Evaluation of Three Anticoagulants Used for Short-Term Storage of Loggerhead Sea Turtle (*Caretta caretta*) Whole Blood

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Abstract: Anemia is a common clinical pathological finding in stranded loggerhead sea turtles (*Caretta caretta*) secondary to trauma and chronic debilitation. These animals may benefit from species-specific blood product administration, yet few studies have evaluated the effects of anticoagulants on loggerhead sea turtle blood storage. Sea turtles in managed collections may serve as healthy blood donors, allowing for short-term blood storage with transfer of blood products within 24 h to rehabilitation facilities. The objective of this study was to compare the packed cell volume (PCV), total solids (TS), electrolytes, glucose, and venous blood gas analytes over a 24 h storage of loggerhead sea turtle whole blood in three anticoagulants: sodium heparin, sodium citrate, and citrate-phosphate-dextrose-adenine (CPDA-1). Blood from eight loggerhead sea turtles at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center (Surf City, NC) was used following routine venipuncture for health assessment. Whole blood was placed into vacutainer tubes containing sodium heparin, sodium citrate, or CPDA-1 and stored at 4–8°C (39–46°F). PCV and TS were evaluated at 0, 3, 6, 12, and 24 h. Blood gas, electrolyte, and glucose analyses using i-STAT CG8+ cartridges were performed at 0 and 24 h. There were no significant differences in PCV and TS over time. At 24 hours, pink plasma was observed in 62.5% (5/8) of CPDA-1 and 12.5% (1/8) of citrate specimens. CPDA-1 and citrate specimens had a significant increase in potassium at 24 h. Both heparin and citrate specimens had a significant decrease in pH and increase in partial pressure of carbon dioxide at 24 h. Sodium values decreased over time in citrate and CPDA-1 specimens. Loggerhead sea turtle blood stored in CPDA-1, and sodium citrate had more significant blood gas and electrolyte changes over 24 h, indicating a greater degree of erythrocyte leakage or lysis. For the purposes of collection and short-term storage of loggerhead sea turtle blood, sodium heparin is preferable.

Key Words: Blood transfusion, *Caretta caretta*, erythrocyte metabolism, loggerhead sea turtle, reptile, transfusion medicine.

Introduction

The loggerhead sea turtle (*Caretta caretta*) is one of five sea turtle species inhabiting the coastal waters of North Carolina (Epperly *et al*., 1995). Loggerheads of the northwest Atlantic from the southeastern United States to the Yucatán Peninsula in Mexico are currently listed as a subpopulation of least concern, whereas listings of other loggerhead sea turtle subpopulations range from near threatened to critically endangered (Ceriani and Meylan, 2015). Free-ranging individuals receive veterinary care for a variety of reasons, including traumatic injury (fishery gear interactions, boat strike, predation), ingestion of foreign material, infectious disease, and hypothermic stunning events. Clinical pathology tests such as complete blood counts, plasma biochemistries, and blood gas analysis are performed to guide appropriate medical therapy. Cases of severe anemia and hypoproteinemia in stranded sea turtles may benefit from blood product transfusions, because these treatments have been reported in other reptile species (McCracken *et al*., 1994; Wack and Anderson, 2004; Gibbons and Darbo-McLeffan, 2009) and anecdotally in sea turtles (Field *et al*., 2017; Norton *et al*., 2017). Sea turtles found in managed collections may serve as healthy blood donors and could be available on an as needed basis for blood donation. Use of these specimens would allow transport and administration of blood products to a recipient within 24 h. Transfusion of sea turtle blood within 6–8 h of collection has been recommended because methods for long-term storage of nonmammalian blood have not been validated (Norton *et al*., 2017). Because sea turtle anemia cases can often be managed successfully with other supportive measures, transfusions have been recommended mainly for cases of severe anemia (PCV less than 5%) (Norton *et al*., 2017), although two Kemp's ridley (*Lepidochelys kempii*) cases experiencing acute declines in PCV not quite to the 5% threshold (21% to 6% and 24% to 7%) following rescue in the Deepwater Horizon oil spill event received homologous blood transfusions (Field *et al*., 2017).

Proper collection, storage, and administration of blood products are essential for the safety of both the donor and recipient. Inappropriate refrigeration, bacterial contamination, improper

filter size, and prolonged storage may result in hemolysis and acute hemolytic transfusion reactions in recipients following administration (Patterson *et al*., 2011; Hann *et al*., 2014). Blood storage requires the use of a blood anticoagulant and preservative to extend erythrocyte viability. Erythrocytes stored at 1–6°C (34–43°F) undergo cellular changes that can ultimately affect cell function and survival (Wardrop, 1995). Erythrocyte metabolism results in decreased cell glucose, increased lactic and pyruvic acid production, decreased pH and ATP (adenosine triphosphate), and alterations in oxygen affinity (Wardrop, 1995; Sparrow, 2010). Blood preservatives aid in maintaining erythrocyte cell function and structure during storage periods. Citrate-phosphate-dextrose-adenine (CPDA-1) is the most common blood product preservative used in veterinary medicine and is superior to other blood preservatives based on the degree of hematological and biochemical changes of mammalian blood specimens over time (Wardrop, 1995; Mudge *et al*., 2004). The addition of adenine and glucose favors continued erythrocyte metabolism more so than anticoagulants alone.

Reptile blood differs from mammalian blood in having nucleated erythrocytes and reacting differently to available anticoagulants. Varying degrees of hemolysis, thrombocyte and leukocyte aggregation, and alterations in hematology values occur when reptilian blood is placed in ethylenediaminetetraacetic acid (EDTA), including loggerhead sea turtles (Muro *et al*., 1998; Harms *et al*., 2000; Hanley *et al*., 2004; Harr *et al*., 2005; Perpiñán *et al*., 2010). As alternatives, sodium heparin, lithium heparin, and sodium citrate are proven to be adequate and less adverse anticoagulants for reptile blood collection in several species (Bolten *et al*., 1992; Muro *et al*., 1998; Hanley *et al*., 2004; Harr *et al*., 2005; Perpiñán *et al*., 2010). Most recently, CPDA-1 and acid-citrate-dextrose (ACD) were evaluated in the storage of American alligator (*Alligator mississippiensis*) blood over time and both deemed acceptable for the storage of blood for up to 35 days (Emerson *et al*., 2014). Loggerhead sea turtle whole blood storage over time and the effects of CPDA-1 on chelonian erythrocytes have not been previously assessed.

The purpose of this study was to evaluate the effects of different anticoagulants on loggerhead sea turtle whole blood stored over 24 h under refrigeration. Sodium heparin, sodium citrate, and CPDA-1 were selected because of their commercial availability as sterile liquid solutions that allow for flexibility in blood volume collection as well as because of their minimal adverse effects on reptile erythrocytes. To evaluate the effects of these solutions on cell integrity, packed cell volume (PCV), total solids (TS), venous blood gas, glucose, electrolytes, and hemoglobin concentration were assessed. It was hypothesized that loggerhead sea turtle whole blood stored in CPDA-1 would have less significant changes in these analytes over time as compared to specimens stored in sodium citrate and sodium heparin attributable to the addition of adenine and dextrose.

Materials and Methods

Animals and housing: Eight loggerhead sea turtles housed at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center (Surf City, NC) were used for this study. Animals were included if body weight exceeded 30.0 kg (estimated blood volume of 8% body weight equaled greater than 2.4 L), PCV greater than 15%, and the animal did not display any overt signs of clinical disease. Although a sea turtle with a PCV of 15% would not be considered a good donor candidate (30% or greater would be preferred), the amount of blood required for this study was well within safe limits. Animals were housed in individual round tanks that ranged from 1.5–2.4 m in diameter. Water temperature ranged from 23.6–27.4°C (median 26.9°C [74.5–81.3°F, median 80.0°F]) and salinity was 25 g/L (parts per thousand [ppt]). Blood collection was performed incidentally during routine physical examinations in September 2014 in which venipuncture was scheduled for health assessment. Body weights and carapace measurements (straight carapace length [notch-tip], straight carapace width) were obtained on the day of blood specimen collection. Cloacal temperature was measured using a thermocouple (Barnant Thermocouple Thermometer, Barnant Co., Barrington, IL) prior to venipuncture.

Specimen collection and storage: For each animal, one 2.0 mL sodium heparin (33 USP Units; dry) and one 2.7 mL 3.2% sodium citrate (0.105M; 0.2 mL; 1:15 dilution [v:v:]) blood collection tubes were used (BD Vacutainers; Becton, Dickinson and Company, Franklin Lakes, NJ). These specimens will be referred to as heparin and citrate, respectively, for the remainder of the article. A 3.0 mL nonadditive vacutainer plastic tube (BD Vacutainers; Becton, Dickinson and Company, Franklin Lakes, NJ) was filled with 0.3 mL of CPDA-1 solution (Sigma-Aldrich, Co., St. Louis, MO) to yield the commercially available and recommended 1:9 (v:v) dilution of CPDA-1 to whole blood (Morrisey, 2010). Each mL of CPDA-1 contained the following: adenine 0.28 mg, citric acid 3.3 mg, dextrose 31.7 mg, sodium citrate 26.3 mg, and sodium phosphate monobasic 2.2 mg.

Animals were removed from their tanks for physical examination and venipuncture under manual restraint. The left dorsal cervical region was aseptically prepared with three alternating scrubs of dilute providone iodine solution and 70% isopropyl alcohol. An 18 gauge 3.8 cm needle and 20 mL syringe were used for venipuncture and a total of 15 mL of whole blood was collected. Approximately 2.0 mL of whole blood was added to the heparin tube, 2.7 mL of whole blood to the citrate tube, and 2.7 mL of whole blood in the tube containing CPDA-1. The remaining blood specimen was placed into lithium heparin tubes (BD Vacutainers; Becton, Dickinson and Co., Franklin Lakes, NJ). The lithium heparin specimen was used for clinical pathology diagnostics (complete blood count [CBC], plasma biochemistry, PCV, TS, and plasma archival storage). All specimen tubes were gently rolled 8–10 times to mix the anticoagulant solution and blood before storage under refrigeration for the remainder of the study period. The thermocouple was used to monitor and maintain storage temperature between 4°C and 8°C.

Specimen analysis: Each anticoagulant blood specimen was used for five different subsamples, representing the five time points evaluated. The first time point of analysis occurred within 5–10 min of specimen collection and was designated as time 0 h. Subsequent evaluations were conducted 3, 6, 12, and 24 h after the initial assessment.

Prior to evaluation, the specimen tube was gently rolled at least 10 times to resuspend the blood specimen. A 1 mL syringe with 27 gauge needle (Terumo Medical Corp., Elkton, MD) was used to aspirate 0.10–0.20 mL of whole blood from the collection tube for analysis. At each time point, two hematocrit tubes were spun at 13,000 × g using a hematocrit centrifuge for 4 min to determine PCV. Plasma color was recorded as either clear or pink for each specimen, and TS was determined using a refractometer.

Table 1. The PCV/TS of loggerhead sea turtle (*Caretta caretta*) whole blood stored in three different anticoagulants over time ($N = 8$). Values listed are the median (range). Statistically significant differences ($P < 0.05$) among anticoagulants at the same time point are marked with a superscript letter. There were no statistically significant differences of values between time 0 and 24 h for any of the anticoagulants.

	Initial specimens (0 h)			24 h specimens		
Analyte (units)	Sodium heparin	Sodium citrate	CPDA-1	Sodium heparin	Sodium citrate	CPDA-1
PCV (%)	31[a,b] (18–39)	29.5[a,c] (17–35)	28[b,c] (16–33)	30.5[a,b] (17–39)	29[a,c] (17–35)	26.5[b,c] (16–31)
TS (g/dL)	4.7[a,b] (3.0–7.6)	4.25[a,c] (2.6–7.2)	4.4[b,c] (3.0–7.0)	4.8[a,b] (3.1–7.6)	4.3[a,c] (2.7–7.0)	4.5[b,c] (3.2–7.0)

[a]Significant difference between sodium heparin and sodium citrate specimens.
[b]Significant difference between sodium heparin and CPDA-1 specimens.
[c]Significant difference between sodium citrate and CPDA-1 specimens.

Venous blood gas, electrolyte, and glucose analysis was performed on all whole blood specimens at times 0 and 24 h using an i-STAT CG8+ cartridge (Abaxis, Union City, CA). The CG8+ cartridges generated the following values at 37°C: pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), sodium (Na), potassium (K), ionized calcium (iCa), and glucose (Glu), and the instrument calculated bicarbonate ion (HCO_3^-) from the Henderson-Hasselbalch equation. The i-STAT analyzer also calculated percent oxygen saturation (sO_2) and base excess (BE), but these values were excluded from analysis because the calculations rely on assumptions of normal hemoglobin and plasma protein concentrations for humans and species-specific blood oxygen affinity are not applicable to sea turtles (Siggaard-Anderson, 1976). I-STAT analysis also allows temperature correction for calculation of pH, pCO_2, and pO_2 at patient temperature, but this was not considered relevant to the question of relative changes of blood gases over time in vitro.

The remaining blood specimens at the end of the 24 h period were centrifuged at 13,000 × g for 8 min. The resulting plasma was placed in a labeled cryovial and stored at 4°C for 24 h. These plasma specimens were then stored at −70°C for 11 months until hemoglobin analysis was performed attributable to personnel availability. Plasma specimens were thawed at room temperature, and gross lipemia was observed in all specimens. To reduce lipemia prior to specimen analysis, high-speed centrifugation was used; a modified technique by Dimeski and Jones (2011). This was completed on plasma specimens obtained from five animals because three animals' plasma specimens were analyzed prior to high-speed centrifugation, and results excluded from statistical analysis. All specimens were centrifuged at 16,000 × g for 30 min. The infranatant was removed using a pipette and centrifuged at 16,000 × g for 30 min. The resulting infranatent was used for analysis. Hemoglobin analysis was performed using the cyanmethemoglobin method (Lewis and Kumari, 2010). Drabkin's reagent (0.6 mL) was combined with 0.2 mL of specimen plasma and allowed to mix for 5 min. Specimen absorbance was evaluated at 540 nm using a GENESYS 10S UV-Visible spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI) and compared against a standard curve for cyanmethemoglobin. All specimens were run in duplicate.

Statistical analysis: Animal information and hematological and blood gas analytes were analyzed using JMP Pro11 (SAS, Cary, NC). Descriptive statistics were performed on animal body weight, straight carapace length, straight carapace width, and cloacal temperature. A Shapiro–Wilk test was performed on the data, and because they were not normally distributed, nonparametric analysis was performed. A Wilcoxon Signed Rank test for pairwise comparison was performed on the blood gas analytes obtained at 0 and 24 h. A Friedman's test was performed for PCV and TS values over time for each anticoagulant specimen. Plasma color change was compared across the three anticoagulants at 24 h by Fisher's exact test and correspondence analysis. Blood gas analytes, electrolytes, PCV, and TS were also compared between anticoagulant specimens at each time point using a Wilcoxon Signed Rank test. This test was also used to compare cyanmethemoglobin values between anticoagulants at 24 h and to time 0 lithium heparin values. P-values less than 0.05 were considered significant.

Results

All study animals were deemed healthy based on physical examination and evaluation of CBC and biochemistry results. The study animals' median body weight was 46.0 kg and ranged from 32.7–100.9 kg. The largest study animal exceeded the measuring equipment capacity (80 cm calipers) to obtain an accurate straight carapace length; thus, aside from this individual, the median straight carapace length (notch–tip) was 70.3 cm with a range of 61.8–73.1 cm. Straight carapace width median was 59.8 cm with a range of 53.4–71.8 cm. Based on morphometric data, seven animals were estimated to be subadults (10–20 yr) and one was considered an adult (20–30 yr) (Scott *et al*., 2012; Avens *et al*., 2013). Cloacal temperature median was 27.4°C with a range of 24.2–28.2°C.

There were no statistically significant changes for any of the three individual anticoagulants over the different time points for PCV or TS. Yet the PCV and TS values significantly differed between all anticoagulants at times 0 hours and 24 h (Table 1). Heparin specimens had a significantly higher PCV compared to both the citrate and CPDA-1 specimens at both 0 and 24 h. CPDA-1 had the lowest median PCV, and citrate had the lowest median TS at all times points.

Blood gas analysis performed at times 0 and 24 h resulted in statistically significant differences over time and among anticoagulants for the analytes evaluated (Table 2). The pH (37°C [98.6°F]) of both the heparin and citrate blood specimens significantly decreased from 0 to 24 h, but no significant changes were seen in the CPDA-1 specimens. Heparin specimens had a significantly higher pH (37°C) at both time points compared to citrate and CPDA-1, but no significant difference was found between citrate and CPDA-1 specimens. The pCO_2 (37°C) sig-

Table 2. Blood gas, electrolyte, and glucose values from iSTAT CG8+ analyses at times 0 and 24 h of loggerhead sea turtle (*Caretta caretta*) whole blood stored in three different anticoagulants over time ($N = 8$). Data presented are median (range). Statistically significant differences ($P < 0.05$) among anticoagulants at the same time point are marked with a superscript letter. Statistically significant differences ($P < 0.05$) between time points for an anticoagulant are marked with an *.

	Initial specimens (0 h)			24 h specimens		
Analyte	**Sodium heparin**	**Sodium citrate**	**CPDA-1**	**Sodium heparin**	**Sodium citrate**	**CPDA-1**
$pH_{(37°C)}$	7.380*[a,b] (7.357–7.468)	7.116*[a] (7.057–7.136)	7.085[b] (6.973–7.106)	7.335*[a,b] (7.307–7.369)	7.092*[a] (7.062–7.105)	7.079[b] (7.021–7.107)
$pCO_{2(37°C)}$ (mmHg)	58.95*[a,b] (44.6–64.8)	96.45*[a] (85.1–106.1)	99.95[b] (77.8–107.8)	68.55*[a,b] (62.9–74.1)	101.5*[a,c] (88.9–118.5)	95.7[b,c] (83.6–105.1)
$pO_{2(37°C)}$ (mmHg)	62[a,b] (49–68)	77[a,c] (71–85)	82.5*[b,c] (74–95)	82.5[a,b] (8–90)	90[a,c] (57–99)	119.5*[b,c] (75–137)
HCO_3^- (mmol/L)	35.95*[a,b] (31.7–40.3)	30.25[a,c] (27.4–34.1)	29.15[b,c] (23.8–32.4)	36.1*[a,b] (32.8–41.6)	29.95[a,c] (26.9–35.8)	29.0[b,c] (22.4–32.5)
Na^+ (mmol/L)	152.5 (150–155)	152* (150–154)	151.5* (147–156)	152[b] (146–154)	151*[c] (147–153)	148*[b,c] (131–155)
K^+ (mmol/L)	3.5[a,b] (3.0–3.9)	2.95[a] (2.6–3.3)	2.9*[b] (2.3–3.4)	3.55*[a,b] (3.2–3.9)	4.05*[a,c] (3.3–7.1)	4.85*[b,c] (3.5–9.0)
iCa^{2+} (mmol/L)	1.05[a,b] (0.93–1.13)	0.25[a] (0.25–0.25)	0.25[b] (0.25–0.25)	1.03[a,b] (0.94–1.18)	0.25[a] (0.25–0.25)	0.25[b] (0.25–0.25)
Glucose (mmol/L)	6.2*[a,b] (5.2–7.1)	5.4[a,c] (4.4–6.3)	30.5[b,c] (22.6–36.2)	5.5*[a,b] (4.8–6.5)	5.0[a,c] (4.6–5.9)	30.5[b,c] (22.8–35.1)

[a] Significant difference between sodium heparin and sodium citrate specimens.
[b] Significant difference between sodium heparin and CPDA-1 specimens.
[c] Significant difference between sodium citrate and CPDA-1 specimens.
* Significant difference between 0 and 24 h.

nificantly increased at 24 h in both heparin and citrate specimens. The heparin pCO_2 (37°C) was significantly lower than the values of the other two anticoagulants. The pO_2 (37°C) significantly increased over time only in the CPDA-1 stored blood specimens. CPDA-1 specimens also had a significantly higher pO_2 (37°C) at both 0 and 24 h as compared to heparin and citrate specimens. The pO_2 (37°C) was significantly different among all three anticoagulants at both time points.

Heparin specimens significantly increased in HCO_3^- at 24 h. There were significant differences in HCO_3^- among all three anticoagulants at both time points, because heparin had the highest value followed by citrate and CPDA-1. There were no significant differences among sodium values at 0 h between the three anticoagulants. Sodium significantly decreased at 24 h in both the citrate and CPDA-1 specimens. Sodium values were significantly lower only in CPDA-1 stored blood specimens at time 24 h as compared to heparin and citrate specimens. At 24 h, 3/8 CPDA-1 specimens had potassium values that exceeded the i-STAT instrument range (>9.0 mmol/L) and were recorded at the upper limit of 9.0 mmol/L. Potassium values were initially significantly higher in heparin specimens as compared to CPDA-1 and citrate specimens at time 0 h. At 24 h, potassium values were significantly different among all three anticoagulant specimens with CPDA-1 specimens having the highest median value (4.85 mmol/L). Both CPDA-1 and citrate specimens had a significant increase in potassium at 24 h.

All of the citrate and CPDA-1 specimens had iCa values below the lower end of the i-STAT instrument range of 0.25 mmol/L, as expected based on the mechanism of citrate anticoagulation. These values were recorded as 0.25 mmol/L for statistical analysis. Heparin specimens had significantly higher iCa values at both time points compared to citrate and CPDA-1. There were no significant changes in iCa values between the time points evaluated in any of the three anticoagulants. Glucose differed significantly at both time points among all anticoagulant blood specimens with CPDA-1 specimens having the highest median value. Only heparin specimens had a significant change in glucose concentrations between time points, with the 24 h specimen being significantly lower than 0 h sample.

No grossly visible hemolysis was evident for any of the anticoagulants at 0 h. At 24 h, pink plasma was observed in more CPDA-1 specimens (5/8) than in citrate (1/8) or heparin (0/8) specimens ($P = 0.02$). There were, however, no statistically significant differences in the cyanmethemoglobin values among the three anticoagulants at 24 h (Table 3). There were also no differences between the 24 h plasma cyanmethemoglobin values of the three anticoagulants when compared to the time 0 lithium heparin cyanmethemoglobin values. Grossly visible lipemia was still present in specimens at the time of evaluation despite high-speed centrifugation.

Discussion

Our study was designed specifically to investigate the 24 h time window that would be feasible for establishing the need for and transport of loggerhead sea turtle whole blood for transfusions

Table 3. Plasma cyanmethemaglobin values from loggerhead sea turtle (*Caretta caretta*) whole blood stored in three different anticoagulants over time ($N = 5$). Hemoglobin analysis was performed using the cyanmethemoglobin method and Drabkin's reagent. Specimen absorbance was evaluated at 540 nm and cyanmethemaglobin was determined using a standard curve. Data presented are median (range). There were no significant differences.

	Initial specimen (0 h)		24 h specimens	
Analyte	**Lithium heparin**	**Sodium heparin**	**Sodium citrate**	**CPDA-1**
Cyanmethemoglobin (mg/mL)	0.372 (0.310–0.604)	0.250 (–0.020–0.520)	0.328 (0.176–1.318)	0.220 (–0.016–0.316)

between facilities within a practical ground transport radius of each other. This time window would also be applicable to other aquatic facilities maintaining healthy donor sea turtles and other nearby rehabilitation facilities that may be in need of blood products. The narrow study time window of 24 h does not allow for the assessment of long-term storage or banking of loggerhead sea turtle blood using the three anticoagulant preparations selected for evaluation. Yet after only 24 h of exposure to three different anticoagulants, statistically significant biochemical changes in loggerhead sea turtle whole blood were documented.

The PCV of the three individual anticoagulants did not statistically differ over time, but differed among each anticoagulant at 0 and 24 h. This was likely attributable to the resultant dilution of the blood specimens based on the preparation of the anticoagulants used (Stokol *et al*., 2000). Heparin specimens had the highest PCV and heparin tubes contained a spray-dried sodium heparin coating that would not dilute these specimens. The citrate blood collection tubes contained 0.2 mL of sodium citrate liquid solution and the CPDA-1 blood specimens had 0.3 mL of CPDA-1 liquid solution, each of which had significantly lower PCV values compared to heparin. The TS values were likely affected by both dilution and the osmolarity of the different anticoagulants. Total solids are determined by the total organic and inorganic solids contained in liquid solution. The anticoagulant solution CPDA-1 has a higher osmolarity of 470 mOsm/L as compared to 3.2% sodium citrate (436 mOsm/L), which likely accounted for the difference in TS between these two specimens (Rock *et al*., 1998).

Erythrocyte metabolism continues following blood specimen collection, which results in cellular and biochemical changes post-collection. For example, continued erythrocyte cellular metabolism produces lactic and pyruvic acid resulting in a decrease in blood pH (Wardrop, 1995; Sparrow, 2010). Unfortunately, lactate was not analyzed in this study. Carbon dioxide is a byproduct of cellular metabolism and would be expected to increase over time. Both heparin- and citrate-stored loggerhead sea turtle blood had statistically significantly decreased pH and increased pCO_2 after 24 h in our study, suggesting continued cellular metabolism in these specimens. Dextrose, included in CPDA-1, may reduce cellular anaerobic metabolism, which produces lactic acid that lowers blood pH over time (Wardrop, 1995; Mudge *et al*., 2004; Sousa *et al*., 2013; Pignon *et al*., 2014). Because the CPDA-1 specimens lacked statistically significant changes in pH and pCO_2 at 24 h, it suggests that this anticoagulant, through the addition of dextrose, may reduce anaerobic metabolism as compared to the citrate and heparin samples.

CPDA-1 specimens had statistically significant increases in pO_2 at 24 h. This is challenging to explain metabolically but may have resulted from increased dissolution of oxygen from within the specimen tubes during resuspension of the specimens prior to analysis. All of the specimens were handled similarly and both the heparin and citrate specimens had a nonsignificant increasing trend in pO_2 over time. Although temperature can affect dissolution of gases in aqueous solutions, all anticoagulant samples were stored in the same location and temperature throughout the study.

The differences in ionized calcium and glucose between the anticoagulants evaluated are most likely attributable to the addition of specific solutes to these anticoagulants. CPDA-1 specimens likely had higher glucose values because of the dextrose component of CPDA-1. Citrate forms insoluble calcium salts when in solution with calcium ions, inhibiting the activation of the clotting cascade through depleting this clotting factor from solution. Blood specimens stored in citrate and CPDA-1, which both contained citrate, had significantly lower concentrations of ionized calcium compared to sodium heparin stored blood specimens. This finding should be taken into consideration when administering whole blood transfusions to hypocalcemic sea turtles, because a non-citrate anticoagulant may be preferable.

Potassium and sodium ions are important intracellular and extracellular ions, respectively. The transport of these ions across intact cell membranes is regulated by specific sodium–potassium transporters and ion channels. Therefore, cell lysis or leakage can lead to increased concentrations of potassium ions and mild dilution of plasma sodium. Increased plasma potassium values with decreased sodium values are documented in human blood stored over time (Wallas, 1979; Moroff and Dende, 1983). Loggerhead sea turtle blood stored in citrate and CPDA-1 had statistically significant increases in potassium concentrations 24 h after collection. These specimens also had statistically significant decreases in sodium concentrations. Although the osmotic fragility of the loggerhead sea turtle erythrocytes was not directly assessed in our study, our findings suggest that loggerhead sea turtle erythrocytes experience cell lysis or leakage after 24 h storage in citrate and CPDA-1. Grossly visible hemolysis occurred at a higher frequency in CPDA-1 specimens at 24 h. Although there were no statistically significant differences in the cyanmethemoglobin values among the 24 h plasma specimens, lipid remaining in the specimens may have affected analysis. The small size of our specimens after completion of other analyses made ultracentrifugation to reduce lipidemia impractical. Damage to erythrocytes prior to transfusion can have marked effects if administered to a recipient (Patterson *et al*., 2011); thus, evidence of cell leakage in a 24 h period suggests that citrate and CPDA-1 may be less suitable for storage of loggerhead sea turtle blood.

There were several limitations to this study, which may have impacted the results. Because of the number of healthy loggerhead sea turtles available at the time of the study, specimen analysis was limited to a low number. This study would have benefited from a larger sample size. In addition, hematologic and biochemical parameters of loggerhead sea turtles are impacted by the animal's age class, body size, and reproductive status (Friar, 1977; Kakizoe *et al*., 2007; Casal *et al*., 2009; Rousselet *et al*., 2013). This includes PCV, TS, glucose, total proteins, and calcium values (Kakizoe *et al*., 2007; Casal *et al*., 2009; Rousselet *et al*., 2013). Although the majority of study animals were of similar body size and estimated age class, additional evaluation of these anticoagulants and blood from varying age classes and confirmed sexes would provide a more thorough investigation. In addition, a longer blood storage period and additional diagnostic tests such as osmotic fragility and methemoglobin analysis on whole blood would provide further insight to the effects of these anticoagulants on loggerhead erythrocytes. Further work on the appropriate dilution between anticoagulant and blood, transport methods, effects of transport temperature, and longer term storage are also warranted.

This study evaluated the effects of loggerhead sea turtle blood storage in three different anticoagulants over time. There was evidence of continued erythrocyte metabolism in both the heparin and citrate stored blood specimens based on acid-base alterations. Statistically significant increases in potassium values in blood stored in CPDA-1 and citrate as well as changes in plasma color suggest that these anticoagulants may be less suitable for the storage of loggerhead sea turtle blood over a 24 h period. Because heparin-stored loggerhead blood specimens revealed no statistically significant changes in potassium or changes in plasma color, this anticoagulant appears suitable for use for short-term storage of loggerhead sea turtle blood.

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