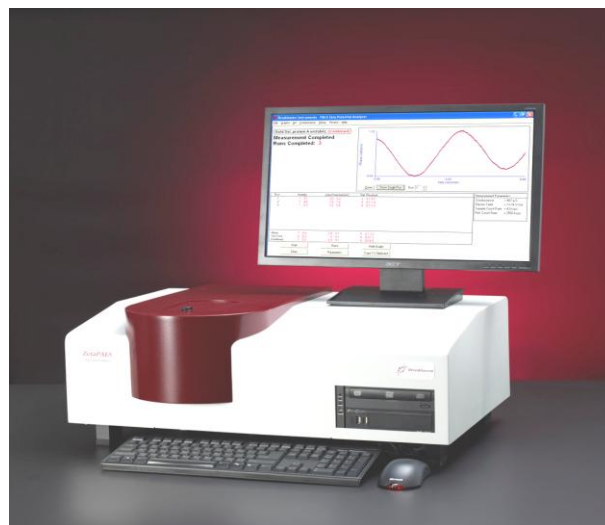
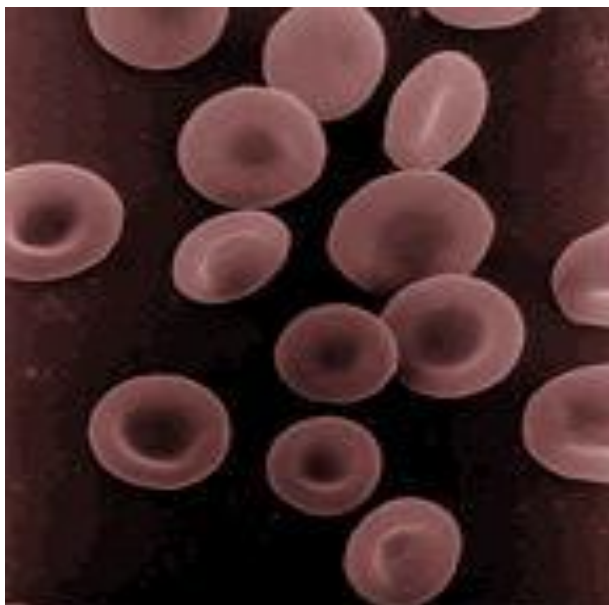


A Review of High Salt Zeta Potential Measurements with the Brookhaven *ZetaPALS*: Red Blood Cells

By Bruce B. Weiner Ph.D., June 2010

Introduction: Zeta potential is a useful property to know for colloids, nanoparticles, and proteins. It is a substitute for the otherwise hard to measure surface charge. The zeta potential is not equal to the surface potential but rather it is the electrostatic potential difference between a rotationally averaged point at the shear, i.e., the slip plane, and an average point out in the liquid. Particles with the same sign zeta potential repel each other and thus maintain dispersion stability. Particles with the opposite sign zeta potential attract each other and thus produce aggregation. And particles with zero or nearly zero zeta potential also aggregate because there is no force strong enough to counteract the always-present attractive forces (ignoring any steric stabilization that may be present).

Two Separate Effects: Zeta potential is determined by two separate effects: charge at the shear plane and free ion concentration (e.g., dissolved salts). In order to monitor the change in zeta potential, one normally adds just enough dissolved salt to keep its effect constant. For



many situations, 1 to 10 mM KNO_3 is sufficient. However, with many biological samples, it is necessary to work at much higher salt concentration, say physiological saline at 155 mM NaCl (0.9% wt/vol).

Problems at High Salt: At such a high salt concentration two main problems arise with respect to the actual measurement. The high conductivity raises the likelihood of Joule heating due to high current. Then, too, such a high ionic strength collapses the electrical double layer, reducing the zeta potential, making it harder to measure. Using PALS¹, phase analysis light scattering, such as the *Brookhaven ZetaPALS*², one has the sensitivity to make measurements under these high salt conditions.

¹ J.F. Miller, K. Schatzel, and B.J. Vincent, *J. Colloid Interface Sci.*, **143**, 532-554, (1991).

² W.W. Tscharnuter, *Applied Optics*, **40**, 3995-4003, (2001).

It is worth emphasizing these are measurements under salt concentration that other instruments, that do not use the PALS principle, fail to make without literally “frying” the sample or the electrode assembly. The electrode assembly is also important as some thin electrodes, supplied by some manufactures peel away from the cell after just a few measurements

Validation at High Salt, RBC's: The question then becomes how to validate such measurements at high salt concentration. Red blood cells (RBC) are a convenient answer since there is a ready supply of this reference material and its electrophoretic mobility (from which zeta potential is calculated) has been known for almost 40 years³. The average is -1.1 mob units ($\mu\text{-cm/V}\cdot\text{s}$, or $10^{-8} \text{ m}^2/\text{V}\cdot\text{s}$). For $\text{pH} > 4.75$, there is apparently no pH dependence.

There is no description of whether this was whole blood or plasma, or the blood type.

Except for the larger white blood cells (WBC) present only in low concentrations (except in the case of inflammation), RBC's are the largest particles in whole blood. They will dominate light scattering and be easily discerned by their characteristic shape under a microscope. An average 7 μm in diameter with a donut-hole shape (see Page 1), they easily stand out.

PALS Measurements Nine Years Ago: The first measurements, using the *ZetaPALS*, were made in May 2001 using 1 drop of whole blood in twice-filtered ($0.2 \mu\text{m}$), 0.9% wt/vol NaCl. The blood type was not recorded. Results: -1.11 ± 0.07 mob units, in excellent agreement with

literature values obtained not with phase analysis laser light scattering but with microscopic techniques.

PALS Measurements Four Years Ago: In September of 2006, 1 drop of Type O+ whole blood in filtered, 2x PBS at pH 7.2 was used. The measured conductance was twice that of physiological saline or 1x PBS. The combined measurements yield -1.09 ± 0.04 mob units using the *ZetaPALS*. Perhaps steric hindrance ameliorates the effect of salt ions at such concentrations.

PALS Measurements Three Months Ago: In March of 2010, 1 drop of Type O- whole blood in filtered, 0.9% wt/vol NaCl was used. The combined measurements yield -1.09 ± 0.06 mob units using a *ZetaPALS*.

Summary: There may or may not be a small differences in average zeta potential between whole blood and isolated RBC's; however, the data reviewed here suggests such differences, if they exist, are smaller than the repeatability in the population and the random measurement error under these difficult conditions (high salt). Nor is there much difference between blood types. So we can conclude that a drop of whole blood in 155 mM NaCl acts like a reasonable reference material for measurements at high salt. Such measurements have been routine for almost a decade using the *ZetaPALS*.

³ A. Zeria and D.J. Wilkins, *Experientia*, **28**, 1435, (1972) as reviewed in “Electrical Phenomena at Interfaces: Fundamentals, Measurements, and Applications”, editors A. Kitahara and A. Watanabe, Marcel Dekker publisher, 398-399 (1994).