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Van de Graaff generator for capillary electrophoresis

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Abstract

A new approach for high voltage capillary electrophoresis (CE) is proposed, which replaces the standard high voltage power supply with a Van de Graaff generator, a low current power source. Because the Van de Graaff generator is a current-limited source (10 µA), potentials exceeding 100 kV can be generated for CE when the electrical resistance of the capillary is maximized. This was achieved by decreasing the capillary diameter and reducing the buffer ionic strength. Using 2 mM borate buffer and a 5 µm i.d. capillary, fluorescently labeled amino acids were separated with efficiencies up to 3.5 million plates; a 5.7 fold improvement in separation efficiency compared to a normal power supply (NPS) typically used in CE. This separation efficiency was realized using a simple set-up without significant Joule heating, making the Van de Graaff generator a promising alternative for applying the high potentials required for enhancing resolution in the separation and analysis of highly complex samples, for example mixtures of glycans.

1. Introduction

Capillary electrophoresis (CE) is one of the most powerful separation techniques in the field of analytical chemistry. Because CE can achieve high separation efficiency and resolution, it has been widely used for the separation of complex chemical mixtures and biological samples [1-4], and has been fundamental to progress made in DNA sequencing since the early 1980s [5, 6]. The separation efficiency in CE - described by the number of theoretical plates (N) - is a function of the electrophoretic mobility of the analyte A, μ_A, the electric field strength, E (i.e. the applied potential, V, divided by the total capillary length, L_{tot}), the capillary length to the detector, L_D, and the diffusion constant of the analyte, D, as demonstrated in **Error! Reference source not found.**:

$$\mathrm{N} = \frac{\mu_A E L_D}{2D} \quad (1)$$

This makes E and L_D the experimental parameters most critical to enhancing separation efficiency, and the system's ability to resolve complex mixtures. The resolution, R_s, between two analytes, A and B, scales with the square root of the applied voltage, as indicated in (1:

$$R_S = 0.177 \frac{\mu_A}{(\frac{\mu_A - \mu_B}{2})} \frac{\sqrt{V}}{\sqrt{D}} \quad (1)$$

where μ_B is the electrophoretic mobility of analyte B.

For the separation of extremely complex samples, the efficiency of the separation method needs to be increased, and so an increase in E is a logical approach. Practically, however, increasing E leads to an increase in current and, consequently, excessive Joule heating [7, 8]. Joule heating results from the fact that the heat generated inside the capillary by the passage of current through it can only be dissipated at the surface, causing a radial temperature gradient across the capillary that compromises separation

efficiency [9]. Additionally, when the rate of heat generation exceeds the rate of dissipation, boiling of the background electrolyte (BGE) results in bubble formation, disrupting the electric field and hence aborting the separation [10, 11].

Joule heating can be minimized by decreasing the current passing through the BGE, which can be achieved by increasing the electrical resistance, for example, by reducing the ionic strength and/or the capillary diameter [12]. Optimization of the conditions is therefore also required as a consequence, since reducing the ionic strength will eventually lead to lower efficiencies due to enhanced dispersion, while narrowing the capillary causes problems for detection (in terms of signal intensity) and increased potential of clogging. Once the separation system has been optimized, more complex samples could be resolved by increasing the L_D while preserving E, which thus requires the application of higher voltages.

This provides a practical limitation, as commercially available high voltage (HV) power supplies suitable for CE are typically limited to NPS, with only a few newer power supplies going up to 60 kV. To circumvent this issue, the group of Jorgenson developed an ultra-high voltage CE system based on a Cockcroft-Walton voltage multiplier design, applying voltages as high as 330 kV across a capillary just under 6 m long [13-15]. The system was applied to the separation of peptides, proteins and DNA, with peptide separations at 580 V/cm yielding separation efficiencies up to 10^7 plates; an order of magnitude greater than realized at the traditional applied potential of NPS. However, this significant achievement comes at a cost in experimental complexity in order to minimize Joule heating through enhanced heat dissipation and to prevent dielectric breakdown of the capillary. During operation, the capillary was immersed in oil and a dedicated sampling interface was developed. The group of Riekkola developed a high voltage CE system capable of working at up to 60 kV [16], minimizing Joule heating by reducing the BGE conductivity using alcohols rather than aqueous buffer. Fast separations with efficiencies up to 220,000 plates were realized, but Joule heating remained problematic and the experimental setup required additional electrical insulation to prevent dielectric breakdown [17-20].

Here, we propose an experimentally simpler alternative by employing a standard, inexpensive Van de Graaff (VDG) generator as a power supply [21]. A VDG is a static electricity generator that can produce extremely high voltages (350 – 900 kV) from the friction between a rubber belt and rollers made from two different materials. Because of their design, these high voltages can only be realized at low currents (10 – 60 µA), making VDG generators unattractive for most applications. For CE, however, low currents imply an inherent advantage of producing minimal Joule heating. In the work presented here, the use of a VDG as power source in CE is explored, maximizing the voltage by maintaining high electrical resistance across the capillary through optimization of the BGE ionic strength and capillary diameter. Using 2 mM borate buffer and a 90 cm long, 5 µm i.d. (inner diameter) fused silica capillary, potentials of up to 104 kV were realized, leading to separation efficiencies of up to 3,480,000 plates using a rather simple and inexpensive experimental setup.

3. Materials and Methods

3.1 Reagents

Sodium hydroxide, sodium tetraborate, fluorescein isothiocyanate isomer I (FITC) and amino acids (L-glutamic acid (Glu), L-glutamine (Gln) and L-alanine (Ala)) were purchased from Sigma-Aldrich (Germany). A 100 mM tetraborate (borax) buffer at pH 9.21 was used as a stock solution for preparing the background

electrolyte (BGE). A sample stock solution containing a mixture of 5 mM of each of the 3 amino acids (AAs) was prepared in BGE. The AAs were labelled with 5 mM FITC and stored in the dark for 12 hours [22]. For the CE experiments, the BGE was serially diluted using purified water (Milli-Q® Direct 8, Merck, Germany) to 10 mM and 2 mM. The sample containing the fluorescently labelled AAs was diluted to 100 µM with purified water prior to use.

3.2 Experimental setup

Fused silica capillary with an i.d. of 5 and 50 µm (375 µm outer diameter) was obtained from Postnova (Germany) and used in 90 cm lengths (length to detector, L_D = 75 cm). The inlet of the capillary was positioned at a sample introduction stage, and the outlet at a reservoir on a detection stage (Fig. 1A). A 10 µA Van de Graaff (VDG) generator (N-100VB, 25 cm dome diameter, Winsco, USA), which generated a negative charge at its dome, was placed near to the detection stage. The detection stage featured a capillary holder that fixed the capillary in place for laser-induced fluorescence (LIF) detection, as well as a stainless steel BGE reservoir (with a volume of approximately 30 mL) suspended beneath the detection stage by four screws, into which the outlet of the capillary was connected via a one-piece fitting (C360-100, LabSmith, USA) (Fig. 1B). To electrically connect the outlet reservoir to the VDG, a platinum wire was wound around one of the screws holding the reservoir in place and then wound to the dome of the VDG. At the sample introduction stage, sample and buffer reservoirs were prepared from 2 mL microcentrifuge tubes for sample injection and separation processes (Fig. 1C). The reservoirs on both the sample introduction and detection stages were located at the same height to prevent hydrodynamic flow due to hydrostatic pressure. For sample injection during VDG experiments, and for performing control experiments, a conventional high voltage power supply (referred to as a "normal power supply" or NPS hereafter) was employed (CZE 2000, Spellman, USA), which was connected to the sample or buffer reservoirs of the sample introduction stage via a platinum electrode. An electric field meter (Statometer III, HAUG GmbH, Germany) was placed 140 mm from the sphere surface of the VDG to serve as a proxy for the applied separation voltage across the capillary. The electric field strengths applied via both the Van de Graaff generator and the conventional power supply were measured in real-time and the data was collected and stored using the supplied software interface (STOFEN, HAUG GmbH, Germany).

For LIF detection, a setup similar to that established by Hernandez et al. [23] was employed. A modified microscope filter cube (DFM1, ThorLabs, Germany) was fitted with a dichroic mirror and bandpass filter sets (MF475-35 excitation filter, MD499 dichroic mirror, MF530-43 emission filter, Thorlabs) (see Fig. S1 in the supplementary information (SI)). A section of polyimide coating was removed from the capillary approximately 15 cm from the outlet using a cigarette lighter to create a detection window, and this was fixed in place in the detection stage. Laser light (nominal wavelength of 488 nm, S3FC488, ThorLabs) was passed through the filter cube, coupled into an optical fiber (M31L10, ThorLabs) via a collimator (F220FC-A, ThorLabs) and focused onto the detection window of the capillary using a microscope objective (Epiplan Neofluar 100×, Zeiss, Germany) that formed part of the detection stage. The emitted light from the fluorescent samples was collected by the same objective and optical fiber setup, then passed through the dichroic mirror into a photomultiplier tube (PMT, H10722-01, Hamamatsu, Japan). The fluorescence signal from the PMT was recorded using an oscilloscope (MPO 6014, Tektronix, Germany).

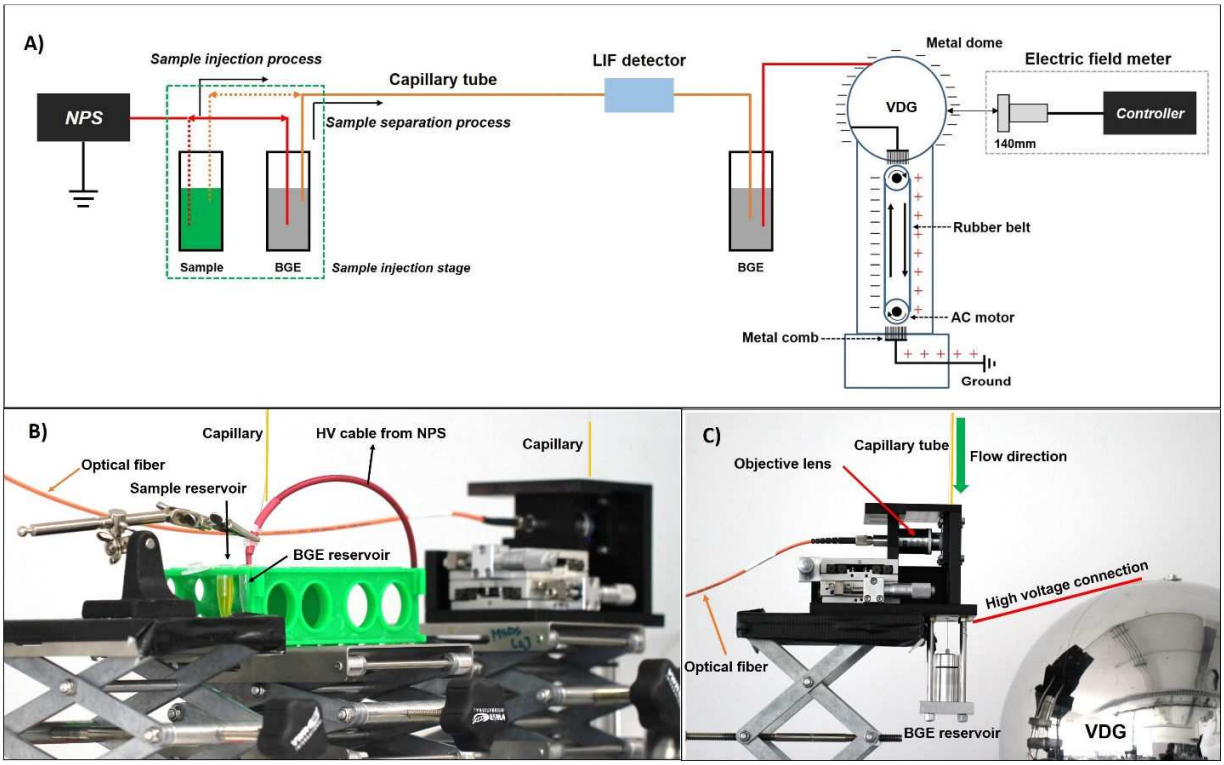

Fig. 1. Experimental setup for Van de Graaff (VDG) generator for capillary electrophoresis. (A) Schematic diagram of the platform, illustrating a capillary tube inserted into a sample or background electrolyte (BGE) reservoir connected to a "normal" DC power supply (NPS) at the capillary inlet, and to a BGE reservoir connected to the VDG at the capillary outlet. The operating principle of the VDG is illustrated on the right, in which a moving rubber belt generates a negative charge that is collected at the dome. Sample injection was achieved electrokinetically using the NPS. In order to measure the electric field strength generated by the VDG, an electric field meter was placed 140 mm away from the dome. (B) For sample injection, the capillary and electrode connected to the NPS for electrokinetic sample injection were temporarily moved to the sample vial and 5 kV was applied for 3 s. After this, the grounded electrode and capillary were moved back to the buffer reservoir. (C) The capillary was aligned with a microscope objective connected to the optical fiber for laser-induced fluorescence (LIF) detection near to the outlet end of the capillary. The outlet of the capillary was inserted into a stainless steel buffer reservoir that was electrically connected to the VDG via a platinum wire.

3.3 Experimental procedure for capillary electrophoresis

The 90 cm long capillary was conditioned with 0.1 M sodium hydroxide via a high pressure pump (L-7100, Hitachi, Japan) for 10 min and washed with deionized water for 10 min. The capillary was then flushed with BGE using the high pressure pump for 10 min and subsequently 30 kV was applied for 10 min to equilibrate under the electric field conditions. For sample introduction, the capillary inlet was manually moved from the BGE reservoir to the sample reservoir, together with the platinum electrode connected to the NPS. For the operator's safety, the VDG dome and other conductive components were touched with a grounded discharge electrode to ensure they were discharged before moving the capillary and electrode. This procedure typically took ~3 s. Sample injection was performed by positioning the capillary inlet and platinum electrode in the sample reservoir and applying 5 kV for 3 s. For the subsequent CE separation, the capillary inlet and platinum electrode were then placed in the BGE reservoir. CE separations were

performed using the VDG and compared to separations conducted using the NPS. Grounding of the experiments with the VDG was achieved by connecting the capillary inlet to the conventional power supply at 0 V, while the VDG was connected to the capillary outlet to apply the high voltage (current limited to 10 μA). For the control experiments, the normal high voltage power supply (NPS) was used to apply +30 kV to the capillary inlet, with the outlet reservoir grounded for separations towards the cathode (the VDG acted as a negative power supply at the opposite end of the capillary).

3.4 Safety of the VDG setup

In previous examples of ultra-high voltage CE, safety features had to be implemented into the setup that complicated the apparatus, e.g. immersion in oil [13,16]. To ensure safety when using the VDG, the apparatus was enclosed in an insulating polymer shield, while a 2 meter "control area" was established around the high voltage setup to ensure that the operator or other persons would not get too close. In order to ensure that the setup was fully discharged prior to the operator handling any of the equipment, the VDG and the other conductive parts of the setup were discharged by temporary connection with a grounded electrode. Despite the large voltages potentially supplied, the VDG used was an educational model that is designed and sold for safe use in high school science courses [24] and limited to an output current of 10 μA. As a rule of thumb, such educational models are only considered potentially dangerous if the dome is larger than 25 cm [25]. Recognising the VDG used here is at the top end of the range considered safe, its stored energy remains well below 1 J, hence a shock is anticipated be "unpleasant and painful" [26], it unlikely to cause actual harm. Therefore, for this prototype apparatus designed to test the feasibility of using a VDG as a voltage source, dedicated safety apparatus or techniques such as oil immersion or insulation were not employed during the experiments described, other than the aforementioned insulation shield. Safety measures to prevent undesirable electrical discharge, along with various other design aspects typically found in CE instruments such as automated injection, could be implemented in future iterations.

4. Results and discussion

With a conventional DC power supply, the output potential is set, and through Ohm's law the current, I, is determined by the ratio of the applied voltage, V, and electrical resistance, R. As long as the instrument-specific current limit has not been reached, the output potential is not affected by the electrical resistance. In CE, minimizing the BGE conductivity is still relevant because reducing the current through the BGE reduces Joule heating. A VDG, in contrast, is a current source, meaning that the current is set by the instrument and the applied potential arises from the product of this current with the electrical resistance. Theoretically, maximizing the electrical resistance of the capillary would result in a maximal voltage output from the VDG. Maximizing the potential supplied by the VDG is hypothesized to yield an optimum separation performance. In the following sections, the effect of capillary diameter and BGE concentration on the separation efficiency and migration times of amino acids in CE is discussed.

4.1 Effect of capillary diameter

The electrical resistance, R, of the capillary tube can be expressed in terms of its length, L_{tot}, cross-sectional area, A (determined by πr^2, where r is radius of capillary tube), and the electrical resistance of the material, ρ, as shown in Equation 3:

$$R = \rho \frac{L_{tot}}{A} = \rho \frac{L_{tot}}{\pi r^2} \quad (3)$$

According to **Error! Reference source not found.**, the total resistance of the capillary tube can be increased by increasing the length of capillary tube or by decreasing its internal diameter (i.d.). Because R scales with the square of the capillary radius, and due to the linear relationship of the separation efficiency and electric field strength (**Error! Reference source not found.**), the capillary i.d. was selected as the first parameter of study. A 10-fold decrease in capillary radius yields a 100-fold decrease in A and is therefore anticipated to increase R by a factor of 100. Thus, according to Ohm's law, at a constant current the voltage should also increase by 100 and in turn improve separation performance, while reducing migration times by a factor of 100. To this end, the effect of inner capillary diameter was investigated.

For this study, the well characterized separation of FITC labeled amino acids (AAs: Gln, Ala, and Glu) was selected using a 2 mM borax buffer (pH 9.21) as a low conductance BGE [27]. The separation performance of capillaries with 50 μm i.d. and 5 μm i.d. was compared (Fig. 2 and SI Fig. S2), with the latter being the narrowest i.d. that was readily available. The separation was first conducted using an NPS to provide a reference to the results obtained using the VDG. Reducing the capillary diameter from 50 μm (Fig. 2A) to 5 μm (Fig. 2B) when using the NPS yielded a reduction in migration times of the AAs by, on average, 1.40 (± 0.14) times, for example with that of Gln decreasing from $t_{50\mu m}$ = 506.2 s to $t_{5\mu m}$ = 388.3 s. This was accompanied by an average increase in separation efficiencies of 6.36 (± 2.66) times for the AAs when decreasing the capillary diameter, in addition to an increase in separation resolution between the AAs by a factor of 1.93 (± 0.31).

The separation performance of capillaries with 50 μm i.d. and 5 μm i.d. was then compared when using the VDG platform, with Table 1 showing the migration times and plate numbers for each AA. As a result of the increase in electrical resistance R upon decreasing the capillary diameter from 50 μm (Fig. 2C) to 5 μm (Fig. 2D), and the subsequent increase in the applied voltage V, the migration times of the AAs were reduced by a factor of 5.34 (± 0.56) to give: t_{Gln} = 115.1 s, t_{Ala} = 118.5 s, and t_{Glu} = 143.4 s. This factor is clearly lower than the theoretically expected 100-fold reduction in migration times based on Ohm's law. It is anticipated this may have been caused by a leak current in the experimental set-up (discussed further in Section 4.3). In addition to the reduction in migration times when reducing the capillary diameter, the number of theoretical plates increased for each of the amino acids. For example, the plate numbers for Gln increased 34.80 times when using the 5 μm capillary ($N_{5\mu m}$ = 3.48 × 10^6) compared to the 50 μm capillary ($N_{50\mu m}$ = 0.10 × 10^6 plates), while those for Ala and Gln increased by 12.67 and 5.84 times, respectively. Together with the separation efficiency, the separation resolution also increased for of the amino acids (Table 2), with an average improvement factor of 4.50 (± 0.44). The signal-to-noise (S/N) ratio was also found to increase for the narrower 5 μm i.d. capillary, as was expected due to the lower volume of injected sample, but the LIF system was sufficiently sensitive for the purpose of this work.

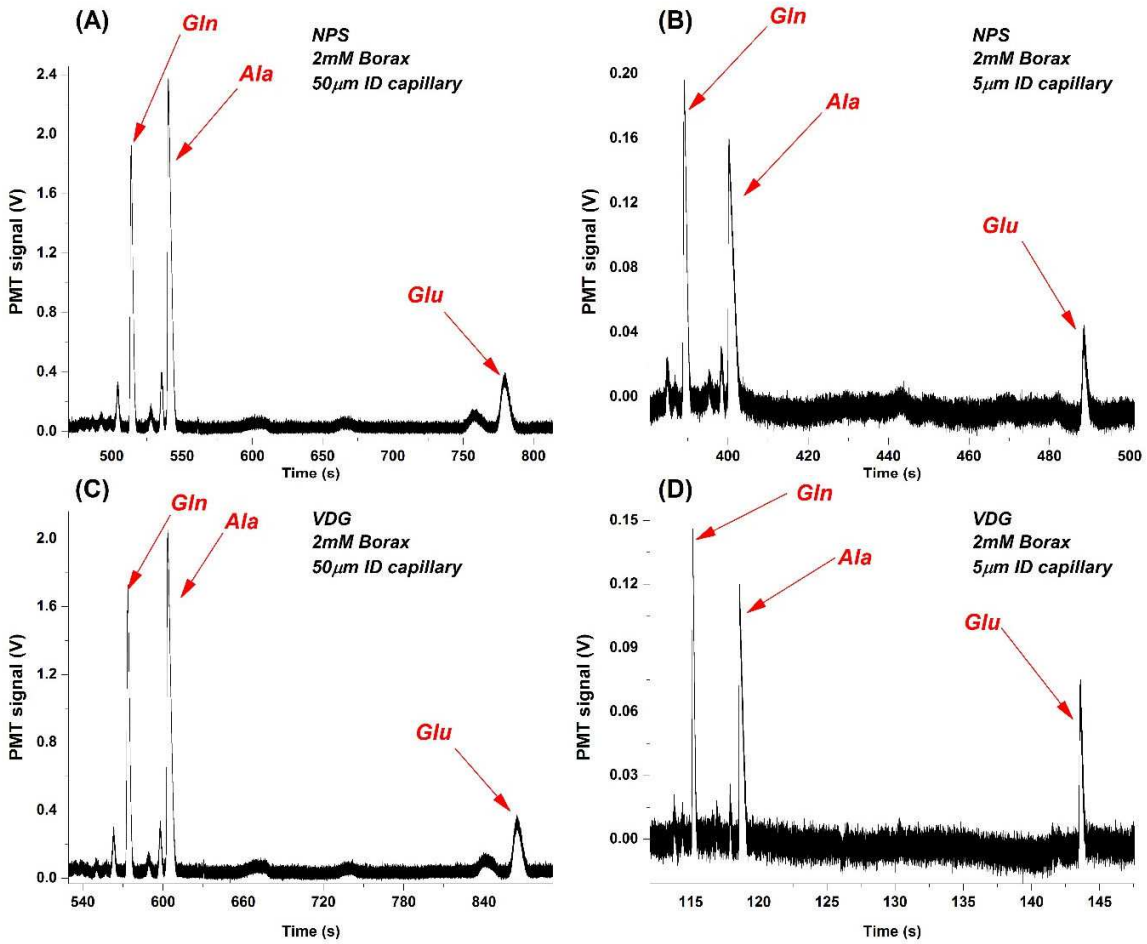

Fig. 2. The effect of capillary diameter on the separation and migration times of three amino acids (AAs) using the VDG CE platform (2 mM borax background electrolyte) and the normal 30 kV power supply (NPS) for comparison. The plots show the separation of AAs achieved with (A) a 50 µm i.d. capillary using the NPS, (B) a 5 µm i.d. capillary using the NPS, (C) a 50 µm i.d. capillary with the VDG, and (D) a 5 µm i.d. capillary with the VDG.

4.2 Effect of BGE concentration

To further enhance output potential of the VDG for enhanced separation efficiency, the effect of BGE concentration on the separation was studied. Experiments were performed using a 90 cm long capillary with a 50 µm i.d.. Initial tests with the NPS alone demonstrated separations with similar migration times (e.g. t_{2mM} = 506.2 s and t_{10mM} = 526.8 s for Gln) and separation efficiencies (e.g. $N_{2mM} = 0.33 \times 10^6$ plates and $N_{10mM} = 0.19 \times 10^6$ plates for Gln) for the 2 mM and 10 mM BGE concentrations. On the other hand, application of the VDG yielded a decrease in the migration times by, on average, a factor of 1.85 (± 0.42) when lowering the BGE concentration from 10 mM to 2 mM borax buffer (Fig. 3, Table 3). This reduction in migration time was accompanied by an increase in separation efficiency (for Gln as an example) from $N_{10mM} = 0.19 \times 10^6$ to $N_{2mM} = 0.94 \times 10^6$ theoretical plates, i.e. a factor of 4.95, when decreasing the BGE concentration (Table 3), while that of Ala increased by 5.89 times from $N_{10mM} = 0.19\times 10^6$ to $N_{2mM} = 1.12\times$

10^6. The separation efficiency of Glu, however, appeared to decrease slightly from N_{10mM} = 0.16 x 10^6 to N_{2mM} = 0.14 x 10^6 plates, but when the efficiencies for the AAs were considered with their migration times, the plate counts per second were notably higher in each case (Fig. 4A). Despite the decreased migration times and mostly increased separation efficiencies, the separation resolution between the three AAs was not found to appreciably increase (Table 4). Reducing the BGE concentration from 10 mM to 2 mM only increased the average resolution by a factor of 1.12 (±0.16), while for the separation of Gln and Ala the resolution was actually slightly reduced.

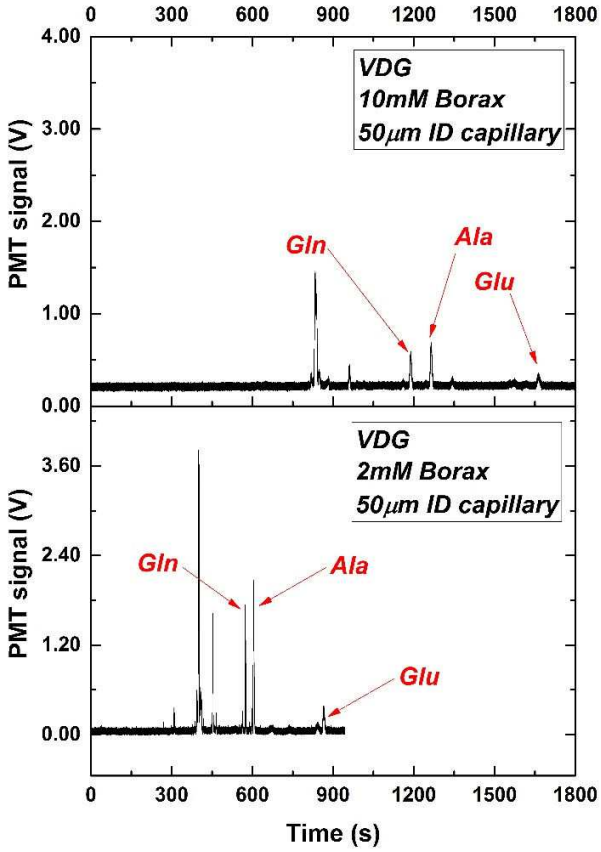

Fig. 3. The effect of background electrolyte (borax buffer) concentration on separation and migration times of amino acids using the VDG CE platform (50 µm i.d. capillary). Electropherograms show separations in (A) 10 mM borax buffer, and (B) 2 mM borax buffer.

Based on the results described above, the maximum output potential from the VDG was expected to be obtained using 2 mM BGE and a capillary i.d. of 5 µm. Indeed, the migration times decreased to 30 % of those achieved using the reference separation at 30 kV (with the conventional power supply) (Table 5), and a separation efficiency of 3.48 × 10^6 theoretical plates was obtained for Glu; an approximately 3.38 fold improvement over the 30 kV reference separation at 1.03 x 10^6 plates. For the other amino acids (Glu and Ala), the separation efficiencies were 1–2.7 times higher ($N_{Ala,VDG}$ = 2.28 × 10^6 and $N_{Gln,VDG}$ = 1.93 × 10^6)

than those obtained using the reference method, and yielding far higher plate counts per second (Fig. 4A). Likewise, the separation resolution of the AAs was found to improve by a factor of 2.38 (± 0.22) when using the VDG platform (Fig. 4B, Table 6) compared to the NPS. The realized separation efficiency for the AAs, however, was still below the $2.7 \times 10^6 – 6.1 \times 10^6$ plates reported by the Jorgenson group for ultra-high voltage CE [13], but was achieved using a far simpler experimental set-up. The repeatability of the migration times obtained with the VDG was good, with relative standard deviations (RSDs) less than 1 % (n = 5) for all amino acids. This is comparable to the repeatability obtained using the NPS (all AAs <1.5 % $RSD_{n=5}$). Electrical breakdown of the capillary was found to occur in all cases where the capillary was exposed to the VDG output for >100 min (data not shown), which may have been caused by, or at least accentuated by, a leak current in the system (see Section 4.3 for further discussion). Despite its impressive results, the application of the ultra-high voltage platform of Jorgenson et al. [13] has been constrained by its significant complexity and expense. Our method, on the other hand, with its very simple and inexpensive setup, significantly lowers the entry barrier for research groups that wish to exploit the high efficiency gains associated with higher voltages, but are deterred by the high initial investment and assembly.

4.3 Prediction and measurement of the applied voltage by the VDG

In CE, the apparent velocity, v_{ap}, of an analyte is a function of its apparent mobility, μ_{ap}, and the applied field strength, E, as shown in Equation 4:

$$v_{ap} = \mu_{ap} E = \mu_{ap} \frac{V}{L_{tot}} \tag{4}$$

From the electropherogram, μ_{ap} can be calculated from the migration time, t_m (Equation 5):

$$\mu_{ap} = \frac{L_{tot} L_D}{V t_m} \tag{5}$$

Assuming μ_{ap} does not vary when using the same BGE, the effective voltage applied by the Van de Graaff generator (V_{VDG}) can be derived from the migration times obtained using the NPS ($t_{m,supplied}$) and the VDG ($t_{m,\,VDG}$), and the potential applied by the conventional power supply ($V_{supplied}$), as illustrated in Equation 6:

$$V_{VDG} = V_{supplied} \frac{t_{supplied}}{t_{VDG}} \tag{6}$$

An electric field meter was used to measure the applied field strength. First, the meter was characterized using the NPS at a controlled temperature (25 °C) and relative humidity (50 % RH) in conjunction with a 90 cm long capillary. As illustrated in Fig. 5A, the field strength for the NPS was around 25 kV/m and was independent of the capillary i.d. (50 µm and 5 µm) and borax concentration (2 mM and 10 mM). Since the electric field meter was positioned 140 mm away from the setup, a correction factor was calculated for the VDG readings to take this into account, based on measurements taken of the NPS. Based on the measurements taken using the electric field meter, the potentials supplied by the VDG were calculated for the 50 µm and 5 µm i.d. capillaries in combination with BGE concentrations of 10 mM and 2 mM borax buffer, and these are summarized in Fig. 5B. The optimal separation efficiency of 3.48×10^6 was obtained with the VDG when using 5 µm i.d. in conjunction with the 2 mM borax buffer, realizing an E of approximately 132 kV/m. This represented an increase in the electric field by a factor of 3.3 compared to the 40 kV/m supplied by the NPS.

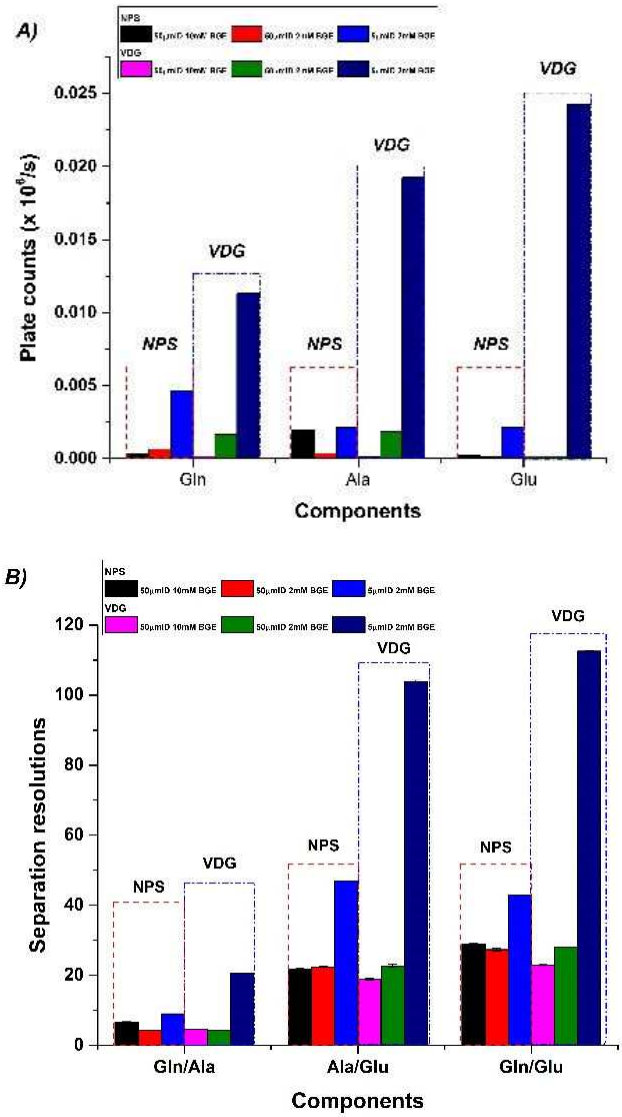

Fig. 4. Comparison of results for the normal power supply (NPS) and the VDG generator with varying background electrolyte (BGE) concentrations (2 mM and 10 mM borax) and internal capillary diameters (5 μm and 50 μm). (A) Separation efficiencies per second for all three amino acids. (B) Separation resolutions between Gln and Ala, Ala and Glu, and Gln and Glu.

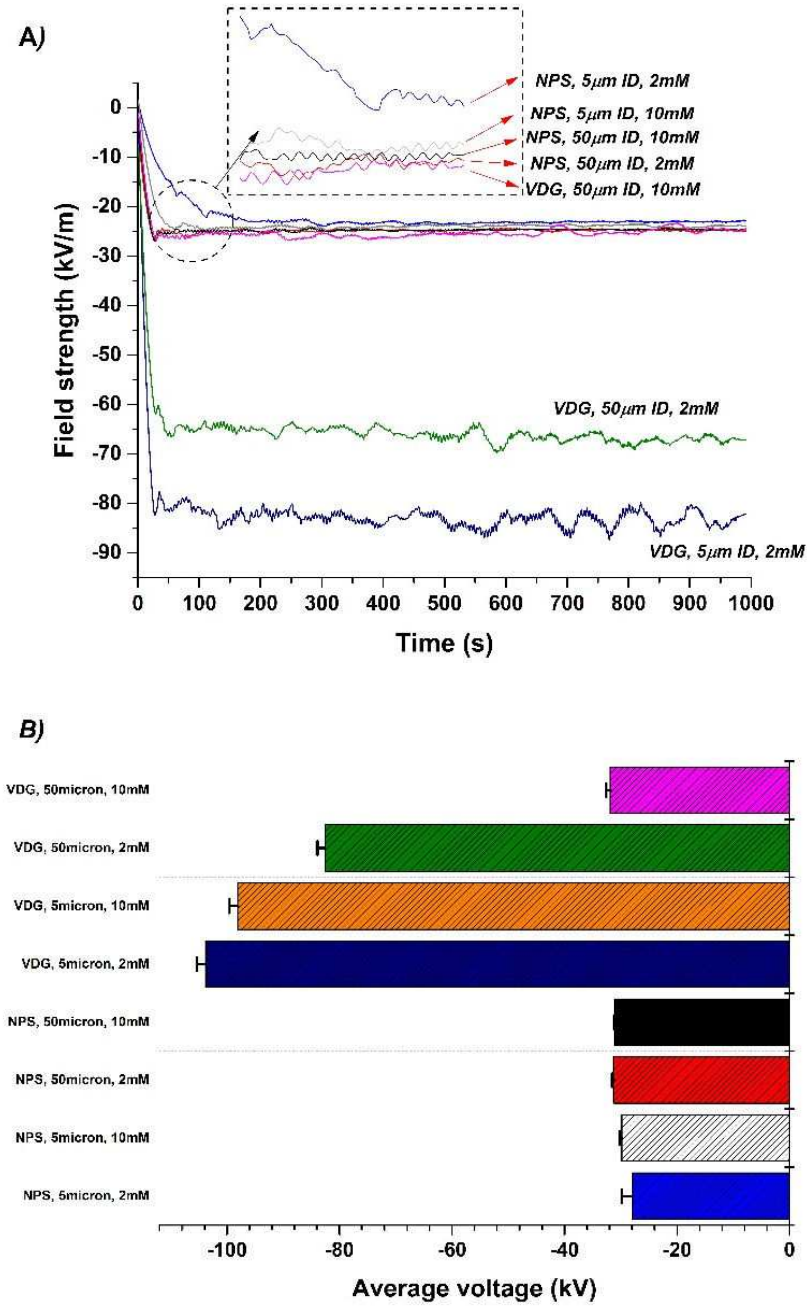

Fig. 5. (A) Electric field strengths, E, of the VDG and NPS for different diameters of capillary tubes and concentrations of BGE, as measured using an electric field meter. (B) Comparison of average voltages for the various combinations of power supply, capillary diameter, and BGE concentration. The average voltage achieved by the VDG was approximately 3.4 times higher than the reference voltage obtained using the NPS.

As predicted by the calculations based on migration times, the measured field strength realized with the VDG (current limited to 10 μA) increased when the reducing the capillary i.d. and the concentration of BGE. For the 10 mM borax buffer, the decrease in i.d. from 50 μm to 5 μm resulted in an increase in E from 32

kV to 104 kV; it was surprising, however, that a similar change in E was not observed when reducing the i.d. for the 2 mM BGE. This may be explained by a leak current. To test this, a current meter was used with the 5 µm i.d. capillary and 2 mM borax, and indicated that only 1.95 – 2.00 µA passed through the BGE. The remaining 8.00 – 8.05 µA may have therefore leaked through the air and the outside of the capillary, compromising the maximum field strength that could be realized, especially at high electrical resistances in the capillary. We anticipate the presence of one or more unidentified parallel resistors in the electronic circuit, limiting the maximum voltage that could be applied across the capillary by the VDG through adjusting the capillary i.d. and the BGE concentration. To achieve even higher voltages, these will have to be eliminated using, for example, measures presented by the Jorgenson group including the immersion of the capillary in an oil tank, or by placing the system in a vacuum chamber. These solutions were not investigated here due to the prototype nature of the apparatus, but could certainly be implemented in future iterations in order to reduce the leak current and, in doing so, further decrease migration times and increase separation efficiency and resolution, whilst also reducing the risk of electrical shock. As it stands, simply combining a VDG with narrow i.d. fused silica capillaries and a low ionic strength BGE enabled the generation of more than 100 kV and allowed separation efficiencies of up to 3.48×10^6 plates in a 90 cm long capillary. An overview of all of the results described here can be found in Tables S1 (migration times and separation efficiencies) and Table S2 (separation resolutions) in the SI.

Compared to previous examples of high voltage capillary electrophoresis using conventional DC power supplies, the Van de Graaff (VDG) generator-based platform developed here represents a far more simple and cost-effective approach. For example, the price range of a commercial HV DC supply is $2,000 to $25,000 (± 30 kV to ± 100 kV). Even for a standard CE separation (performed at 30 kV), a VDG, which retails at around $665, may be a cost-effective alternative to a conventional power supply. However, the user would have to be prepared to routinely determine the applied potential. Additionally, the choice in BGE is limited to those for which the electrical resistance of the BGE-filled capillary exceeds 3×10^9 Ω; the equivalent of a 90 cm long, 50 µm i.d. capillary containing 10 mM borax buffer to maintain equivalent performance to an NPS.

5. Conclusions

An alternative high voltage capillary electrophoresis platform was developed based on the use of a Van de Graaff (VDG) generator to supply the separation potential. Previous high voltage CE techniques have required the use of complex setups or non-aqueous mobile phases in order to avoid problems with Joule heating. By moving away from a voltage supply to a current source, excessive Joule heating was avoided through the inherent current limitation. A 5 µm i.d. capillary with a 2 mM borate buffer of BGE provided sufficiently high electrical resistance to allow the 10 µA limited VDG to generate 104 kV. Separation efficiencies of up to 3.48×10^6 plates were achieved, an improvement factor of around six compared to an NPS under the same conditions. These initial results demonstrate great potential for achieving rapid, high quality separations using an economic and easy-to-use platform. Future work will include the optimization of various separation parameters, including injection plug size, replacement of the sample injection system to eliminate the need for an NPS, and the characterization of the system using neutral markers. An important challenge is to minimize the current leak that presently limits the potential applied, and increases the risk of an unpleasant electrical shock. Furthermore, the presented set-up is amenable to many of the design and safety features typically found in commercial CE instruments, including shielding of the operator from electric shock and automated sample injection. Application areas for the high

efficiency, high resolution separations are expected in the analysis of complex chemical and biological samples, such as for chiral molecule and glycan analysis.

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Table 1 Comparison of migration times and separation efficiencies of three amino acids using between a 50 μm inner diameter (i.d.) capillary and a 5 μm i.d. capillary with the VDG platform (n = 5). The background electrolyte (BGE) consisted was 2 mM borax.

	Migration times (s)		Migration time reduction factor	Separation efficiency (x 10^6 plate counts)		Separation efficiency improvement factor
	50 μm i.d. capillary	5 μm i.d. capillary		50 μm i.d. capillary	5 μm i.d. capillary	
Gln	571.5 ± 13.0	115.1 ± 0.7	4.97	0.33 ± 0.014	1.93 ± 0.006	5.84
Ala	600.8 ± 12.7	118.5 ± 0.7	5.07	0.18 ± 0.010	2.28 ± 0.010	12.67
Glu	857.8 ± 19.5	143.4 ± 1.1	5.98	0.10 ± 0.003	3.48 ± 0.005	34.80

Table 2 Separation resolutions between amino acids when using different capillary inner diameters with the VDG platform (n = 5), and the improvement factor as the diameter was reduced. The background electrolyte (BGE) consisted was 2 mM borax.

	50 μm i.d. capillary	5 μm i.d. capillary	Improvement factor
Gln/Ala	4.19 ± 0.09	20.48 ± 0.06	4.89
Ala/Glu	22.66 ± 0.46	103.83 ± 0.45	4.58
Gln/Glu	28.02 ± 0.74	112.54 ± 0.16	4.02

Table 3 Comparison of migration times and separation efficiencies when using the VDG platform with 10 mM borax and 2mM borax as the BGE (n = 5). The capillary diameter was 50 μm.

	Migration times (s)		Migration time reduction factor	Separation efficiency (x 10^6 plate counts)		Separation efficiency improvement factor
	10 mM borax	2 mM borax		10 mM borax	2 mM borax	
Gln	1190.4 ± 29.1	571.5 ± 13.0	2.08	0.19 ± 0.003	0.94 ± 0.014	4.95
Ala	1264.8 ± 30.7	600.8 ± 12.7	2.11	0.19 ± 0.003	1.12 ± 0.010	5.89
Glu	1666.7 ± 36.2	857.8 ± 19.5	1.94	0.16 ± 0.002	0.14 ± 0.003	1.00

Table 4 Separation resolutions between amino acids using 10 mM and 2 mM borax as the BGE (n = 5), with a capillary diameter of 50 μm.

	10 mM borax	**2 mM borax**	**Improvement factor**
Gln/Ala	4.44 ± 0.06	4.19 ± 0.09	0.94
Ala/Glu	18.84 ± 0.26	22.66 ± 0.46	1.20
Gln/Glu	23.17 ± 0.38	28.02 ± 0.74	1.23

Table 5 Comparison of the performances of the VDG platform and the NPS (n = 5). The BGE was 2 mM borax, and the capillary diameter was 5 μm.

	Migration times (s)		**Migration time reduction factor**	**Separation efficiency (x 10^6 plate counts)**		**Separation efficiency improvement factor**
	NPS	**VDG**		**NPS**	**VDG**	
Gln	388.3 ± 0.6	115.1 ± 0.7	3.37	1.79 ± 0.003	1.93 ± 0.006	1.08
Ala	399.9 ± 0.8	118.5 ± 0.7	3.37	0.86 ± 0.002	2.28 ± 0.010	2.65
Glu	487.2 ± 1.4	143.4 ±1.1	3.40	1.03 ± 0.003	3.48 ± 0.005	3.38

Table 6 Separation resolutions between amino acids when using the NPS compared to the VDG platform (n = 5). The BGE was 2 mM borax, and the capillary diameter was 5 μm.

	NPS	**VDG**	**Improvement factor**
Gln/Ala	8.96 ± 0.01	20.48 ± 0.06	2.29
Ala/Glu	46.95 ± 0.10	103.83 ± 0.45	2.21
Gln/Glu	42.81 ± 0.12	112.54 ± 0.16	2.63