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Development of cellulose paper testing strips for quick measurement of glucose using chromogen agent

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ABSTRACT

Cellulose paper based glucose test strips were successfully prepared using 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA) as the chromogen agent. Cellulose paper is a good substrate for carrying chromogen agents and other chemicals so that the quantitative analysis can be done based on the colorimetric chemistry. The color intensity of the developed compounds, which was measured as the differential diffusive reflectance of the test strip at 510 nm, was correlated to the glucose concentration of the sample solutions in the range of 0.18–9.91 mg/ml. These colorimetric test strips could be conveniently used, do not have to use an electronic device, and would have potential applications in the home monitoring of blood glucose for people with diabetes.

1. Introduction

The normal glucose concentration in the human blood stream is in the range of 60–95 mg/dl (Langer, Yogev, Xenakis, & Rosenn, 2005). Frequent and convenient house monitoring of blood glucose concentration is of critical importance to people with diabetics to prevent both short- and long-term complications from hyperglycemia (Allemann, Houriet, Diem, & Stettler, 2009; Clar et al., 2010; Hu, 2009; Lockwood, Xue, & McArthur, 2010; Towfigh et al., 2008). Diabetics who use insulin may need to test their blood glucose 3 to 10 times a day, to determine the use of their insulin doses (Fonseca, Foyt, Shen, & Whitcomb, 2000).

There are many blood glucose test strips available on the market today, such as Accu-Check® Aviva and Compact Plus, OneTouch® Ultra2, FreeStyle® Freedom, and Ascensia® Contou,and all of them use plastic material as the substrate (Griffey, Wilhite, Heacook, & Dietzen, 2007; Jday-Daly et al., 2011; Kuo et al., 2011; Lyon et al., 2009; Sonmez et al., 2010; Weise et al., 2008). These test strips have built-in electrodes, a capillary structure and reactive chemicals on the plastic substrate, and have to be used together with an electronic device called glucometer that is based on electrochemical methods (Lehmann, Kayrooz, Greuter, & Spinas, 2001; Welschen et al., 2005). Usually, users would have to establish the calibration curve for the test strips (Johnson, Fry, Flatland, & Kirk, 2009). Moreover, these plastic based test strips are relatively expensive to make due to the complexity of the structure (Chen, Wu, Wu, Wu, & Jaw, 2004; Dunn, Eastman, & Tamada, 2004).

"Dip-and-read" type of test strips without the need of electronic device, such as glucometer would be more convenient and less costly for the end users (Fang, Gao, Yan, Wang, & Zhou, 2005; Lourenco et al., 2011). Recently, the use of biodegradable lignocellulosic fibers, for the production of value-added products, for example, nano-crystalline cellulose (NCC), received much attention (Alemdar & Sain, 2008; Jahan, Saeed, He, & Ni, 2011). Cellulose paper is biodegradable and is potentially an excellent substrate for carrying polymers/chemicals. Compared with plastic materials such as PE, PP, PVC and CTA, paper is from renewable and sustainable forest resources and can carry functional chemistry, for example, polypyrrole conductive polymers (Huang, Kang, & Ni, 2005, 2006). In earlier studies, paper based test strip were developed for the determination of heavy metals, such as chromium in water samples (Kong & Ni, 2009a, 2009b). The objective of this paper is to determine the potential of developing cellulose paper-based test strips for blood glucose concentration monitoring. Limited studies have been reported in the literature on paper

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based glucose test strips. A few studies have suggested that chromogen reagents are the key factors affecting the sensitivity and reproducibility of test strips for measuring glucose concentration. For example, 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA) was employed as the chromogen to replace phenol in the detection of high-density cholesterol (Trinder & Webster, 1984), and the sensitivity was improved by 4–5 times, and the molar absorptivity increased from 6000 to 29,400. TBHBA was also employed in the measurement of high-density lipoprotein cholesterol (HDL-C) (Moshides, 1988) and free haemoglobin in the cerebrospinal fluid. Cui et al. have reported that the dosage of chromogen reagents could be reduced to 1/6 by using TBHBA in blood glucose testing (Cui, Yao, & Li, 1992). It has also been reported that TBHBA was used as the chromogen reagent in measuring the glucose concentration in saliva, but requiring a charge coupled device (CCD) as the detector (Liu, Wang, Zuo, & Pu, 2006).

In this study, we aimed to develop a paper based test strip for quick and easy blood glucose measurement using TBHBA as the chromogen reagent. The glucose concentration can be read directly from the color intensity of the paper strips, based on a color-glucose concentration chart, but without the need of an electronic device.

2. Experimental

2.1. Materials and instruments

The paper substrate used for preparing the test strip was quantitative filter paper with medium porosity from Fisher Scientific (Q8 No. 09-790E). Gelatin was purchased from Fluka. Glucose oxidase (from Aspergillus niger Type X-S, lyophilized powder), peroxidase (from horseradish Type VI, essentially salt free, lyophilized powder, 250–330 units/mg solid), 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA, 97%), 2,4-dichlorophenol (DCP, 99%), 3,3′,5,5′-tetramethyl-benzidine (TMB, 99%) and 4-aminoantipyrine (4-APP) were purchased from Sigma–Aldrich. Other chemicals were reagent grades from Sigma–Aldrich. Double distilled water was used for preparing the chemicals.

A Genesys 10-s UV–Vis spectrophotometer (Thermo Electro Co, USA) was used to determine the absorbance of the solution. A Datacolor ELREPHO 4230 spetrophotometer was used for the measurement of the reflectance of the test strips.

2.2. Methods and procedures

The filter paper was pre-extracted with acetone in a Soxhlet extractor for 4 h. The extracted filter paper was dried at room temperature in a fume hood, and then cut into 4.0 cm × 2.5 cm strips. 1.2 ml of 5% gelatin, 0.25 ml enzyme (16.2 mg glucose oxidase and 1.2 mg peroxidase per 10 ml in double distilled water, denoted as C_{EM}), 1.05 ml of citric acid buffer solution, 2.0 ml of TBHBA (5 mg/ml), 0.5 ml 4-APP (20 mg/ml) were mixed in a 50-ml glass vial to make a soaking solution. Then, the pre-cut paper strips were soaked with the solution for 5 min and dried at room temperature. The prepared test strips were sealed in a plastic bag and stored in dry place.

For the testing experiments with the prepared paper based test strips, 5 μl of glucose solution of various concentrations were dropped onto the prepared test strips with a 20-μl micro syringe. A circular red spot of 10 mm in diameter was formed on the test strip in about 1 min. The color intensity of the red spot was then determined by measuring its diffusive reflectance at 510 nm with a 9 mm diameter aperture adapter on a Datacolor ELREPHO spectrophotometer. The lower the diffusive reflectance at 510 nm, the stronger the intensity of the red spot on the test strips. For each sample, five replicates were conducted and the average result was reported.

Table 1
Comparison of the performance of TBHBA, TMB and DCP, as chromogen agents for the resulting paper based glucose test strips.

Chromogen agent	TBHBA	TMB	DCP
Solubility in water at pH 4–5	High	Low	Low
Color formed upon reaction with glucose	Red	Bluish green	Pink
Response (minimum ΔR at 1.08 g/l glucose concentration)	−22.6	−5.3	−6.7
Response time (time to minimum ΔR)	1 min	2 min	3 min
Repeatability (relative standard deviation)	3%	10%	10%
Storage stability of the test strips (at 3 °C)	>3 month	>3 month	<1 month

The change in reflectance was calculated using Eq. (1):

$$\Delta R = R_c - R_0 \quad (1)$$

In Eq. (1), R_0 and R_c are the reflectance of a paper test strip before and after reaction for 2 min with a sample glucose solution of concentration c in g/l.

The preparation of the paper testing strips using DCP and TMB as the chromogen agent followed the same as above, except that DCP/APP or TMB was used, instead of TBHBA/APP. For DCP/APP and TMB, the color intensity of the paper testing strips was determined at 550 nm and 685 nm, respectively.

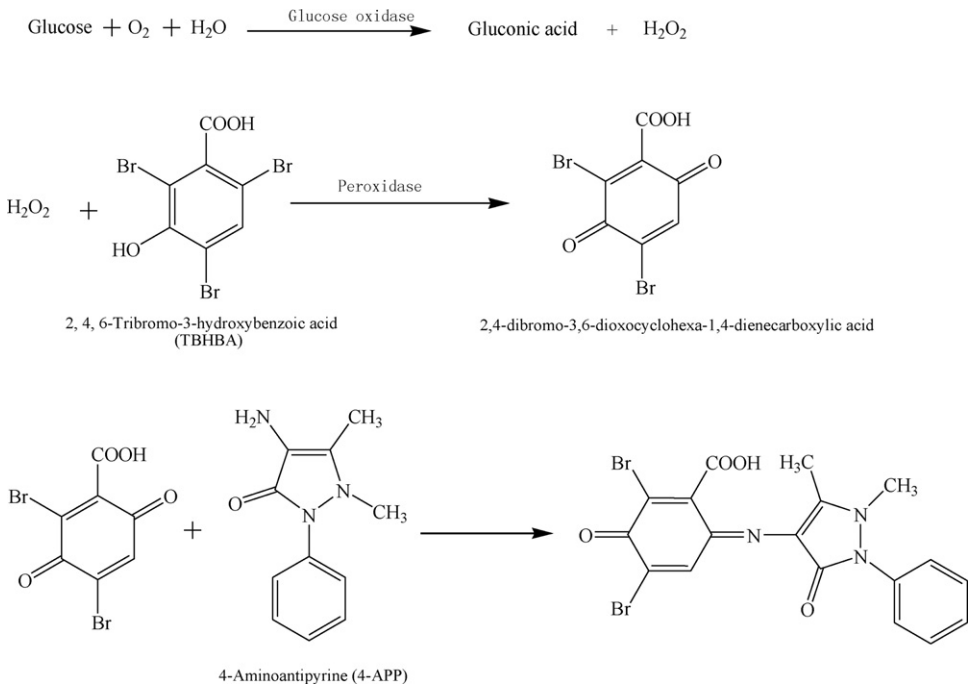
3. Results and discussion

3.1. Chromogen agents

The paper based glucose test strips developed had three key components, namely chromogen, enzymes and gelatin. They were prepared in a mixture, which was then applied to a paper substrate by soaking/dipping. For industrial applications, this can be done readily using a surface application set-up, such as sizing press, in the papermaking process.

Glucose oxidase and peroxidase are the commonly used enzymes to catalyze the reaction of glucose with the chromogen agent. These enzymes work in a narrow pH range of 4–5. The function of gelatin is to stabilize and enhance the catalytic activity of the enzymes, and to control the diffusion rate of sample solution to match the overall color-forming process. Identification of an appropriate chromogen agent is one of the critical steps in the development of paper test strips for measuring glucose concentrations. There are different chromogen agents available, but most of them are generally not suitable for paper strips. For example, DCP and TMB have too low solubility in water (e.g. DCP: 0.44 g/l at pH 5; TMB: 0.075 g/l at pH 5) in the pH range interested in this study. In contrast, TBHBA has good water solubility: 90 g/l at pH 5. More importantly, we found that TBHBA can be attached/fixed firmly onto the cellulose fibers of the paper substrate via charge interaction, due to the fact that cellulose fibers bear negative charges while TBHBA/4-APP do have positive charges.

Table 1 compares the performance of TBHBA as the chromogen agent, with those of TMB and DCP, and the paper test strips were prepared by the 1-step method, as described in Section 2. These three chromogens display different color in reaction to glucose: red, pink and bluish green for TBHBA, DCP and TMB, respectively. TBHBA loaded test strips had very good response in terms of visual color intensity to the glucose concentration, with good repeatability and stability. The color intensity was measured as the differential reflectance (ΔR) at the peak absorption wavelength (510, 535 and 665 nm for TBHBA, TMB and DCP, respectively). The higher



Scheme 1. The reaction mechanism of glucose with TBHBA and 4-APP.

the color intensity, the lower the ΔR, due to the light absorption of the colored substance developed from the reaction of the glucose–chromogen–APP system. The response of the TMB loaded test strips was much lower compared with that of the TBHBA loaded test strips, and the repeatability of the testing results was also not as good, probably due to its low water solubility that caused uneven distribution of TMB on the paper. DCP was found to be unsuitable for the paper based glucose test strips. As shown in Table 1, DCP gave low response with poor testing repeatability and short shelf life to the paper based glucose test strips. Among the three chromogen agents we have investigated, TBHBA appears to be the best suited for our purpose. The reaction mechanism of glucose with TBHBA and 4-APP was shown in Scheme 1 (Cui et al., 1992). In the first step, glucose reacts with oxygen and water to produce hydrogen peroxide. Subsequently, the formed hydrogen peroxide will react with TBHBA to produce 2,4-dibromo-3,6-dioxocyclohexa-1,4-dienecarboxylic acid, which in turn reacts with 4-APP to yield a compound in red color (Cui et al., 1992).

When the TBHBA/enzymes/gelatin mixture was applied to the original filter paper, we noticed that a light red color developed on the test strips, probably due to contaminants of low molecular weight reducing carbohydrates such as sugars and starch, which are present in the paper. This background color had absorption in the same range (450–550 nm) as the sample, causing decreased sensitivity of the test strips. However, we found that an acetone pre-extraction of the filter paper before the application of TBHBA/enzyme/gelatin mixture can effectively resolve this problem.

Fig. 1 shows the visual color changes of the paper based test strips prepared with the TBHBA/enzyme/gelatin mixture on the pre-extracted filter paper, in response to the glucose concentration of the samples. It can be seen that a red color spot of about 10 mm in diameter was formed on the test strip when a sample drop (5 μl) was applied, and the red color intensity increased with the increasing glucose concentration. It is evident that the prepared test strips had good response to the change in the glucose concentration.

The diffusive reflectance of the red spot in Fig. 1 was scanned in the visible light wavelength range on an ELREPHO spectrophotometer meter with a 9 mm aperture. Differential reflectance (ΔR) was obtained from the difference of the diffusive reflectance spectra of the paper test strip before and after testing using the prepared test strip. As shown in Fig. 2, the differential reflectance spectra indicated an absorption peak at about 510 nm due to the formation of red color. More importantly, ΔR at 510 nm increased with increasing glucose concentration in the sample solution, which can be used to establish a correlation between ΔR and the sample glucose concentration.

3.2. Enzymes and gelatin

In addition to the chromogen agent, enzymes (glucose oxidase and peroxidase) and gelatin are also needed to the preparation of the paper based glucose test strips. The enzymes catalyze the oxidation of glucose, while gelatin protects the enzymes from deactivation by forming complexes with glucose (Lourenco et al., 2011). The activity of these enzymes has significant effect on the sensitivity and reproducibility of the test strips for the determination of glucose concentrations. Glucose oxidase and peroxidase are stable when stored at low temperature (−20 °C for glucose oxidase and 3 °C for peroxidase). However, in aqueous solution, the enzymes can lose their activity easily even at room temperature.

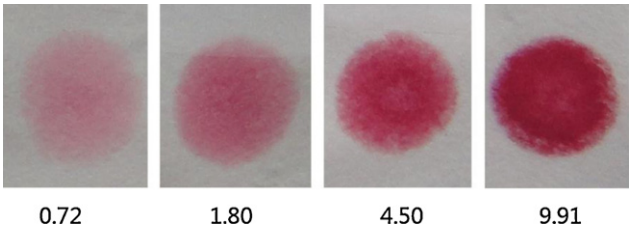


Fig. 1. Visual color intensity change in response to the change in glucose concentration of the paper test strips loaded with TBHBA (The pictures were taken with a digital camera 2 min after the sample glucose solutions were applied to the test strips. The numbers below were the glucose concentrations in mg/ml of the sample solutions).

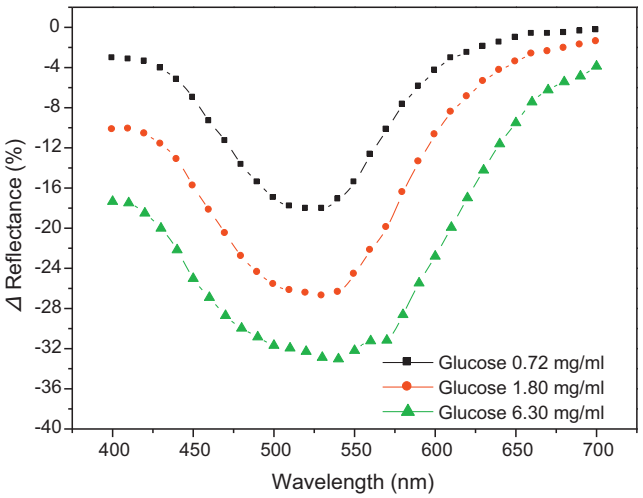


Fig. 2. Differential reflectance spectra of the paper test strips loaded with TBHBA upon reaction with sample glucose solutions of different concentrations.

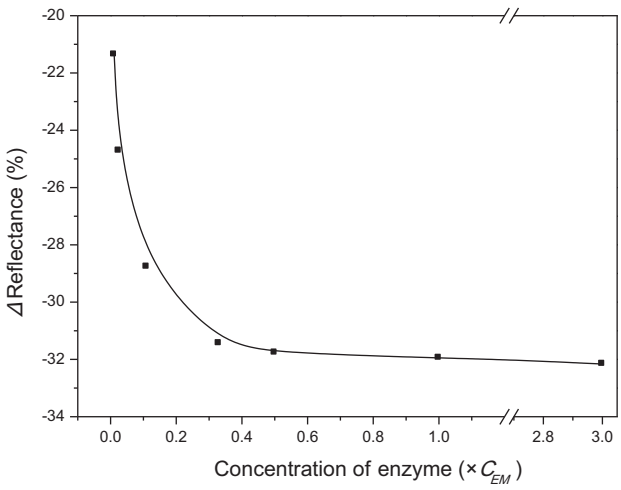


Fig. 3. Effect of enzyme dosage on the ΔR after reaction with a sample solution of 6.30 g/l glucose concentration (gelatin amount: 1.2 ml, 5%; totally solution volume: 5 ml).

We found that in the process of preparing the paper based glucose test strips, a significant amount of the enzyme activity of the glucose oxidase and peroxidase were lost even the process was performed at room temperature (about 23 °C). To solve this problem, we used citric acid as the pH buffer to control the pH in the range of 4–5, and used gelatin to stabilize the enzymes in the preparation of the paper based glucose test strips. Gelatin molecules contained a large number of hydroxyl groups that can form hydrogen bonds with the amino and carboxylic groups of the enzymes, thus inhibiting its spontaneous or heat-induced deactivation. A number of publications have reported that the addition of polymers, such as ethylcellulose, polyethyleneglycol, polyvinylpyrrolidone, polystyrene, hydrolyzed polyacrylonitrile and polyoxyethylene can enhances the stability of the enzymes (Alfani, Cantarella, Cirielli, & Scardi, 1984; Greco & Gianfreda, 1985; Leiriao, Fonseca, Taipa, Cabral, & Mateus, 2003; Srirangsan, Kawai, Hamada-Sato, Watanabe, & Suzuki, 2011). It has been proposed that the interaction between the enzyme and polymer results in formation of complexes that improve enzyme stability (Lane et al., 2011; Lee et al., 2010). The effect of polyoxyethylene (POE) on the activity and thermal stability of some enzymes is well known: the improved thermal stability is attributed to the interaction between the enzyme and POE (Godjevargova, Vasileva, & Letskovska, 2003). A more recent study showed that hydrolyzed polyacrylonitrile (HPAN) can offer superior stabilizing effect to glucose oxidase due to the interaction between the carboxylic groups and amino groups of HPAN with those of the enzymes (Godjevargova et al., 2003). With citric acid and gelatin as stabilizer in the preparation paper test strips, the enzyme dosages can be decreased while the sensitivity is maintained. As shown in Fig. 3, the maximum sensitivity was obtained at about 0.4 times C_{EM}.

In addition to stabilizing the enzymes, another function of gelatin was to control the penetration/diffusion rates of a sample solution on the paper test strips to match the colored adduct formation process, in order to obtain a uniform colored spot on the test strip for the maximum sensitivity and repeatability. We determined the effect of gelatin dosage on the formation of the colored spot. When the gelatin dosage was too low, the liquid penetrated/diffused faster than the colored compound formation process, resulting in lower color intensity in the center portion and higher color intensity on the edge. On the other hand, if the gelatin dosage was too high, the liquid could not spread uniformly to form a circular shape. Under the conditions of this study, a range of 4–5 g/m^2 gelatin was found to be a good compromise, in terms of color uniformity, sensitivity and repeatability.

Table 2
Effect of elapse time on the response of the paper test strips measured as the ΔR after reacting with a sample solution of 0.72 mg/l glucose concentration.

Elapse time	ΔR	Elapse time	ΔR
30 s	−13.29	20 min	−18.35
1 min	−18.33	40 min	−18.36
2 min	−18.33	60 min	−18.38
3 min	−18.33	80 min	−18.42
10 min	−18.33	100 min	−18.45

3.3. Response time, calibration curve and shelf life of the paper based test strips

The color development of the prepared paper based test strips to glucose was fast. As seen in Table 2, the ΔR reached minimum in about 1 min, indicating that the overall color formation process to generate the colored adducts can be completed within 1 min. Equally important is the fact that the developed color is stable, because the color intensity did not change over a relatively long period of time (e.g. 100 min).

In Fig. 4, the ΔR was plotted against the logarithm of the glucose concentration to establish a calibration curve based on the experimental data obtained. It can be seen that there was a good correlation between the ΔR and the glucose concentration, with a regression correlation coefficient of 0.9866.

When stored in a cool/dark place, the prepared paper test strips was quite stable. As shown in Table 3, the results of the test strips

Table 3
Effect of storage time on the response of the paper test strips measured as the ΔR after reacting with a glucose solution of 4.5 g/l in concentration.

Storage time	Storage temperature		
	3 °C	25 °C	50 °C
6 h	−31.95	−31.98	−14.76
1 days	−31.98	−31.92	−10.56
2 days	−31.92	−31.95	−1.14
3 days	−31.95	−31.86	–
14 days	−31.86	−31.86	–
1 month	−31.89	−31.83	–
2 months	−31.83	−31.86	–
3 months	−31.80	−31.65	–

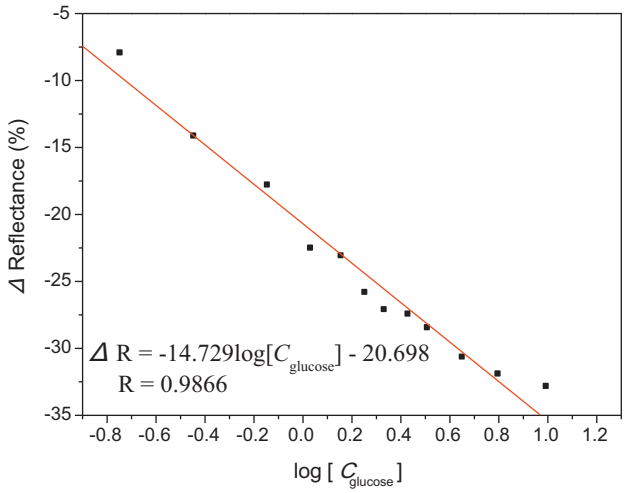


Fig. 4. Correlation between the ΔR of the test strip and the log of the glucose concentration.

remained constant when tested in a 3-month period when the storage temperature was at 3 or 25 °C. However, at a higher storage temperature (e.g. 50 °C), the test strips lose their function quickly, which is apparently due to the thermal-induced deactivation of the enzymes (Altikatoglu, Basaran, Arioz, Ogan, & Kuzu, 2010).

4. Conclusions

Paper based glucose test strips were successfully prepared using 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA) as the chromogen agent, glucose oxidase and peroxidase as the enzyme catalysts, and gelatin as the enzyme stabilizer. The results indicated that TBHBA is superior to DCP or TMB as the chromogen. The developed red color intensity, which was measured as the differential diffusive reflectance of the test strip at 510 nm, was correlated to the glucose concentration of the sample solutions in a range of 0.18–9.91 mg/ml. When stored in a cool/dark place, the prepared paper test strips was stable. This paper strip has high sensitivity and may be a useful tool to monitor the glucose concentration in blood.

References

Alemdar, A., & Sain, M. (2008). Isolation and characterization of nanofibers from agricultural residues – Wheat straw and soy hulls. *Bioresource Technology*, *99*, 1664–1671.

Alfani, F., Cantarella, M., Cirielli, G., & Scardi, V. (1984). The use of synthetic-polymers for preventing enzyme thermal inactivation. *Biotechnology Letters*, *6*, 345–350.

Allemann, S., Houriet, C., Diem, P., & Stettler, C. (2009). Self-monitoring of blood glucose in non-insulin treated patients with type 2 diabetes: A systematic review and meta-analysis. *Current Medical Research and Opinion*, *25*, 2903–2913.

Altikatoglu, M., Basaran, Y., Arioz, C., Ogan, A., & Kuzu, H. (2010). Glucose oxidase-dextran conjugates with enhanced stabilities against temperature and pH. *Applied Biochemistry and Biotechnology*, *160*, 2187–2197.

Chen, Y. Y., Wu, Y. T., Wu, C. C., Wu, S. M., & Jaw, F. S. (2004). Development of wireless blood glucose meter and diabetes self-management system. *Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, *26*, 3384–3386.

Clar, C., Barnard, K., Cummins, E., Royle, P., Waugh, N., & Assessmen, A. H. T. (2010). Self-monitoring of blood glucose in type 2 diabetes: Systematic review. *Health Technology Assessment*, *14*, 1+.

Cui, Y. L., Yao, G. L., & Li, L. (1992). Determination of blood glucose using 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA) as the chromogen agent. *Journal of Clinical Laboratory Science*, *10*, 24–25.

Dunn, T. C., Eastman, R. C., & Tamada, J. A. (2004). Rates of glucose change measured by blood glucose meter and the GlucoWatch Biographer during day, night, and around mealtimes. *Diabetes Care*, *27*, 2161–2165.

Fang, Y. J., Gao, Z. X., Yan, S. L., Wang, H. Y., & Zhou, H. Y. (2005). A dip-and-read test strip for the determination of nitrite in food samples for the field screening. *Analytical Letters*, *38*, 1803–1811.

Fonseca, V., Foyt, H. L., Shen, K., & Whitcomb, R. (2000). Long-term effects of troglitazone – Open-label extension studies in type 2 diabetic patients. *Diabetes Care*, *23*, 354–359.

Godjevargova, T., Vasileva, N., & Letskovska, Z. (2003). Study of the thermal stability of glucose oxidase in the presence of water-soluble polymers. *Journal of Applied Polymer Science*, *90*, 1393–1397.

Greco, G., & Gianfreda, L. (1985). The use of synthetic-polymers for preventing enzyme thermal inactivation – Some comments. *Biotechnology Letters*, *7*, 65–68.

Griffey, M. A., Wilhite, T. R., Heacook, J., & Dietzen, D. J. (2007). Clinical evaluation of the Bayer Ascensia (R) Contour (R) blood glucose monitoring system in neonates. *Clinical Chemistry*, *53*, A216–A217.

Hu, J. (2009). The evolution of commercialized glucose sensors in China. *Biosensors & Bioelectronics*, *24*, 1083–1089.

Huang, B., Kang, G. J., & Ni, Y. H. (2005). Electrically conductive fibre composites prepared from polypyrrole-engineered pulp fibres. *Canadian Journal of Chemical Engineering*, *83*, 896–903.

Huang, B., Kang, G. J., & Ni, Y. (2006). Preparation of conductive paper by in situ polymerization of pyrrole in a pulp fibre system. *Pulp & Paper – Canada*, *107*, 38–42.

Jahan, M. S., Saeed, A., He, Z. B., & Ni, Y. H. (2011). Jute as raw material for the preparation of microcrystalline cellulose. *Cellulose*, *18*, 451–459.

Jday-Daly, I., Augereau-Vacher, C., De Curraize, C., Fonfrede, M., Lefevre, G., Lacour, B., et al. (2011). Multicenter evaluation of the reliability of five blood glucose monitoring systems. *Annales De Biologie Clinique*, *69*, 55–61.

Johnson, B. M., Fry, M. M., Flatland, B., & Kirk, C. A. (2009). Comparison of a human portable blood glucose meter, veterinary portable blood glucose meter, and automated chemistry analyzer for measurement of blood glucose concentrations in dogs. *Javma-Journal of the American Veterinary Medical Association*, *235*, 1309–1313.

Kong, F., & Ni, Y. (2009). Determination of Cr(VI) concentration in diluted samples based on the paper test strip method. *Water Science and Technology*, *60*, 3083–3089.

Kong, F. G., & Ni, Y. H. (2009). Development of cellulosic paper-based test strips for Cr(Vi) determination. *Bioresources*, *4*, 1088–1097.

Kuo, C. Y., Hsu, C. T., Ho, C. S., Su, T. E., Wu, M. H., & Wang, C. J. (2011). Accuracy and precision evaluation of seven self-monitoring blood glucose systems. *Diabetes Technology & Therapeutics*, *13*, 596–600.

Lane, S. M., Kuang, Z. F., Yom, J., Arifuzzaman, S., Genzer, J., Farmer, B., et al. (2011). Poly(2-hydroxyethyl methacrylate) for enzyme immobilization: Impact on activity and stability of horseradish peroxidase. *Biomacromolecules*, *12*, 1822–1830.

Langer, O., Yogev, Y., Xenakis, E. M. J., & Rosenn, B. (2005). Insulin and glyburide therapy: Dosage, severity level of gestational diabetes, and pregnancy outcome. *American Journal of Obstetrics and Gynecology*, *192*, 134–139.

Lee, E. H., Tsujimoto, T., Uyama, H., Sung, M. H., Kim, K., & Kuramitsu, S. (2010). Enhancement of enzyme activity and stability by poly(gamma-glutamic acid). *Polymer Journal*, *42*, 818–822.

Lehmann, R., Kayrooz, S., Greuter, H., & Spinas, G. A. (2001). Clinical and technical evaluation of a new self-monitoring blood glucose meter: Assessment of analytical and user error. *Diabetes Research and Clinical Practice*, *53*, 121–128.

Leiriao, P. R. S., Fonseca, L. J. P., Taipa, M. A., Cabral, J. M. S., & Mateus, M. (2003). Horseradish peroxidase immobilized through its carboxylic groups onto a polyacrylonitrile membrane – Comparison of enzyme performances with inorganic beaded supports. *Applied Biochemistry and Biotechnology*, *110*, 1–10.

Liu, L. L., Wang, H. Z., Zuo, S. Y., & Pu, X. Y. (2006). Automatic enzymic assay of saliva glucose with 2,4,6-tribromo-3-hydroxybenzoic acid as chromogen on biochemical analyzer. *Chongqing Yixue*, *35*, 1552–1553, 1556.

Lockwood, C., Xue, Y. F., & McArthur, A. (2010). Self-monitoring of blood glucose in type 2 diabetes mellitus: A systematic review of economic evidence. *Journal of Advanced Nursing*, *66*, 1931–1936.

Lourenco, N. M. T., Osterreicher, J., Vidinha, P., Barreiros, S., Afonso, C. A. M., Cabral, J. M. S., et al. (2011). Effect of gelatin-ionic liquid functional polymers on glucose oxidase and horseradish peroxidase kinetics. *Reactive & Functional Polymers*, *71*, 489–495.

Lyon, M. E., Baskin, L. B., Braakman, S., Presti, S., Dubois, J., & Shirey, T. (2009). Interference studies with two hospital-grade and two home-grade glucose meters. *Diabetes Technology & Therapeutics*, *11*, 641–647.

Moshides, J. S. (1988). Enzymic determination of the free cholesterol fraction of high-density lipoprotein in plasma with use of 2,4,6-tribromo-3-hydroxybenzoic acid. *Clinical Chemistry (Winston-Salem, NC)*, *34*, 1799–1804.

Sonmez, A., Yilmaz, Z., Uckaya, G., Kilic, S., Tapan, S., Taslipinar, A., et al. (2010). The accuracy of home glucose meters in hypoglycemia. *Diabetes Technology & Therapeutics*, *12*, 619–626.

Srirangsan, P., Kawai, K., Hamada-Sato, N., Watanabe, M., & Suzuki, T. (2011). Stabilizing effects of sucrose–polymer formulations on the activities of freeze-dried enzyme mixtures of alkaline phosphatase, nucleoside phosphorylase and xanthine oxidase. *Food Chemistry*, *125*, 1188–1193.

Towfigh, A., Ronnanova, M., Weinreb, J. E., Munjas, B., Suttorp, M. J., Zhou, A., et al. (2008). Self-monitoring of blood glucose levels in patients with type 2 diabetes mellitus not taking insulin: A meta-analysis. *American Journal of Managed Care*, *14*, 468–473.

Trinder, P., & Webster, D. (1984). Determination of HDL-cholesterol using 2,4,6-tribromo-3-hydroxybenzoic acid with a commercial CHOD-PAP reagent. *Annals of Clinical Biochemistry*, *21*(Pt 5), 430–433.

Weise, A., Scherze, J., Grenningloh, M., Schondorf, T., Hauth, W., Lipfert, B., et al. (2008). Comparative evaluation of the stada glucocheck monitoring device. *Diabetes Stoffwechsel Und Herz*, *17*, 25–31.

Welschen, L. M. C., Heine, R. J., Bloemendal, E., Stalman, W. A. B., Nijpels, G., Bouter, L. M., et al. (2005). Self-monitoring of blood glucose in patients with type 2 diabetes who are not using insulin: A systematic review. *Diabetes Care*, *28*, 1510–1517.