Noninvasive measurement of pH in platelet concentrates with a fiber optic fluorescence detector

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BACKGROUND: Stored platelets (PLTs) are metabolically active, resulting in a decrease of pH during storage. The pH of PLT concentrates (PCs) is recognized as a measure of quality, and pH limits are set by regulatory bodies. A pH sensor was built into a PLT storage container, and the feasibility of testing pH using a noninvasive fluorescent measurement method was evaluated.

STUDY DESIGN AND METHODS: A citrated polyvinylchloride (PVC) PLT storage container with pH sensor insert was made and evaluated for biocompatibility during PLT storage and on pH reading accuracy, reproducibility, and durability. A noninvasive fluorescence reader was tested versus syringe-based sampling and subsequent measurement with a blood gas analyzer (BGA). The effect of interfering substances in plasma on the accuracy of this optical measurement was tested. Calibration and accuracy of the pH sensor were determined in both phosphate-buffered saline and in PCs.

RESULTS: The citrated PVC storage container with pH sensor insert showed good storage properties for 300 mL of pooled buffy coat PLTs in plasma over 7 days. The pH sensor was easy to use and tracked pH22 in the range of 6.2 to 7.8 over 11 days of storage. Accuracy in PCs was 0.08 pH units measured at 22°C when calibrated against a BGA.

CONCLUSION: The storage container with integrated pH sensor and noninvasive reader allows pH of PCs to be tracked over time in a noninvasive manner.

ABBREVIATIONS: BGA(s) = blood gas analyzer(s); PC(s) = platelet concentrate(s); pKa = acid dissociation constant.

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self-calibrating pH electrodes in a closed system, which also measure dissolved oxygen and carbon dioxide. BGAs are very accurate (precision within 0.02 pH units), but require constant calibration, careful sample handling with syringes, and rapid analysis. The sampling technique is critical and can give rise to variable pH readings in different laboratory settings. In addition, different analyzers have been shown to give different pH values in PLT-rich plasma, with variations of up to 0.14 pH units. Therefore, even BGA measurements must be controlled and the type of analyzer precisely described.

A method that does not require invasive sampling to measure pH in PC would have great advantages. Optical sensors for measuring pH are well known. Certain aromatic organic compounds change color with pH and can be immobilized on solid supports to form “pH paper.” These colorimetric indicators are easy to use, but color changes can be difficult to distinguish accurately and can be masked by colored analytes (interfering substances). Fluorescent pH indicators have also been immobilized on solid supports and generally are more sensitive in comparison to the simple colorimetric indicators. The improved sensitivity of fluorescent indicators allows the solid support to be miniaturized and has been used as an advantage in the development of fiber optic sensor devices for measuring pH, CO₂, and O₂ parameters in blood.

Especially valuable for optical pH measurement are fluorescent dyes where the ratio of emission intensity at two distinct wavelengths varies, changing with pH. Dual-emission pH-sensitive dyes must be carefully chosen since they are most accurate at their acid dissociation constant (pKₐ). Toward this end, we developed a dual-emission dye with pKₐ ~ 6.5 and immobilized it to solid supports to prepare a miniaturized pH sensor for PC. This article presents the preclinical studies related to development of this noninvasive, pH-sensing PLT storage container. The pH can easily be measured at any time during storage with the system and the integrated sensor has no effect on the in vitro properties of PC. Therefore, instead of quality control (QC) on a limited number of PCs after outdating, it will be easy to check all PCs and to identify low-pH PLT units during the storage/distribution process up to the point of transfusion into patients.

**MATERIALS AND METHODS**

**Materials**

Annexin 5A–fluorescein isothiocyanate (FITC) was from VPS-Diagnostics (Hoeven, The Netherlands) and CD62P-FITC was from Beckman Coulter (Woerden, The Netherlands). Glutaraldehyde 25% was from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

**Preparation of PLT storage containers with pH sensor inserts**

Structure and synthesis of the ratiometric fluorescent dye, conjugation to human serum albumin (HSA), immobilization of the protein conjugate to nitrocellulose membranes, and the configuration of the sensor disks and plastic holders has been described elsewhere. Briefly, the carboxylic acid form of the fluorescent dye was activated with carbodiimide and reacted with lysine residues in HSA at pH 8.5. The labeled HSA (~3 fluors : HSA molecule) was purified by ultrafiltration and immobilized to nitrocellulose membrane disks in phosphate-buffered saline (PBS; pH 7.4). After thorough washing, the 90-mm-diameter disks were dried and used to punch hundreds of 3-mm-diameter sensor disks. A single 3-mm sensor disk was held in a hollow tip and trapped against the clear polycarbonate window of an injection-molded insert tube (Fig. 1). The window serves as a sterile barrier and allows light to pass in and out of the bag for the fluorescent readings. The polycarbonate membrane retainer ring and insert tube are attached together, pinching the disk in place. These three-piece pH inserts were either tested for sensor performance in open tube assays or incorporated into PLT storage containers prepared from polyvinylchloride (PVC) film with butyryl trihexyl citrate (BTHC). To allow insertion of the pH sensor, a 3-cm-long × 5-mm-inside-diameter opaque blue PVC sleeve was welded into the seam of the container. The containers were also equipped with 30-cm pigtail tubing, a sampling bag, and two twist-
Fig. 2. pH1000 fluorescence reader. To read pH, the pH-sensing PLT storage container is placed in the cradle and the sensor tube is clicked onto the probe.

off ports to allow pH measurement by standard (invasive) syringe-based methods. PLT storage containers with the pH sensor insert were packaged in gas-permeable outer pouches. The containers were ethylene oxide sterilized and CE marked (Council of Europe; approval for sale in Europe) for use in transfusion medicine as Class IIb medical devices.

**pH measurements by BGA**

pH was measured with a commercially available BGA (Rapidlab 860 or Bayer 348, Siemens Medical Solutions Diagnostics, Breda, The Netherlands) according to the manufacturer’s instruction. The BGAs were frequently calibrated (one point calibration every hour and at least daily a two-point calibration) and before use checked for performance with RapidQC Complete Levels 1, 2, and 3 (Siemens). All PLT storage containers used were equipped with sterile sample-site couplers to allow samples of PC to be removed with a syringe. pH measurement of the sample was at 37°C. If applicable, for conversion of pH_{37} values to pH_{22} values, the Severinghaus equation derived for whole-blood measurement was used.13

**pH measurement by noninvasive sensor method**

The containers equipped with the fluorescent pH sensor were tested using a fiber optic reader (BCSI pH1000, Blood Cell Storage, Inc. [BCSI], Seattle, WA) as shown in Fig. 2 and was operated using the method described in the BCSI pH1000 operator’s manual. The pH1000 reader is CE marked as a Class I in vitro diagnostic and the underlying technology is described elsewhere.11 The sensor insert in the storage container was gently slid over the fixed probe on the reader until the sensor window clicked into position against the probe face. The reader menu on the keypad was set for pH reading and fluorescent data were collected automatically by pushing the enter button. The green LED flashed and the emitted fluorescent signals at 568 and 600 nm were measured and the data transformed to give a pH value (either pH_{22} or pH_{37}) as described below. The actual pH measurement time is 0.3 second.

**Accuracy of pH sensor in standard buffers**

To evaluate the accuracy of pH measurement with the pH sensor inserts, storage containers with pH sensor inserts were equipped with sample-site couplers and filled with 15-mL volumes of PBS buffers over a range of pH (five bags at each pH; nominally 6.2, 6.5, 6.8, 7.0, 7.4, and 7.8). The containers were positioned so that the sensors were fully wet and pH was measured after 48 hours of soaking with the pH1000 reader.

**Calibration of the pH sensor for PLTs in plasma**

To create a lookup table for PCs in plasma that covers the desired pH range, expired (older than 5 days) apheresis PLTs from Puget Sound Blood Center (Seattle, WA) were sterile docked to BCSI storage containers from four different production lots. Each PLT unit was transferred via the pigtail tubing into a BCSI container, mixed well, and placed in a PLT incubator (Helmer, Noblesville, IN) to equilibrate for 1 to 4 hours. pH readings were performed after equilibration on both the BCSI pH1000 and a Bayer Rapidlab 348 BGA and then once daily on both instruments until readings were below the limit of detection of the BGA (pH 6.0).

**Effect of interfering substances in plasma on ratiometric fluorescence pH assay**

BCSI pH sensors were exposed to 1-mL samples of plasmas containing various types of potential interfering substances. Each plasma sample was acidified in open polypropylene tubes with variable amounts of hydrochloric acid to achieve a range of pH. Fluorescent ratio measurements were obtained with a prototype pH1000 reader after 1 hour of soaking. Blood gas pH was measured for the same samples at the same time. Four different color plasma types were evaluated: 1) normal clear gold plasma, 2) creamy white plasma from a lipemic donor, 3) greenish plasma from a female donor on contraceptives, and 4) pink plasma containing lysed red blood cells (RBCs).

**Preparation of PCs**

PCs for the in vitro quality studies were prepared from overnight (15-20 hr) held whole blood via the pooled buffy coat method, essentially as described before.14 Pools from five buffy coats were centrifuged and the PLT-rich supernatant was transferred across a leukoreduction
filter (Compostop CS, Fresenius HemoCare, Emmer-Compascuum, The Netherlands) to an empty 1.3-L PVC-BTHC storage container (Compoflex F730, Fresenius HemoCare) or 1.3-L PVC-BTHC storage container with pH sensor (BCSI) with the use of a Compomat G4 (Fresenius HemoCare). For some paired experiments 2 units were combined and split over a Compoflex F730 and a BCSI container.

Sampling and measurement of PCs during storage

The PCs were stored at 22 ± 2°C horizontally shaking with one cycle per second (PLT incubator, Helmer). Samples were taken with a syringe via a sampling-site coupler (Codan, Deventer, The Netherlands) under aseptic conditions on Days 2, 4, 7, and 9 of shelf life (meaning Days 1, 3, 6, and 8 of PC storage, because PCs were prepared after 15- to 20-hr storage of whole blood) and analyzed for various PLT quality parameters. Additional samples for pH determination were taken on Days 3, 8, 10, 11, and 12 of shelf life.

Measurement of PLT parameters

PLT counts were measured with a commercially available hematology system (Advia 2120, Siemens Medical Solutions Diagnostics) as described by the manufacturer. PLT morphology was analyzed by judging the swirling score15 and by the microscopic characterization of the different forms of PLTs, that is, discoid, discoid with dendrites, balloons, or spheres in fixated (1% glutaraldehyde) samples, resulting in a so-called Kunicki score.16

The expression of the activation antigen CD62P and the binding of annexin 5A was measured essentially as described earlier.17 The mitochondrial membrane potential was determined as described by Verhoeven and colleagues18 with the fluorescent dye JC-1 (Molecular Probes, Eugene, OR) and analysis on a flow cytometer (FACScan, BD Biosciences, Franklin Lakes, NJ). The JC-1 signal was then calculated as ratio of the FL2 and FL1 fluorescence (FL2/FL1).

The amount of total nucleotides was analyzed in perchloric acid extracts of PLTs as previously described.19 The neutralized samples were kept at −70°C until high-performance liquid chromatography (HPLC) analysis. The nucleotides in the extracts were separated by an anion-exchange HPLC method. Column was a prepacked partisphere partisil-5 SAX cartridge (125 × 4.6 mm i.d., Whatman, Clifton, NY).

RESULTS

pH1000 fluorometer and sensor insert design

A dual-channel fiber optic fluorometer was designed and built to measure a pH-sensitive fluorescent sensor membrane in a PLT storage container. The membrane is in contact with PC and easily interrogated through a viewing window in the container. The fluorescence-based pH measurement method requires no sampling of the container contents. The sensor is similar in size to a standard port of a blood bag (Fig. 1), but has a 1/8-inch-diameter hole that the fiber optic probe of the pH1000 slides into. The tip of the insert serves as a retaining ring to trap the membrane disk against a leak-proof plastic window, inside the PLT storage container. To ease pH measurements, a device was built consisting of a tray to put the container on and a keypad with LCD display for communication (Fig. 2). The gently curved platform inclines the container so that the sensor remains wet and positions the sensor holder in front of the fiber optic sample probe. The fluorometer contains a pulsed LED excitation source (500-540 nm) and measures two emission wavelengths (568 and 600 nm, both 10-nm bandwidth). The fluorometer contains a lookup table to convert fluorescence ratio into pH and shows the measured pH over the range 6.2 to 7.8 (at 22 or 37°C). The actual measurement data can also be transferred to a computer or blood bank information management system and via a bar code reader coupled to the unit identification number.

Accuracy of fluorescent pH sensor in PBS

The accuracy of the pH1000 system (reader and containers with pH sensor insert) in PBS buffer is shown in Fig. 3. The data were generated from a collection of 35 bags with incorporated pH sensors and filled with various PBS buffers. This assay shows fluorescence ratio data versus BGA pH and allows calculation of a line of best fit (a calibration curve). The 600-nm/568-nm fluorescence intensity ratio changes from 1.6 ratio units at pH 6.2 to approximately 3.6 ratio units at pH 7.8. A change in pH of 1.6 units corresponds to a change in fluorescence ratio units of 2.0; therefore, 1 ratio unit = 0.8 pH units. The sensor is clearly most precise from pH 6.2 to 6.4.
consistent with the pKₐ of the fluorescent dye and behavior of other fluorescence-based pH sensor assays. At pH 6.2, 1 standard deviation (SD) in the fluorescence assay was 0.025 ratio units (precision = 0.02 pH units). At higher pH the ratiometric assay was less precise (Fig. 3, x-axis). For example at pH 7.4, 1 SD is 0.1 pH units. Hence a precision of 0.1 pH units is measured in this assay over the range of pH 6.2 to 7.8. The intraassay variation for a single sensor’s reading at a given time is 0.005 ratio units at 1 SD. The interassay variation between sensors is, at 1 SD, on the order of 0.03 ratio units below pH 7.4 and 0.07 ratio units above pH 7.4. The interassay variation clearly determines the precision.

The standard device for calibration of the pH measurements was the Bayer Rapidlab BGA. Unlike the fluorescent pH sensor, precision was equally good for the BGA across the measurement range of pH 6.2 to 7.8 (Fig. 3, y-axis). The BGA self-calibrates every 20 to 60 minutes with two control buffers, pH 7.382 and pH 6.838, that are pumped through the electrode pH sensor. pH readings of four significant figures are displayed with the BGA but three significant figures are reliable (1 SD = 0.02 in standardized buffers, data not shown). By contrast, the pH sensor in the pH1000 system does not require control buffers, but simply uses the calibration curve in the form of a lookup table to translate fluorescent ratio units to pH.

**Calibration of pH sensor for PLTs in plasma**

To create a lookup table for the pH1000 system for the accurate conversion of fluorescence ratio into pH for PLTs in plasma, we used multiple measurements of PC during storage with the BGA. The calibration curve for PC (Fig. 4) was different than the calibration curve generated with standard PBS buffers (Fig. 3). It is not clear why there is a difference between these sample types, but it is clear that calibration for reading pH in PC must be done with the same matrix as the storage solution. The lookup table in the device is critical to the ultimate accuracy of the pH readings and is different for PC in plasma or plasma/additive solution mixtures.

**Effect of interfering substances**

The effect of colored impurities in human plasma on the fluorescence ratio assay was examined. The plasma of PCs can be lipemic, slightly red by presence of RBCs or free hemoglobin, or colored green by the presence of contraceptives. Worst-case samples of plasma were selected and artificially acidified. The effect of these impurities on the fluorescence ratio was not significant. Agreement between sensor fluorescence ratio and blood gas pH at 37°C was calculated by comparing the differences as described by Bland and Altman. This is shown in Fig. 5 and indicates a high accuracy (mean difference, 0.00) with a precision of 0.06 pH units at 1 SD.

**In vitro quality of pooled PLTs after storage in containers with pH sensor inserts**

In total 24 leukoreduced products were prepared, with 16 products stored in the container with integrated pH sensor (BCSI container; prototype version of the sensor) and 8 products in a standard approved storage container (Compoflex F730 container) to be used as control. Table 1 shows that the PCs in both types of container were very similar (all p values > 0.05). After leukoreduction, the number of WBCs was well below 1 ¥ 10⁶ in all units (as determined by fluorescent Nageotte counting). These units were used to measure changes in in vitro quality parameters during storage of PC in plasma. The storage performance of the new BCSI container with pH

![Fig. 4. Calibration curve of pH sensor in the BCSI container. The pH1000 fluorescent reader and pH sensor insert were calibrated using sterile BCSI containers filled with PC samples as described under Materials and Methods. The total number of fluorescent readings was 94.](image-url)

Fig. 5. Effect of interfering substances on fluorescence ratio pH assay. Various plasma units with possible interfering instances were artificially acidified with hydrochloric acid to a pH range and measured in open tube with inserted pH sensor. After equilibration, the fluorescence ratio and BGA measurement were obtained and for the various samples the difference between the measured pH (BCSI pH – blood gas pH) was plotted against the mean pH from the two methods.
Table 1. Composition of PCs in two different PLT containers*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>BCSI container</th>
<th>Compoflex F730 container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume PC</td>
<td>mL</td>
<td>0.144</td>
<td>0.144</td>
</tr>
<tr>
<td>PLT concentration</td>
<td>×10^9/mL</td>
<td>0.516</td>
<td>1.227 ± 127.2</td>
</tr>
<tr>
<td>Total PLTs</td>
<td>×10^9</td>
<td>0.646</td>
<td>415 ± 41.6</td>
</tr>
<tr>
<td>Total WBC</td>
<td>×10^9</td>
<td>0.447</td>
<td>0.58 ± 1.09</td>
</tr>
<tr>
<td>PLT recovery based on buffy coat pool</td>
<td>%</td>
<td>0.003</td>
<td>78 ± 4.9</td>
</tr>
<tr>
<td>Filtration time</td>
<td>sec</td>
<td>0.479</td>
<td>330 ± 41.0</td>
</tr>
<tr>
<td>Total PLTs in buffy coat pool</td>
<td>×10^9</td>
<td>0.115</td>
<td>509 ± 129.5</td>
</tr>
</tbody>
</table>

* Values are given as the mean ± SD; BCSI container n = 16 and F730 container n = 8. p Values are given for an unpaired t test.

Table 2. Quality parameters during storage of PC in two different PLT containers*

<table>
<thead>
<tr>
<th>Shelf life</th>
<th>Container</th>
<th>Kunicki score</th>
<th>% Discoid</th>
<th>Swirl</th>
<th>CD62P mean %</th>
<th>Annexin V mean %</th>
<th>JC-1 FL2/FL1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>BCSI</td>
<td>320 ± 11.4</td>
<td>60.4 ± 3.3</td>
<td>3.0 ± 0.1</td>
<td>6.3 ± 1.5</td>
<td>3.0 ± 1.6</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>F730</td>
<td>318 ± 10.4</td>
<td>60.0 ± 4.1</td>
<td>3.0 ± 0.1</td>
<td>6.5 ± 1.8</td>
<td>2.7 ± 0.8</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Day 4</td>
<td>BCSI</td>
<td>305 ± 13.2</td>
<td>54.4 ± 5.4</td>
<td>2.8 ± 0.3</td>
<td>9.6 ± 2.3</td>
<td>6.0 ± 1.6</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>F730</td>
<td>293 ± 18.1</td>
<td>48.8 ± 7.4</td>
<td>2.9 ± 0.2</td>
<td>9.6 ± 2.2</td>
<td>5.7 ± 1.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>BCSI</td>
<td>275 ± 14.0</td>
<td>41.7 ± 5.6</td>
<td>2.6 ± 0.3</td>
<td>14.7 ± 2.6</td>
<td>12.3 ± 2.4</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>F730</td>
<td>269 ± 13.5</td>
<td>40.0 ± 5.3</td>
<td>2.5 ± 0.4</td>
<td>11.7 ± 1.8</td>
<td>12.3 ± 3.0</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>Day 9</td>
<td>BCSI</td>
<td>246 ± 13.8</td>
<td>30.3 ± 5.5</td>
<td>2.0</td>
<td>17.4 ± 3.7</td>
<td>14.7 ± 3.4</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>F730</td>
<td>246 ± 21.0</td>
<td>30.6 ± 7.8</td>
<td>2.0</td>
<td>14.3 ± 3.1</td>
<td>15.0 ± 3.2</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SD; BCSI container n = 15 and F730 container n = 8. All p values for comparison between the containers were >0.05 in an unpaired t test.

Table 3. pH values during storage of PC in two different PLT containers*

<table>
<thead>
<tr>
<th>Day</th>
<th>pH1000†</th>
<th>BGA</th>
<th>F730 container, BGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.958 ± 0.139</td>
<td>7.021 ± 0.026</td>
<td>7.024 ± 0.040</td>
</tr>
<tr>
<td>2</td>
<td>7.123 ± 0.167</td>
<td>7.162 ± 0.033</td>
<td>7.154 ± 0.031</td>
</tr>
<tr>
<td>3</td>
<td>7.200 ± 0.177</td>
<td>7.174 ± 0.070</td>
<td>7.184 ± 0.035</td>
</tr>
<tr>
<td>4</td>
<td>7.235 ± 0.154</td>
<td>7.145 ± 0.105</td>
<td>7.177 ± 0.052</td>
</tr>
<tr>
<td>7</td>
<td>7.172 ± 0.190</td>
<td>7.081 ± 0.059</td>
<td>7.098 ± 0.049</td>
</tr>
<tr>
<td>8</td>
<td>7.147 ± 0.196</td>
<td>7.025 ± 0.070</td>
<td>7.044 ± 0.058</td>
</tr>
<tr>
<td>9</td>
<td>7.069 ± 0.155</td>
<td>6.879 ± 0.093</td>
<td>6.965 ± 0.071</td>
</tr>
<tr>
<td>10</td>
<td>6.973 ± 0.172</td>
<td>6.861 ± 0.092</td>
<td>6.873 ± 0.084</td>
</tr>
<tr>
<td>11</td>
<td>6.879 ± 0.205</td>
<td>6.775 ± 0.108</td>
<td>6.831 ± 0.086</td>
</tr>
</tbody>
</table>

* Values are given as pH0.5; mean ± SD; BCSI container n = 15 and F730 container n = 10.
† Combined values from three different prototype batches of pH sensor inserts.

sensor insert was compared to a standard container (Compoflex F730 container). Both PLT storage containers were made from PVC-BTHC and had a similar capacity for PLT storage. Table 2 shows that no significant differences (unpaired t-test; all p values >0.05) were found between the two containers with respect to in vitro PLT quality up to Day 9 of shelf life, as measured by swirling score, Kunicki score, percentage of discoid PLTs, percentage of PLTs positive for CD62P and annexin 5A, and mitochondrial activity depicted as FL2/FL1 ratio. During storage, the normal gradual decline for the in vitro parameters was found in both types of container. Over the whole storage period the PLT count remained constant in both containers (data not shown). The amount of glucose consumption was 0.888 mmol per 10^12 PLTs per day in the BCSI container and 0.852 mmol per 10^12 PLTs per day in the Compoflex F730 container, and both containers showed similar lactate production. The absolute amount of intracellular adenine nucleotides on Day 1 was also similar, with about 10% decrease for ATP and 25% for ADP from Day 2 to Day 7 in both storage containers. At the end of the storage period, all PCs were checked for sterility. One unit in a BCSI container was found to be contaminated, resulting in an aberrant pH profile (early decrease) and in vitro quality parameters at the end of storage and had therefore to be excluded from the analysis, because it would have prevented the evaluation of the possible effect of the pH sensor insert.

Accuracy of fluorescent pH sensor in PCs

The performance of a preproduction prototype pH sensor was measured in the units from the above studies on in vitro PLT quality on a daily basis. The pH was measured by BGA and/or the pH1000 system. Three different prototype batches of BCSI container were examined and the mean pH values measured are shown in Table 3 (first two columns). Good correlation was found for the BCSI container between the two pH values measured on a daily basis.
basis. The pH sensor continued to give readings for up to 12 days of shelf life in PC, indicating good stability of the fluorescent membrane (readings were stopped after 12 days). The changes in pH during storage of PLTs in the Compoflex F730 container as measured with the BGA were very similar to those found for the prototype BCSI containers (Table 3, last column). Figure 6 shows the comparison of BGA and pH1000 pH during storage of PC as measured with the final design of the pH1000 system as used for CE certification. The new sensor design and the final lookup table derived from the calibration curve in Fig. 4 resulted in accuracy in PC of 0.01 pH units with a 95% confidence interval [CI] and prone to sampling errors. We and others believe that there is an urgent need for standardized methods of pH measurement.

The pH1000 measurement system records pH without sampling by use of the noninvasive fluorescence assay. The ratiometric fluorescent dye used in this application has an ideal pk a (~6.5) for accurate measurement of the pH 6.2 to 6.4 lower-limit cutoff for acceptable PLT quality. Owing to the ratiometric approach, the system has minimal disturbance by interfering substances affecting the fluorescence excitation and emission intensity. Sterilization by ethylene oxide of the BCSI containers with pH sensor insert did not affect accuracy and precision (data not shown). The system is engineered in such a way that possible user errors are prevented as much as possible, although user errors will always happen. Use of the system as instructed will result in the claimed accuracy of pH readings at 95% confidence. The ratiometric approach is also making the system more robust compared to fluorescence emission measurements.

Albumin was chosen as a macromolecular linker to coat the surface of the porous substrate, passivating the surface to prevent fouling with other proteins or interfering substances. The 0.22-μm-pore-size nitrocellulose blot membranes were chosen as the porous substrate since these membranes are biocompatible and are designed to bind proteins avidly. Recombinant HSA (grown in yeast) was used in production to prevent the possibility of contamination from human- or animal-donated products. The other materials used in the PLT storage container with pH reading insert are common to blood bag manufacturing. The PVC-BTHC material is used in construction of similar containers like the Compoflex F730 container, which was used as a control for in vitro PLT storage performance. The two container types performed identically, indicating that the pH sensor had no effect on the in vitro quality of the stored PC in the container. The pH sensor membrane can be in contact with PC for at least 11 days with frequent measurements without lower accuracy or precision. The biocompatibility of the sensor materials was confirmed by lack of toxicity or sensitization in ISO-approved assays (basis for CE mark) and by thorough in vitro testing with PC.

Daily pH measurements recorded with both the pH1000 measurement system and a BGA with invasive sampling gave similar shape curves during storage of PC. As shown in Table 3, starting pH7 (Day 2 of shelf life) is approximately 7.0. As the stored PLT suspensions are slowly agitated in the incubator, dissolved carbon dioxide (carbonic acid) leaves the solution, causing an initial rise in pH that peaks on Day 3. After the dissolved carbon dioxide (from the donor’s metabolism) leaves the plasma, the effects of acidic components from metabolism of the stored PLTs become apparent. After Day 3, the pH of the stored PLTs slowly decreases as carbon dioxide and lactic
acid overwhelm the buffering capacity of the plasma. At the end of storage (allowed shelf life is 7 days in The Netherlands) the pH of the PLTs remained above pH 6.8. However, one of the factors known to influence pH is bacterial growth in PC. The pH1000 system has already been used to measure the effect of bacterial growth on the pH profile during storage of PC.23,24

Breathable PLT storage containers are made by various manufacturers and from various plastic films and may have variable pH profiles. In any event, noninvasive pH measurement using the pH1000 system was successful, and by choosing an opaque sleeve made from a material compatible with the bag film it is feasible to incorporate the same pH sensor in PLT containers made from other standard materials such as polyolefin25 or different PVC plasticizers such as diundecyl phthalate or trimethyl mellitate.25

In comparison to the invasive syringe-based sampling method required for the BGA, the pH1000 fluorometer was easy to use. After sliding the pH insert over the sample probe, a single button push reads pH with a flash of light. The instrument was designed with optical filters that were optimized for the excitation and emission wavelengths of the dyes and the ratiometric assay was shown to give accurate results in PBS buffer studies and in leukoreduced PCs in plasma.

In summary, the noninvasive pH1000 measurement system provides a rapid and accurate pH measurement for use in transfusion medicine. pH profiles during storage can be easily obtained with the pH1000 measurement system. Poor-quality PLT units would likely be discovered if noninvasive pH measurements were performed on all PLT transfusions. Just before PLT transfusions.

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CONFLICT OF INTEREST

M.W. Reed, S. Geelhood, L.M. Barker, and R. Pfalzgraf are all employed by Blood Cell Storage, Inc. (BCSI), the company that developed and sells the pH1000 device that is the subject of this article. P. Harris, as an employee of Micron Engineering, is a paid consultant of BCSI. D. de Korte, as an employee of Sanquin Blood Supply Foundation, is a member of the Scientific Advisory Board of BCSI.

REFERENCES


23. Barker LM, Zanassy OZ, Reed MW, Geelhoed SJ, Pfalzgraf RD, Cangelosi GA. Daily non-invasive fluorescence ratio measurement of platelet concentrate pH can detect the presence of bacteria. Transfusion 2006;46S:54A.

