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High Resolution Non-contact Fluorescence Based Temperature Sensor for Neonatal Care

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Abstract

To date, thermistors are used to continuously monitor the body temperature of newborn babies in the neonatal intensive care unit. The thermistor probe is attached to the body with a strong adhesive tape to ensure that the probe stays in place. However, these strong adhesives are shown to increase microbial growth and cause serious skin injuries via epidermal stripping. The latter compromises the skin's ability to serve as a protective barrier leading to increase in water loss and further microbial infections. In this article a new approach is introduced that eliminates the need for an adhesive. Instead, two kinds of fluorophores are entrapped in a skin friendly chitosan gel that can be easily wiped on and off of the skin, and has antimicrobial properties as well. A CCD camera is used to detect the temperature dependent fluorescence of the fluorophore, tris(1,10-phenanthroline)ruthenium(II) while 8-aminopyrene-1,3,6-trisulfonic acid serves as the reference. This temperature sensor was found to have a resolution of at least 0.13°C.

Keywords

temperature sensor; neonatal care; fluorescence; non-contact

1. Introduction

Compared to adults, newborns are particularly vulnerable to temperature extremes. If heat loss is not preempted, newborns could suffer the ill effects of cold stress or hypothermia. Depending on the gestational age, mortality may increase by 10% for each degree Celsius (°C) that a baby's body temperature is below 36°C.[1] Conversely, at relatively high temperatures, infants may be subjected to the dangers of heat stress or hyperthermia. Both types of thermal stresses, hypo- and hyperthermia can be a significant cause of morbidity and mortality in this vulnerable population.[1],[2] Thus, in the hospital setting, infant incubators are designed to keep the infant in an environment of constant temperature. Nonetheless, providing optimal thermal conditions to the newborn require the body temperature to be measured. To date, wired thermistors are conventionally employed, that are attached to neonate's skin using a strong adhesive to keep them in place. However, adhesive use on the thermistor probe cover is not an innocuous intervention. Investigations have shown increased microbial growth beneath probe covers, some of which have proved to be pathogenic.[3] Others have found skin impairment ranging from chemical sensitivities to prolonged mechanical force on the skin from some adhesives. [4] Adhesives can irritate the skin by occlusion or by altering the skin morphology via epidermal stripping. [5][6] The removal of adhesively attached skin temperature probes can result in "Skin tears" in which

shear or frictional forces separate the dermis from the epidermis. These tears can compromise skin barrier function, cause a marked increase in trans-epidermal water loss, and in many cases, disrupt the skin's ability to protect against microorganism invasion.[7] Especially for extremely premature neonates, the adhesive poses a high potential risk of injury due to their immature skin. Studies have shown that at 24 weeks gestation, premature neonates have little stratum corneum and attenuated rete ridges. Their skin is red, wrinkled, translucent, and gelatinous in appearance. They lack subcutaneous tissue; therefore, their dermis is lying directly over the muscle.[8] Consequently, skin stripping secondary to adhesive dressing and/or tape removals can result in full-thickness tissue loss. Even at 36 weeks, the epidermal and dermal layers of the neonates' although structurally similar to the adult are just up to 60% as thick as an adult.[9] As the consequence epidermal stripping secondary to tape and adhesive dressing removal is most common in neonates born before 27 weeks' gestation and is the primary cause of skin breakdown in the NICU (Neonatal Intensive Care Unit).[10][11] Given the neonate's attenuated rete ridges, adhesive products typically bond more aggressively to the epidermis than the epidermis does to the dermis.[12] Consequently, epidermal stripping is not only a source of discomfort, but can also lead to other morbidity in very low birth weight neonates and those who are immune-compromised.

Skin friendly adhesives that can hold the wired probe in place while minimizing trauma to the skin have not yet been developed. There are weaker adhesives, but they don't adhere well, especially in high humidity environments. Alternative temperature probes that don't require adhesives are nonexistent. In addition, remote thermal-imaging systems that may offer non-adhesive, non-contact features and a resolution of 0.3°C are still too expensive for general use in the NICU.

In this paper, we present fluorescence-based temperature sensing as an alternative to current practice. In this technique, the temperature sensing fluorophore is incorporated in a soft hydrogel that can then be applied gently on the infant skin. Unlike the heavy wired probe, the sensing gel is the probe itself and does not need adhesive tape to hold it in place. Therefore, the adhesion strength can be minimized to such an extent that the gel adheres to the skin strongly enough not to fall off, but can also be gently wiped off at the end of the measurements. Slightly acidic water (pH 5.5) dissolves and can readily remove the gel from the skin surface. The adhesion strength of the hydrogel has been reported to be 0.094 N*mm⁻¹[13]. In comparison, the acrylic based tape (3M Scotch 928 ATG) which can be easily peeled off from ordinary paper is even stronger at 0.14 N*mm⁻¹ [14].

A variety of fluorophores have been found to show high sensitivity to temperature. The fluorescence intensities and decay times of these compounds decrease with increasing temperatures and can be measured with a high degree of accuracy. Thus, many fluorophores have been utilized as luminescent temperature probes.[15] However, most of these are not suitable for neonatal healthcare applications. As an example, alexandrite crystals were found to be sensitive between 15-45°C, and the phosphorescence decay time decreases from 300 to 220 μs within this range. The long decay time makes it easy to be precisely determined with low cost instrumentation. While these properties may seem appropriate for neonatal applications, alexandrite crystals cannot be ground to a fine powder for application on baby skin. Additionally, the grinding process creates defects on the crystal structure rendering alexandrite non-luminescent. Many other materials also show strong temperature sensitivity over the desired range of temperatures, such as zinc sulfide, and lanthanide phosphors such as La₂O₂S:Eu. The lanthanide phosphors also show desirable lifetime changes as the temperature changes.[16]-[18] Unfortunately, all of these fluorophores are excitable by UV light which is potentially harmful to neonate skin.

In contrast to the UV-excited materials, there are classes of fluorophores that are excitable by visible light such as ruthenium(II) tris(1,10-phenanthroline) (Ruphen) and ruthenium(II) tris(bipyridine) (Rupby). These dyes are not only highly luminescent but their luminescence intensity and decay times are also very sensitive to temperature. Moreover, investigations by other researchers indicate that these compounds might be nontoxic and incapable of penetrating human skin.[19]-[21]

These non-UV excited dyes have been widely employed as temperature probes in various research fields. Fluorescence-based temperature probes have a variety of advantages over thermoelectric probes, including immunity to high electromagnetic fields and the capability for long distance measurements. For instance, fluorophores can be employed in thermal convection studies in both huge bioreactors and micro-sized lab-on-a-chip environments. [22] Furthermore, since light transmission requires no conducting medium, the probe and the photodetector need not to be in direct contact; hence, remote detection can be realized. Due to the advantages of luminescence sensors, a number of fluorescence-based temperature probes have been developed.[16][20][23]

In general, there are three ways to measure the temperature using luminescence sensors: steady state fluorescence intensity measurements, ratiometric intensity measurements, and luminescence decay time measurements. The simplest method is to measure the steady state intensity. However, for long term measurements the intensity can drift along with the photobleaching of the fluorophore, the movement of the probe and fluctuation of the ambient light. The other two techniques, however, are not as susceptible to this problem of drift. The decay time technique utilizes the decay time of the fluorophore as the temperature sensitive parameter. The decay time is the time span required for the dye at the excited state to return to the electronic ground state. This transition is temperature dependent. Since it is an intrinsic property of the dye molecule, the temperature measurement based on the decay time technique is practically independent of the fluorophore concentration. However, the technique requires fast optoelectronics and can be pricey. The ratiometric approach, on the other hand, takes the intensity ratio of two emission bands of the fluorophore system to represent the temperature. Assuming the photobleaching effect has the same impact on the emission bands, the ratio is unaffected, thereby making the system less prone to drift. As no high speed measurement is required this approach is suitable for using relatively inexpensive digital camera with low frame rate as the detector.

In this paper we present our feasibility studies on a dual fluorophore system for the ratiometric determination of temperature. Our approach is based on the temperature dependent fluorescence of tris(1,10-phenanthroline)ruthenium(II) with 8-aminopyrene-1,3,6-trisulfonic acid as the reference probe. The measurement is carried out using a low frame rate CCD camera.

2. Development of the sensor system

2.1. Preparation of temperature sensing gel

Chitosan (medium molecular weight, Sigma) is dissolved in 0.1 M acetic acid (Sigma) to make a 2% w/v gel. To each gram of this gel, 0.5g glycerol is added. To prepare the gels containing the fluorophores, 0.1 g of silica gel adsorbed with either tris(1,10-phenanthroline)ruthenium(II) (Ruphen) or 8-aminopyrene-1,3,6-trisulfonic acid (APTS) is added to 1g of the chitosan. These fluorophore containing gels are then spread on the front side of a thermal block.

2.2. Hardware

A benchtop fluorimeter (Eclipse, Varian) was employed for fluorescence spectral analysis. The temperature sensor setup (Figure 1,2) consists of a thermal block with a peltier element for the attachment of the temperature sensing gel, a CCD camera (Pike F145, Allied Vision Technologies), a blue (475nm) emitting LED (XR7090BLU, Cree) with driver electronics made in house containing the current source module (LTM8040, Linear Technology) and the microcontroller (dsPIC33FJ128GPX02, Microchip). The temperature of the thermal block can be manually set precisely and controlled via a temperature controller (LDT-5910, ILX Lightwave). The CCD camera is connected to the PC via Firewire, which both transmits the image data to and receives control commands from the PC.

Apart from this interface, the camera has an additional multifunction IO-port. One of these functions is to send out a signal representing the time the exposure is made. This signal is used to switch the LED. The LED is switched on and off by the CCD camera as follows: the IO-port of the camera is connected to two external interrupt pins on the microcontroller of the electronic circuit driving the LED. The first interrupt responds to the rising edge of the camera output signal. The microcontroller activates the current source by setting its PWM input pin at high, which then turns the LED on. The second interrupt is responsive to the falling edge and turns the LED off. Further, the microcontroller is programmed to turn on the LED on every second camera signal. This enables the camera to take alternately one image with LED light and one image with no LED light, which is crucial for the cancellation of signal offset caused by ambient light. This cancellation process is described in the software section below.

2.3. Software

The control and image analysis software for the sensor system is built on the Labview programming platform (Labview 2010 + Vision module). The software's key steps are described in the flow chart in Figure 3. At the start of the program, the camera is set at continuous acquisition mode (30 frames per second), exposure time of 16ms and resolution 640×480. Two sequential frames are acquired (the hardware ensures the first frame is with the LED off and the second – with the LED on). In this way, the ambient light is captured in the first frame, while the second frame captures the ambient light plus the fluorescence. Pixel values of the first frame are then subtracted from the second frame; thus the signal offset caused by the ambient light is eliminated and the pure fluorescence is obtained. Then, rectangles with the images of the fluorescent gels are extracted. The coordinates of the upper left and lower right corner of the gels images are selected manually. Average pixel intensity of all pixels in these rectangles is calculated for both Ru(phen) and APTS gels. Then, the ratio of the Ru(phen) and APTS is linearly scaled to temperature. The values are subjected to 90 point median filter, which minimizes possible influence of rapid changes in illumination (i.e. people walking around the incubator). The temperature value is displayed on a virtual digital display and as a time graph. The user has an option to vary the exposure time and parameters of the digital filter, as well as to archive the results.

3. Results and discussions

The fluorophores Ru(phen) and APTS are an ideal fluorophore pair for ratiometric temperature measurement. In Ru(phen), the ruthenium ion Ru(II) forms an octahedral complex with three phenanthroline molecules. As shown in Figure 5a the complex has a strong and broad absorption band extending from ultraviolet to green region with a maximum at around 450nm. This absorption band is due to the very prominent metal to ligand charge transfer transition (MLCT) to the triplet MLCT state that is energetically slightly lower than the triplet d-d state. The transition from the MLCT state to the ground

state results in emission of light (590nm) whereas the promotion to the d-d state by thermal energy leads to the radiationless decay to the ground state. These two processes compete at any given temperature. Hence, less fluorescence emission is observed at higher temperatures and vice versa. APTS, on the other hand, is a polycyclic aromatic compound consisting of 4 fused benzene rings. Its excitation spectrum ranges from ultraviolet to blue light. The excitation maximum is at around 420nm in aqueous solution, whereas the emission peak is located at 500nm. As shown on figures 5a and 5d, both dyes can be excited by blue light which is harmless to skin and, therefore, acceptable for our application. Additionally, an inexpensive but efficient LED can be employed as the single excitation light source. This not only simplified the instrumental setup, but also avoided the errors caused by two excitation light sources whose emission eventually fluctuated or drifted at different rates. As the emission peaks of Ruphen (Figures 5b-c) and APTS (Figures 5e-f) are about 90nm apart, each can be easily isolated with the appropriate filters. Thus, the two dyes can be incorporated together into the gel and their fluorescence signatures effectively discriminated. For the final application on neonates mixing the dyes together in one gel will be crucial. Because babies tend to twist and turn, the location of the sensing gel can change from the point of view of the camera resulting in a variation of the fluorescence recorded. However, as long as the two dyes are combined in one sensing gel the ratio of their fluorescence will not change. Moreover, the fluorescence of these dyes is only slightly affected by pH (Figures 5b and 5e). This is important as the skin of neonates at different gestational ages have varying pH levels. More importantly for our purposes, ambient temperature has varying impact on the fluorescence of the two dyes. On the one hand, the fluorescence of Ruphen is extremely sensitive to temperature. As shown in Figure 5c, the fluorescence intensity of Ruphen drops drastically by 75 per cent when the ambient temperature is changed from 25°C to 50°C. The significant temperature sensitivity in the physiological range makes Ruphen an ideal body temperature probe. On the other hand, the same temperature change has virtually no impact on the fluorescence of APTS (Figure 5f). Thus, APTS makes a very good reference probe for Ruphen in ratiometric temperature measurements.

For the temperature measurements using the digital camera, corrections have to be made for the presence of ambient room light. This can be achieved by taking pairs of images where one image is taken with the LED on and one image taken with the LED off. The brightness of the images captured by the camera is determined by the brightness of the ambient light combined with the fluorescence light from the fluorophores. When the ambient light is excessive, this usually causes a huge offset making the detection of the fluorescence light from the temperature probes insignificant. However, by subtracting the image with LED on from the image with the LED off, the ambient light can be mathematically eliminated. This results in an image that shows only the fluorescence caused by the LED excitation.

Using the hardware described in the Experimental Section, we determined the effect of temperature on the sensing gels. Note, that all measurements were carried out not in the darkness; the probe is exposed to room lights scattered sun light as the windows were not shaded. In spite of ambient light the measurements were effected. Within the physiologically relevant temperature range, the ratio of the fluorescence intensity of Ruphen and APTS correlates linearly to the temperature the sensing gel on the thermal block (figure 6). The intercept of the calibration curve is 0.932 (± 0.0116) and the slope is $-0.01587 (\pm 3.13E-4)/^{\circ}\text{C}^{-1}$. The maximum standard deviation of the data points is $5.00E-4$. Experimental data suggest that the probe has a resolution of better than 0.1°C . However, this is impossible to prove with our test equipment since both the reference probe (thermistor) and the temperature controller have a nominal resolution of 0.1°C .

As a final test on the suitability of the temperature sensor to continuous use in the NICU, the long term stability was investigated. According to our collaborating pediatric expert (NICU, University of Maryland), neonates including premature babies in the NICU are bathed every day. Hence, the probe is required to perform satisfactorily for a maximum period of one day. The stability of the sensing gel is determined by the resilience of the fluorophores to photobleaching. The photobleaching rate is an intrinsic property of the fluorophores and is influenced by presence of oxidants such as oxygen. Since our ratiometric probe employs two different dyes, we expected their bleaching rates to differ from one another. In figure 7, the fluorescence of APTS drops almost linearly by 25% from its original value after 85000 measurements at a rate of about 1 sec^{-1} (24h). In contrast, the fluorescence of Ruphen remains virtually unchanged. This divergence could lead to a significant drift of the measurement signal if no compensation measures are undertaken. To correct this discrepancy a compensation loop is implemented in the signal processing software. The compensation algorithm takes into consideration the exposure time of the dye to the blue LED excitation and compensates for the drop of the APTS fluorescence. It is also assumed that the ambient oxygen concentrations are constant within the duration of the experiments. As can be seen in Figure 7, the software compensated for the decrease in fluorescence of APTS during the 85000 measurements to a satisfactory extent. The average compensated value is 1189.9 and its standard deviation is ± 4.21 .

Measurements were carried out to test the accuracy, response time and stability of the sensor. The experiments were conducted inside the closed baby incubator at ambient light. The NIST approved thermistor of the incubator served as the reference probe. As the gel layer is not more than 0.1mm thick, the heat transfer was very fast, so that the probe responded virtually instantly to temperature changes. The temperature measured by the sensing gel and the thermistor were virtually identical. When holding the temperature at a certain value for about 3 hours, the sensing gel showed a fluctuation of less than $\pm 0.05^\circ\text{C}$. The drift over a period of one day is no more than $+0.15^\circ\text{C}$.

4. Conclusion

The presented work has proven the feasibility of the wireless optical temperature sensor that fulfills the resolution requirement in the NICU and that eliminates the need for the detrimental adhesive. For future work, the two dyes will be incorporated into one gel to make the probe invulnerable to movement of the infant. The software will be fine-tuned, and the sensing gel formulation optimized to minimize the photobleaching rate of APTS. Additionally, more toxicity tests will be conducted prior to clinical trials.

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References

1. Chandon, S.; Baumgart, S. Temperature regulation of the premature infant. In: Taeusch, HW.; Ballard, RA.; Gleason, C.; Avery, ME., editors. Schaffer and Avery's diseases of the Newborn. 6. Philadelphia: Elsevier Health Sciences; 2004. p. 364-371.
2. Baumgart S. Current concepts and clinical strategies for managing low-birth-weight infants under radiant warmers. Medical Instrumentation. 1967; 21:23–26. [PubMed: 3675736]
3. Marples RR, Richardson JF, Seal DV, Cooke EM. Adhesive tapes in the special care baby unit. Journal of Hospital Infection. 1985; 6:398–405. [PubMed: 2868037]

4. Hoath, SB.; Maibach, HI. Neonatal Skin Structure and Function. 2. New York, NY: Marcel Dekker, Inc; 2003.
5. Rutter N. Clinical consequences of an immature barrier. *Semin Neonatol.* 2000; 5:291–297.
6. Rutter N. The immature skin. *Br Med Bull.* 1988; 44:957–970. [PubMed: 3076838]
7. Blackburn S, DePaul D, Loan L, Marbut K, Taquino L, Thomas K, Wilson S. Neonatal thermal care, part II. Microbial growth under temperature probe covers. *Neonatal Network.* 2001; 13:19–23. [PubMed: 12144211]
8. Eichenfield L, Hardaway C. Neonatology dermatology. *Curr Opin Pediatr.* 1999; 11:471–474. [PubMed: 10555601]
9. Campbell JM, Banta-Wright SA. Neonatal skin disorders: a review of selected dermatologic abnormalities. *J Perinat Neonat Nurs.* 2000; 14:63–83.
10. Malloy-McDonald MB. Skin care for high risk neonates. *J WOCN.* 1995; 22:177–182.
11. Association of Women's Health, Obstetric and Neonatal Nurses (AWHONN). Neonatal skin care, Evidence-based clinical practice guideline. Association of Women's Health, Obstetric and Neonatal Nurses (AWHONN). 2001 Jan.
12. Lund C. Prevention and management of infant skin breakdown. *Nurs Clin N Am.* 1999; 34:907–920.
13. Khan TA, Peh KKh, Ch'ng HS. Mechanical, Bioadhesive Strength and Biological Evaluations of Chitosan films for Wound Dressing. *J Pharm Pharmaceut Sci.* 2000; 3:303–311.
14. Scotch ATG Tape 924 • 926 • 928 • 969 • 976 • 970XL, Data sheet. Aug.2000
15. Khalil G, Lau K, Phelan GD, Carlson B, Gouterman L, Callis J, Dalton L. Europium beta-diketonate temperature sensors: Effects of ligands, matrix, and concentration. *Review of Scientific Instruments.* 2004; 75:192–206.
16. Zhang, ZY.; Grattan, LS. *Fiber Optic Fluorescence Thermometry.* Springer-Verlag; New York: 1994.
17. Lam H, Rao G, Loureiro J, Tolosa L. Dual optical sensor for oxygen and temperature based on the combination of time domain and frequency domain techniques. *Talanta.* 2011; 84:65–70. [PubMed: 21315899]
18. Borisov MB, Klimant Ingo. Blue LED Excitable Temperature Sensors Based on a New Europium(III) Chelate. *J Fluoresc.* 2008; 18:581–589. [PubMed: 18288591]
19. Dobrucki JW. Interaction of oxygen-sensitive luminescent probes [Ru(phen)3]2+ and [Ru(bipy)3]2+ with animal and plant cells in vitro. Mechanism of phototoxicity and conditions for non-invasive measurements. *Journal of photochemistry and photobiology B.* 2001; 65:136–144.
20. Kocincova AS, Borisov SM, Krause Ch, Wolfbeis OS. Fiber-Optic Microsensors for Simultaneous Sensing of Oxygen and pH, and of Oxygen and Temperature. *Anal Chem.* 2007; 79:8486–8493. [PubMed: 17948966]
21. Baleizao C, Nagl S, Schaeferling, Nerberan-Santos MN, Wolfbeis OS. Dual Fluorescence Sensor for Trace Oxygen and Temperature with Unmatched Range and Sensitivity. *Anal Chem.* 2008; 80:6449–6457. [PubMed: 18651755]
22. Swarts JW, Janssen AEM, Boom RM. Temperature effects during practical operation of microfluidic chips. *Chemical Engineering Science.* 2008; 63:5252–5257.
23. Paez G, Strojnik M. Erbium-doped optical fiber fluorescence temperature sensor with enhanced sensitivity, a high signal-to-noise ratio, and a power ratio in the 520, –530-and 550 – 560-nm bands. *Applied Optics.* 2003; 42:3251–3258. [PubMed: 12790476]

Figure 1.

Experimental setup of the temperature measurement. The temperature of the fluorescent probe (D) is set via the temperature controller (E). The LED (A) is switched on by the camera (B) itself when acquiring an image. The camera is controlled by the PC (C) via Firewire connection. The controlling and image analyzing software is built on a Labview platform.

Figure 2.
Switching the LED

Figure 3.
Flow chart of the control and image analyzing software.

Figure 4.

Photographic image of the experimental setup. The whole system was setup inside the incubator. Its thermistor served as the reference temperature probe and was placed in a drill hole inside the aluminum heating block.

Figure 5.

Excitation and emission spectra of Ruphen and APTS. 5a and 5d are the excitation spectra of Ruphen and APTS, respectively. 5b and 5e are the emission spectra as a function of pH, and 5c and 5f are the emission spectra of Ruphen and APTS, respectively, at different temperatures. The temperature measurements were carried out in aqueous solutions at pH5. All of the spectra are collected on a benchtop fluorimeter.

Figure 6.

Response of the ratiometric probe to temperature. For each temperature shown in the figure a minimum of 300 measurements were carried out.

Figure 7.

The fluorescence of APTS and Ruphen decreases at different rates when illuminated for many hours. The fluorescence of Ruphen remains virtually unchanged after 85000 measurements while the fluorescence of APTS drops by 25% within the same period of time.