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Short communication

Sodium dodecyl sulfate solution is an effective between-run rinse for capillary electrophoresis of samples in biological matrices

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Abstract

It is common practice in capillary electrophoresis to perform some sort of capillary washing step(s) between separations. In many analyses little consideration is given to optimization of the wash, and typically a rather standard washing procedure is used involving a few minutes wash with 0.1 *M* NaOH followed by a few minutes reconditioning with the run buffer. As an alternative to this procedure, we have investigated the use of wash solutions containing sodium dodecyl sulfate (SDS). This type of wash has been used in the analyses of both small molecules and proteins, with encouraging results. After the SDS wash, the electroosmotic flow has been shown to be restored to values close to normal in a capillary which had previously been coated with plasma proteins. Separation efficiency for a test compound (dextromethorphan) is improved if an SDS rather than a HCl–NaOH wash is used after injection of plasma. In a direct-injection analysis of plasma proteins using a pH 10 borate buffer, an SDS-based washing procedure (total time, 1 min) gave better migration-time reproducibility than an NaOH-based wash, which took 5 min in total.

1. Introduction

Most commercial capillary electrophoresis (CE) instruments offer automated capillary washing steps as part of a standard CE separation method. The rationale for this, as explained in one instrument manual, is that "Non-specific (i.e. adsorptive) interactions can be minimized by removing all traces of contaminants from the capillary surface. . . .this is accomplished by washing the capillary with a caustic solution (0.1 *M* NaOH) before every sample injection" [1]. While this is a reasonable general recommendation, there are cases when other wash procedures may be preferable. In this article we shall show that for bioanalytical separations, rinsing the capillary with a solution containing sodium dodecyl sulfate (SDS) can be a useful alternative.

SDS is widely used to create a micellar phase in one of CE's more commonly used modes, micellar electrokinetic capillary chromatography (MEKC) [2]. MEKC has been successfully used with direct injection of biological fluids such as plasma [3–6]. SDS plays a dual role in these

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assays. As well as providing a micellar pseudophase, it also binds to the proteins, denaturing them (even at sub-millimolar concentrations [7]) and giving them a net negative charge which considerably reduces their tendency to adhere to the walls of the fused-silica capillary. A concentration of 10 m*M* of SDS present in the run buffer is effective at keeping the capillary clean when plasma samples are being analysed by direct injection [6]. Indeed, for this type of assay it may be even be disadvantageous to use a vigorous between-run washing step; Nakagawa et al. [3] reported improved coefficients of variation for the electroosmotic flow velocity when a wash step with 1 *M* NaOH was omitted. Because of the well-known characteristics of SDS as a protein denaturant [7], and its proven effectiveness in preventing adsorption of proteins in direct injection assays [3–6], we decided to investigate its use in a wash solution, to remove adsorbed proteins between separations. In this article, a variety of experiments which illustrate the utility of SDS for capillary washing are described.

2. Experimental

CE separations were carried out using an Applied Biosystems 270A-HT (Foster City, CA, USA) integrated CE system. Capillaries of 375 μm O.D. and of 50 μm I.D. were obtained from Polymicro Technologies (Phoenix, AZ, USA) and cut to the desired lengths. The polyimide coating on the capillary was removed from near one end to provide a detection window. Injections were made by applying a vacuum (17 kPa) to the anodic end of the capillary. Detection was by on-capillary UV absorbance measurements at 200 nm. Data was analysed using a Spectra-Physics (San Jose, CA, USA) Datajet integrator, and stored on a personal computer running Spectra-Physics Winner System software. Unless otherwise stated, all chemicals were of analytical grade, and were obtained from local suppliers. Pooled plasma was obtained from a local hospital, and stored frozen at −20°C until use. Deionized water for all solutions was prepared using a Milli-Q50 ultrapure water system (Millipore, Montreal, Canada).

Analyses of dextromethorphan were performed using a buffer consisting of 50 m*M* solutions of sodium phosphate and sodium tetraborate, which were mixed together to give a measured final pH of 8.5. For most separations, urea was added, to a concentration of 8 *M*. The separations were carried out on an 80.5-cm capillary (61.5 cm to the detector), at a capillary oven temperature of 25°C. The separation potential was 20 kV, giving a current of 25 μA. A 100 μg ml^{-1} solution of dextromethorphan was diluted 1:1 with water or human plasma before hydrodynamic injection for 1.5 s. Between run washes were performed at 68 kPa with 0.1 *M* NaOH for 3 min followed by 3 min with the separation buffer. Other capillary treatment and cleaning is described in the results section.

Plasma proteins were analyzed using a pH 10, 60 m*M* borate buffer, prepared by taking 371.0 mg of $Na_2B_4O_7 \cdot 10H_2O$, and 52.7 ml of 0.1 *M* NaOH solution, made up to 100 ml with water. The separations were carried out using a 43-cm long capillary (22 cm to the detector), at an oven temperature of 30°C. The separation potential was 20 kV, with a current of 51 μA. Between-run washes were performed at 68 kPa with either 0.1 *M* NaOH (1 min) followed by run buffer (4 min), or with run buffer with 200 m*M* added SDS (0.5 min) followed by run buffer alone (0.5 min).

3. Results and discussion

3.1. Dextromethorphan analysis

Dextromethorphan as a test compound was repeatedly analyzed in aqueous solution or spiked into plasma. Under the experimental conditions used, the plasma samples caused contamination of the capillary surface, as revealed by changes in the separation efficiency for dextromethorphan and changes in the electroosmotic flow. A standard between-run wash with 0.1 *M* NaOH was inadequate to completely remove adsorbed species. After a series of plas-

ma samples were analysed, a more vigorous wash procedure was employed, and a series of runs with aqueous samples were then performed to monitor the effectiveness of the wash.

Fig. 1a shows the separation of an aqueous standard sample of dextromethorphan. The peak width at half height is ≈ 0.03 min, and the electroosmotic flow-time (t_{eo}) is 11.0 min. The second electropherogram (b) shows a separation of dextromethorphan in plasma. This is the third of a series of three consecutive injections of this sample. The peak width at half height for dextromethorphan is ≈ 0.24 min; t_{eo} increased with each injection (16.0, 17.5 and 20.9 min, respectively), indicating continuing contamination of the capillary surface. This is despite the presence of 8 *M* urea in the separation buffer, which should be enough to cause denaturation of the proteins, and a between-run wash with 0.1 *M* NaOH.

To try to remove the adsorbed plasma components, a more vigorous wash method was used. This consisted of a 20-min rinse with 1 *M* NaOH, with the capillary oven temperature increased to 50°C, followed by a 10-min wash with run buffer. The normal pre-run wash was then performed, followed by injection of an aqueous dextromethorphan standard; this gave a peak width of 0.07 min, and a t_{eo} of 12.7 min

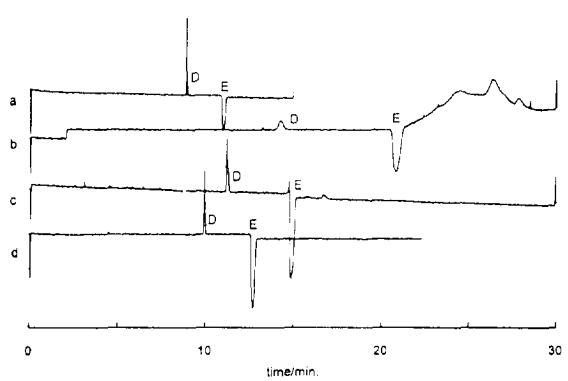

Fig. 1. Separation of dextromethorphan (D) in an aqueous sample in a new capillary (a); in plasma (b); after injection of plasma, followed by a capillary wash with 1 *M* HCl and 1 *M* NaOH (c); after injection of plasma, followed by a capillary wash with 50 m*M* SDS (d). The electroosmotic flow point is marked E.

(electropherogram not shown). The relatively slow electroosmosis indicates that the cleaning procedure was not completely effective. As an alternative, the capillary was then rinsed for 10 min each with 1 *M* HCl and 1 *M* NaOH, with the capillary oven temperature set at 50°C. Fig. 1c shows the separation of an aqueous dextromethorphan standard after this procedure. The half-width for the dextromethorphan peak is 0.08 min, and t_{eo} is 15.0 min. Consecutive analyses revealed a steadily increasing electroosmosis. After leaving the capillary in the run buffer solution overnight, it was found that t_{eo} had decreased to 11.34 min, with a half width for the dextromethorphan peak of 0.04 min (electropherogram not shown). This is quite similar to the measurements made before injecting plasma onto the capillary. From the above results it seems that a wash with 0.1 *M* NaOH at 30°C is inadequate for removing adsorbed plasma components. A more vigorous approach, using 1 *M* HCl and 1 *M* NaOH at 50°C is effective, but the capillary requires considerable re-equilibration time.

The capillary was then challenged with dextromethorphan in plasma once more, and as before a reduction in electroosmotic flow and an increase in peak width was noted, electropherograms being similar to that shown in Fig. 1b. The capillary was then washed for 10 min with the run buffer containing as an additive 50 m*M* SDS, and the aqueous dextromethorphan standards were then re-analyzed. A typical electropherogram is shown in Fig. 1d. Peak width was 0.05 min, and t_{eo} was 12.8 min; t_{eo} was stable over two runs (12.8 and 12.9 min, respectively). Better peak widths are achieved directly after the SDS wash, compared to after the HCl–NaOH wash; t_{eo} is similar. Addition of 0.5 m*M* SDS to the run buffer resulted in an immediate increase in the electroosmotic flow, with a t_{eo} of 11.2 min, and a reduction in the peak width to 0.03 min. Analysis of a plasma sample (no SDS in the buffer), followed by a 10-min wash with 50 m*M* SDS in the buffer, followed by analysis in the urea-containing buffer with 0.5 m*M* SDS added gave identical t_{eo} and peak width to the preceding runs, i.e. 11.2 min and 0.03 min. The

effect of SDS in the run buffer at this submicellar concentration can be explained as being due to the common property of surfactants to form a monolayer at the solution–surface interface [7]. In this way the ζ-potential is maintained since any adsorbed material not removed by the washing procedure is covered by the anionic surfactant.

Comparison was also made of electropherograms before and after plasma samples were injected when urea was not present in the buffer. Before analysis of plasma samples, t_{eo} was 7.5 min, and the dextromethorphan peak width was just 0.02 min. After analysis of plasma samples, the capillary was washed with SDS as described above. After being left overnight, the peak width was 0.03 min, and t_{eo} was 7.8 min. Since the peak widths can only be measured in 0.01 min increments using the present equipment, it cannot be said that there is any significant difference in the measured peak widths.

The results of these experiments with SDS as a wash solution suggest that it is as effective as the 1 *M* HCl–1 *M* NaOH wash, and considerably more effective than just a 0.1 *M* NaOH wash alone. However, improved performance was achieved by including a sub-millimolar concentration of SDS in the run buffer, suggesting that residual surface contamination still existed.

3.2. Plasma protein analysis

The analysis of plasma samples in a variety of buffers with an SDS content varying from 0 to 200 m*M* was investigated to determine the effect of SDS concentration on the separation window in direct-injection analyses [6]. In these studies, multiple analyses with direct injection of plasma were performed under a variety of conditions; using a 60 m*M*, pH 7 phosphate buffer, the electroosmotic flow in a fused-silica capillary was found to decrease, as expected, after repeated injections of plasma samples (Fig. 2). After four direct injections of plasma, the electroosmotic flow reached a stable value, about 40% of the initial flow velocity. Switching to a buffer containing 10 m*M* SDS, the electroosmotic flow immediately increased, and by the third analysis,

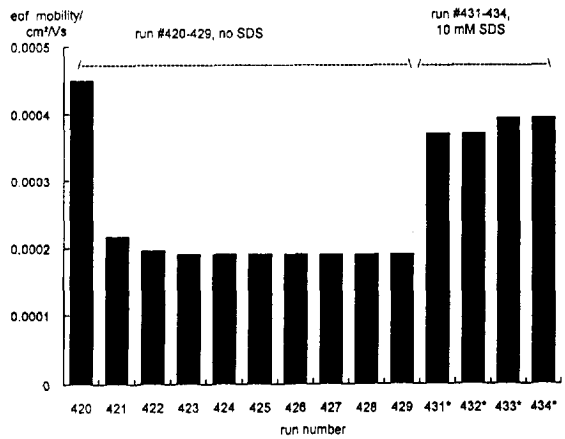

Fig. 2. Rapid reduction followed by a stabilization in electroosmotic flow on injection of a series of plasma samples using a 60 m*M* pH 7 phosphate buffer with no SDS (run nos. 420–429). Addition of 10 m*M* SDS to the run buffer for the subsequent four injections (run nos. 431–434) results in a rapid increase in the electroosmotic flow to a stable value close to that before analysis of plasma. Between run washes were made with the run buffer.

had returned to 90% of its original value (Fig. 2). No special cleaning procedure was performed, so the increase in electroosmosis must be attributed to cleaning of the capillary by the 10 m*M* SDS in the separation buffer. This observation prompted us to investigate the use of SDS as an alternative to a NaOH wash in the analysis of plasma proteins. Details of this assay are reported elsewhere [8], but here we wish to discuss the effectiveness of the washing procedure. Two alternative capillary washing procedures were compared; an SDS/buffer rinse, and a rinse with 0.1 *M* NaOH followed by separation buffer similar to that used in previously reported separations of plasma proteins [9]. A pH 10 run buffer was chosen to reduce protein–capillary interactions [10], thus at this pH, contamination of the capillary surface is less severe in comparison to the previously described experiments using dextromethorphan at pH 8.5. However, protein adsorption is not completely eliminated; if only run buffer is used to wash the capillary between analyses, electroosmosis is almost halved after five injections of plasma.

Details of the migration time and peak area

Table 1
Migration time and peak-area data with two different washing procedures

Component	Migration time (min)		Peak area (arb. units)	
	Mean	C.V. (%)	Mean	C.V. (%)
0.5-min rinse with 200 mM SDS in run buffer, then 0.5-min rinse with run buffer (n = 17)				
HSA	2.24	0.4	$1.10 \cdot 10^6$	1.3
α_1-Globulin	2.08	1.0	$1.25 \cdot 10^5$	9.5
α_2-Globulin	1.92	0.4	$8.67 \cdot 10^4$	7.1
β-Globulin	1.78	1.1	$8.98 \cdot 10^4$	5.8
γ-Globulin (a)[a]	1.68	0.6	$6.14 \cdot 10^4$	12.8
γ-Globulin (b)[a]	1.52	0.5	$2.71 \cdot 10^5$	2.3
1-min rinse with 0.1 M NaOH, then 4-min rinse with run buffer (n = 19)				
HSA	2.03	1.6	$1.11 \cdot 10^6$	2.3
α_1-Globulin	1.87	1.7	$8.12 \cdot 10^4$	11.6
α_2-Globulin	1.60	1.1	$1.47 \cdot 10^5$	5.2
β-Globulin	1.49	1.1	$1.24 \cdot 10^5$	6.1
γ-Globulin	1.44	1.3	$3.40 \cdot 10^5$	1.3

[a] The slightly slower migration when SDS was used lead to recognition of a shoulder on the globulin peak as a separate entity by the automated integration process, hence the two globulin entries a and b.

reproducibility obtained with the two different capillary washes are shown in Table 1. Using SDS, the total washing time was 1 min (0.5 min run buffer + 200 m*M* SDS, 0.5 min run buffer only), while with NaOH the total washing time was 5 min (1 min 0.1 *M* NaOH followed by 4 min run buffer). Considering first the migration time data, it can be seen that the migration time of each component is shorter (due to a stronger electroosmotic flow, $t_{eo} \approx 1.1$ min with NaOH wash, $t_{eo} \approx 1.15$ min with SDS wash), and the migration time reproducibility is significantly poorer when an NaOH wash is used instead of an SDS wash. The poorer reproducibility with the NaOH wash may be explained by variation in the electroosmotic flow and hysteresis effects with changes in pH [11]; during the NaOH wash the change in pH within the capillary from 10 to 13 causes a modification of the capillary surface, and the subsequent 4 min rinse with run buffer is inadequate to allow complete re-equilibration. Indeed, when a shorter buffer rinse was used, the electroosmotic flow was lower ($t_{eo} \approx 1.2$ min) and the migration time reproducibility was even worse. For example, with a 1-min NaOH wash followed by a 1-min buffer rinse, the migration time of HSA was 2.36 min, with a coefficient of variation (C.V.) of 3.6%. In comparison to the NaOH wash, minimal re-equilibration time is needed with the SDS rinse procedure.

The C.V.s for the peak-area data are similar for both wash procedures. In this analysis, heterogeneity of the plasma proteins and surface interactions give rise to considerable broadening and incomplete resolution. Thus small changes in the electropherograms from run to run can lead to relatively large variations in peak area due to integration errors, masking any effects due to the washing procedure.

Based on the slight differences in electroosmotic flow and migration times, it is difficult to say whether the SDS wash or NaOH wash is the most effective procedure for removing adsorbed plasma components–both procedures seem to be useful. However, the SDS wash allows a much faster analysis. When optimized for analytical speed, the time taken for separation of all the protein peaks was < 2.5 min. With the SDS rinse, the capillary washing procedure added only 1 min to the total analysis. With the NaOH

rinse, the capillary washing time and re-equilibration took twice as long as the actual separation.

Surfactants other than SDS, both anionic and cationic, are capable of the same cooperative combination with water soluble proteins which leads to denaturation [7], and in principle they could be used as wash solutions. However, since SDS is quite effective in this role, and is cheap and widely available, it is questionable as to whether investigation of other surfactants is worth the effort. Cationic surfactants are unlikely to be generally useful because of problems in achieving reproducible electroosmotic flow due to significant changes in the interfacial ζ-potential caused by the adsorbed surfactant [12].

4. Conclusions

SDS may usefully be employed as a wash detergent when analysing samples which cause contamination of fused-silica capillaries due to adsorption of proteins. In such cases, a wash with SDS is more effective than the typically-performed wash with 0.1 *M* NaOH. The major advantage of the SDS wash step is that the overall wash process is quicker, since less re-equilibration time is needed to achieve a stable electroosmotic flow.

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