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# Comparison of Conductance to Volume Equations: the Gain Coefficient α

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*Abstract* — **The conductance catheter technique is used to measure real-time pressure and volume data in a beating heart. There are three competing equations for transforming the raw conductance signal into volume: (1) Baan's equation, (2) The cuvette equation (i.e. Relative Volume Units), and (3) Wei's equation. This paper explores the accuracy of these three equations compared to ultrasound echo in mice, and discusses the reason for discrepancy regarding both Baan's equation and the cuvette equation. We conclude that Wei's equation is the most accurate, because its nonlinear mapping yields volumes in the range of physiologic norms.**

## I. Introduction

Blood volume measurement using conductance catheters has long been the most convenient way for scientists to perform hemodynamic assessment *in vivo* in animals. The field was pioneered by Jan Baan and co-workers, who performed the first experiments in dogs and derived volume from the conductance measured *in vivo* in 1981[1]. Since Baan's early contributions, variations on his conductance to volume equation and calibration methods have appeared, leading to confusion among researchers as to which equation to use, and what the advantages and disadvantages of each equation are. Among these equations, a defining difference is the method for calibration of the gain coefficient, α. Baan's equation uses a stroke volume measurement to calibrate the value of α. The cuvette equation, which is a simplified version of Baan's equation, is a linear mapping between conductance and the volume measured by a conductance catheter in non-conducting cuvettes of known volume, and assumes a constant value of $\alpha = 1$. Wei's equation, which emphasizes the nonlinear relationship between conductance and volume, introduces a variable gain term $\alpha(G_B)$ dependent on the blood conductance $G_B$. This paper explores the differences among the different blood conductance to volume equations and points out their strengths and weaknesses using *in vivo* murine heart data and ultrasound echo as the standard of comparison.

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## II. Methods

### A. Admittance measurement instrumentation

All experiments used the instrumentation outlined in detail in other publications [2-5]. Briefly, a tetrapolar measurement of complex admittance (both magnitude and phase) is made using a 1.2 Fr catheter designed for the size of a mouse heart. AC current at a frequency of 20kHz is injected into the ventricle via the outer two electrodes and the resulting potential difference between the inner two electrodes is measured and compared to the current stimulus to derive the necessary magnitude and phase signals.

### B. Parallel Conductance

A difficult problem in this measurement is the removal of the contribution of the non-blood structures in the heart such as the surrounding myocardium. This problem has been discussed in detail previously [3, 6], and is much more acute in the small mouse heart than in large animals. All equations in this paper deal with the blood conductance only, after the parallel conductance ($G_P$) has already been removed, and the blood conductance is denoted $G_B = G - G_P$ to fit with common terminology. Currently, the hypertonic saline technique originally suggested by Baan is most commonly used to remove a constant value of parallel conductance from the total conductance signal when using the cuvette equation or Baan's equation [7, 8], and the admittance technique is most commonly used to determine the parallel conductance contribution when using Wei's equation [3, 4, 6]. Table 1 below summarizes the method of removal of $G_P$ used with each equation.

Table 1: $G_P$ calculation

| Equation | $G_P$ calculation |
|---|---|
| Cuvette | Hypertonic Saline |
| Baan's | Hypertonic Saline |
| Wei's | Admittance Technique |

The techniques for both hypertonic saline and for the admittance method can be used interchangeably with all equations, because they only affect the $G_P$ term.

### C. Conductance to Volume Equations

Three separate calibrations of the measurement of conductance were performed to determine their affect on the final volume measurement. These calibrations all implement different equations to define the relationship from conductance to volume, and are summarized below:

*1) Cuvette equation*

A plexiglass cuvette block was drilled to have 7 holes of diameters ranging from 1.6mm to 4.39mm. Given that the length between the voltage contacts is L=4.5mm, these holes correspond to volumes which span the expected range of volumes in a beating mouse heart (from 9µl to 68µl). Manufacturer specifications for calibration of conductance catheters suggest filling the cuvette volumes with heparanized blood to deteremine the conductance to volume relationship. The relationship between the conductance measured by the catheter and the volume in these heparanized blood filled cuvettes is then determined by a linear fit, and the slope is used as the value K in the following equation:

$$V = K(G - G_P) \quad (1)$$

where V is volume (µl), K is the calibration constant determined by cuvette calibration which is equivalent to $\rho L^2/\alpha$ in Baan's equation (2) below. G is the measured conductance (µS), and $G_P$ is the parallel conductance (µS) determined using the hypertonic saline technique. In these studies, heparanized mouse blood was not used for the calibration, because heparin changes the conductivity of blood, which renders the calibration inaccurate. Instead, hypertonic saline of conductivity equal to blood drawn at the end of the experiment was used.

*2) Baan's equation*

Baan's equation utilizes the technique of matching the stroke volume (SV) to a reference (usually the integral of flow in the aorta measured by ultrasonic flow probe). This technique ensures that at least the SV will be correct in the final PV loop. Baan's equation is:

$$V = \frac{\rho L^2}{\alpha}(G - G_P) \quad (2)$$

where ρ is the blood resistivity (Ohm-m), L is the length between the voltage measurement electrodes (m), and α is a constant designed to correct for the stroke volume

$$\propto = \frac{\rho L^2 (G_{BED} - G_{BES})}{SV} \quad (3)$$

where $G_{BED}$ is the blood conductance at end diastole (S), $G_{BES}$ is the blood conductance at end systole (S), and $G_{BED} - G_{BES}$ is the measured conductance range at steady state (S), and SV is the concurrently measured stroke volume (L).

*3) Wei's equation*

Wei's equation is very much like Baan's equation, and can be expressed as Equation 2, with two exceptions: 1) The parallel conductance $G_P$ is usually calculated dynamically using the admittance technique to account for wall motion during the cardiac cycle (and is therefore non-constant [6]), and 2) α is a function of the blood conductance

$$\alpha(G_B) = 1 - \frac{G_B(t)}{\gamma} \quad (4)$$

where γ is a constant defined as the larger positive root of the quadratic equation:

$$A\gamma^2 + B\gamma + C = 0 \quad (5)$$

whose constant coefficients are defined as:

$$\begin{aligned} A &= SV - \rho L^2 (G_{BED} - G_{BES}) \\ B &= -SV(G_{BED} + G_{BES}) \\ C &= SV \cdot G_{BED} \cdot G_{BES} \end{aligned} \quad (6)$$

*D. Stroke Volume Sensitivity*

The most prominent difference among the three equations is the calculation of the α term. Because the only other parameters involved in determining α are $G_{BED}$ and $G_{BES}$ (which are dependent on the conductance measurement itself), it is reasonable to quantify the sensitivity of the final volume measurement to the stroke volume. The volume range was calculated for all mice while varying the stroke volume in order to determine the sensitivity of both admittance and conductance volume calculations to an arbitrary stroke volume input.

For example, in each mouse the inputs of blood resistivity, end systolic and end diastolic conductance, and length between the voltage electrodes were measured and input, while stroke volume was swept at an arbitrary value and the volume range plotted against the varied stroke volume.

*E. Murine Studies*

The Institutional Animal Care and Use Committees at the University of Texas Health Science Center at San Antonio and the University of Texas at Austin approved all experiments. A total of 8 mice were studied. The background strain was C57BlkS/J, ages 3-4 months, 26.5 ± 3.5g. Mice were placed on a heated, temperature controlled operating table for small animals (Vestavia Scientific, Illinois). Experiments were performed at a murine body temperature of 37°C.

Mice were anesthetized by administration of 1-2% Isoflurane and were allowed to breathe spontaneously with 100% supplemental $O_2$. The right carotid artery was entered and a tetra-polar micro-manometer catheter (Scisense, Inc., London, Ontario) was advanced into the left ventricle (LV) of the intact beating mouse heart. The right jugular vein was cannulated as well for later administration of hypertonic saline to determine steady state parallel conductance. A total of n = 8 mice were studied. The position and placement of the tetra-polar catheter in the LV was guided by simultaneous imaging with a transthoracic echocardiogram (VisualSonics, Toronto, Ontario). Instantaneous LV pressure- volume (PV) relations were monitored to assure that a physiologic loop was obtained before acquiring data, and final acceptance of the catheter position was based upon both echocardiography and the appearance of the loop. Data acquisition consisted of simultaneous LV pressure, conductance at 20 kHz, and admittance at 20 kHz, and echocardiographic images for later calculation of end-

diastolic volume (EDV), end-systolic volume (ESV), and stroke volume (SV).

With the catheter centered in the LV, a bolus of 3% hypertonic saline was administered n = 3 times per mouse via the right jugular vein, for later determination of steady state parallel conductance, as previously described [2].

The mouse was euthanized via blood removal from an LV apical syringe, and the blood maintained in a water bath at 37°C. A custom tetra-polar surface probe was placed onto the surface of the blood, and blood conductivity was determined. This value of blood conductivity was used in all equations.

## III. Results

### A. SV sweep data

The results of the SV sweep in one mouse are shown below. The data shows that the stroke volume inflates both the end systolic and end diastolic volume values to correct for the stroke volume found using echo when using Baan's equation, but do not significantly change the systolic volume when using Wei's equation. In the study, the stroke volume was 26.8 ± 7.2 µl, and at this stroke volume it is apparent that Baan's equation would overestimate both $V_{ED}$ and $V_{ES}$ by a significant amount.

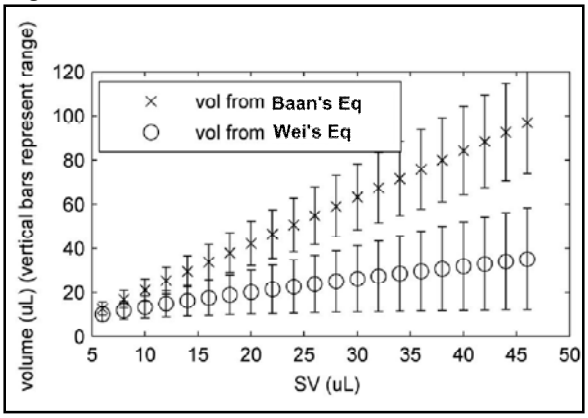


Fig. 1: Volume ranges for varied stroke volume (SV) input. Cuvette results are not shown in this graph because they do not depend on SV. Note that Baan's equation produces unnaturally large volumes when corrected for SV, contrary to Wei's equation.

### B. PV loops from all equations

The PV loops that were generated by the three equations using the same conductance/admittance data are shown below. Notice that Wei's equation comes closest to the ultrasound echo standard, and Baan's equation overestimates the volume as predicted by Fig. 1 above. The cuvette volume underestimates the echo standard consistently, mostly because there is no way to correct the stroke volume. The end systolic volume is actually more accurate for the cuvette equation than it is for Baan's equation, which can be seen more clearly by the Bland-Altman plot of Fig. 3.

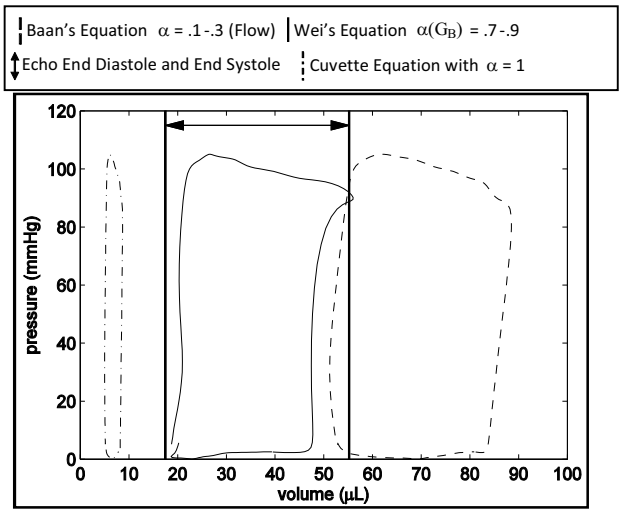


Fig. 2: Representative PV loops from each of the three equations. The echo reference is shown in solid vertical bars.

### C. Bland-Altman Plots

A Bland-Altman plot was calculated to help visualize the data for all mice. The plot shows that Baan's equation overestimates both EDV and ESV by 20.96 ± 38.42 µl, while Wei's equation underestimates both by -6.64 ± 24.3 µl. The cuvette calibration equation is on par with Wei's equation for ESV, with an error of -7.54 ± 16.23 µl, but significantly underestimates the EDV with an error of -29 ± 15.33 µl. This result is significantly larger error with respect to Wei's equation ($p<0.03$).

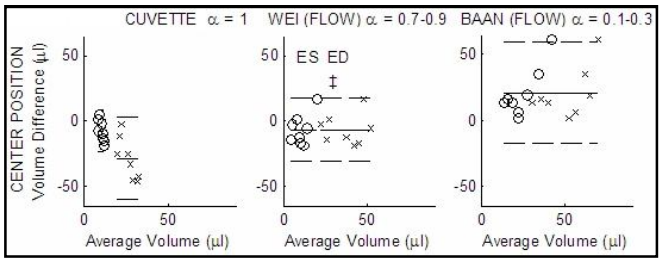


Fig. 3: Bland-Altman plot summarizing the volume error vs. average volume for all mice using each calibration method. The 'x' marks represent $V_{ED}$ and the 'o' marks represent $V_{ES}$. ‡ Less error than cuvette calibrated volume.

## IV. Discussion

### A. "Nonlinearity" of Wei's equation

It is a common misconception that because Wei's equation is nonlinear, that it is somehow less useful to use this more complicated equation in mice, because the benefit is minimal due to the field shape being more approximately linear in smaller animals. While it is true that the relationship between conductance and volume can be approximated as linear in any size animal, absolute volume error increases quickly when correcting for stroke volume with Baan's equation.

### B. Scaling of the "gain term" α

In the Bland-Altman plot, it is possible to see that the α

term is quite different in all three volume plots. This is the cause of varied results when using each of the three conductance to volume equations. A recent paper [8] describes the $1/\alpha$ term as a "gain term," which is a good explanation of why in Fig. 2. we see a gross overestimation of the true echo volume. In order to improve estimates of SV, Baan's equation inflates all values (not just stroke volume) to compensate. Likewise, because the gain term is small in the cuvette equation ($1/\alpha \approx 1$), the resulting $V_{ED}$, $V_{ES}$, and SV are small, and this study proves this underestimation error to be significantly larger than when using Wei's equation ($p<0.03$).

### C. Scalability to larger animals

It is future work to prove these concepts in larger animals, but modeling studies suggest that the concepts scale with animal heart size and catheter size [9]. It is important to note that just because the error in mice increases with larger SV input, one cannot make assumptions about the measurement not being useful in larger animals. Larger animals require a larger catheter affecting the "L" in the equation, which will affect the catheter cell constant.

## V. Acknowledgment

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