

Capillary zone electrophoresis is a method of choice for the measurement of acidity constants of a variety of compounds including pharmaceuticals due to its unique features. In this study the effect of temperature variations on the measurement of pK_a of some weak acid and base compounds was determined. The new method that employs internal standard developed by Fuguet et al [12] was used in this study but was applied to temperatures of 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C

The maximum voltage applied to the electrophoretic runs at all temperatures was decided based on the linearity of the relationship between voltage and intensity with regards to Ohm's law thus reducing the Joule heating effects. The linearity was defined by an $r^2 \geq 0.999$. Based from this the maximum voltage was found to be 18 kV in all the buffers prepared.

The internal standard used was chosen based on the similarity of its pK_a with the pK_a of the compound under study. Using this, the effect of temperature on the measured pK_a was assessed by applying injections to the CZE at various temperature conditions. All the experimental pK_a values were calculated using the literature pK_a values of the internal standard at the corresponding temperature and then were compared with the literature pK_a of the analyte also at the same temperature. Generally all the results have small difference from the literature values. Exceptions are acridine and 4-tertbutylphenol. For acridine the errors can be attributed to the wrong literature values while for 4-tertbutylphenol the errors can be due to its possible degradation as well as of the internal standard in basic media used in the electrophoresis. Comparability of the results with the literature values at different temperatures may indicate well-controlled temperature conditions inside the capillary. These results support the wide application of these new method to give reliable results even at high temperatures of analysis when the internal standard and the analyte are of similar pK_a values.

During the determination of the pK_a of a compound sometimes the temperature in the instrument fluctuates. In this study by using an analyte and an internal standard with similar ΔH values, the temperature range at which the computed pK_a of the analyte remains within a certain limit of error was established. The van't Hoff law states the

dependency of equilibrium constants with temperature. If both the analyte and the internal standard have similar ΔH they will have similar van't Hoff plots and accordingly similar response with variations in temperature. It follows that taking the data for the analyte obtained from different temperatures and the literature pK_a of the internal standard at 25°C, it is expected that the difference between the literature and the experimental pK_a of the analyte will not change at any temperature employed within a certain limit of error. Results of the research show that when the internal standard and the analyte have ΔH that are close to each other, the computed differences between the literature pK_a (at a specific temperature) from the calculated pK_a (at various temperatures) are not significant. On the contrary when the difference in the ΔH is large then the differences in the calculated pK_a of the analyte are correspondingly large.

To summarize, reliability of the results of the pK_a determination at different temperatures is attainable when the pK_a of the internal standard used is close to the pK_a of the analyte. In addition, the similarity in the ΔH is a useful basis to evaluate the effect of temperature changes with the pK_a during the determination at a specified temperature condition.