that the proposed strategy has the potential to be applied in the monitoring of anti-diabetic drugs. Typically, some additional methods are used to remove the sample matrix prior to analysis, such as solid-phase extraction techniques, but this process is complex and time consuming. In contrast, the increments of PGM readout used in this study allows us to use one touch with the sample to obtain the background readout and subtract it, which can be realized easily by an untrained person. This method does not require immobilization of the enzyme, chemical modification of the substrate, and customization of the PGM, highlighting an easily accessible and user-friendly method for the monitoring of acarbose and miglitol.

Table 2. Recovery studies of acarbose and miglitol in normal human serum by the PGM method ($n = 3$).

Anti-Diabetic Drugs	Added (μM)	Found (SD) (μM)	Recovery (%)
Acarbose	10.0	9.0 (1.1)	89.6
	20.0	20.3 (4.8)	101.6
	30.0	34.3 (4.2)	114.5
Miglitol	10.0	9.4 (1.6)	93.9
	20.0	19.6 (1.9)	98.2
	30.0	31.9 (2.0)	106.5

4. Conclusions

In this study, a simple and portable acarbose and miglitol detection method was developed based on α -glucosidase catalyzing the hydrolysis of AA-2G in PGM for the first time. The developed method does not require the design and manufacturing process of PGM nor any modification of enzyme or substrates. Furthermore, this strategy enjoys sensitivity by taking advantage of the two pathways to generate a PGM-detectable signal. The glucose is catalyzed by FAD-dependent glucose dehydrogenase in glucose test strips, and AA can trigger the reduction of $\text{K}_3[\text{Fe}(\text{CN})_6]$ to $\text{K}_4[\text{Fe}(\text{CN})_6]$ through redox reaction, which is conceptually different from the PGM methods for other non-glucose analyte detection. However, this method also has some disadvantages. First, the limited detection

range of PGM of 1.1–33.3 mM may result in a narrow linear range of analyte. Second, the developed method has limited detection capability for trace-level analytes. Third, it is difficult to apply this method to the simultaneous analysis of multiple analytes. Finally, the determination of the anti-diabetic drugs in human serum sample by the developed PGM method was achieved with good recovery. In short, this study provides a simple and portable bioanalytical method for the monitoring of acarbose and miglitol using PGM, which can be of great potential in therapeutic monitoring.

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Institutional Review Board Statement: Normal human serum used in this study was purchased from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China, which is a biological product. Thus, not applicable to Ethics statement.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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