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Note

Reverse iontophoresis of lithium: electrode formulation using a thermoreversible polymer

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Abstract

This work investigated the use of a thermoreversible gel as a collector vehicle in reverse iontophoresis applications. A 20% (w/w) aqueous gel of Pluronic F127 was a suitable receptor medium to be used at the cathodal chamber. In vitro iontophoresis experiments investigated the simultaneous extraction of lithium (analyte of interest) and sodium (used as an internal standard) into either a control buffer or a gelled receptor. The gelification process at room temperature provided a suitable consistency and contact with the skin surface during the iontophoresis experiments. Subsequent cooling of the gelled solution to 4 °C allows an easy recovery of lithium and sodium for later quantification. Both the lithium extraction fluxes and the lithium to sodium ratio of extraction fluxes were linearly related to the subdermal lithium concentration. On the whole, the results show that thermoreversible polymer solutions offer a simple and convenient way to handle samples in reverse iontophoresis studies.

Keywords: Pluronic F-127; Thermoreversible polymer; Reverse iontophoresis; Lithium; Non-invasive monitoring

1. Introduction

Reverse iontophoresis has been proposed as an alternative procedure for non-invasively sampling drugs through the skin. Lithium is a first-choice mood stabilizer for preventing relapses in bipolar patients; further, it is the only mood stabilizer for which a preventive effect on suicidal risk has been demonstrated [1]. Regular lithium monitoring is required to ensure efficacy and to prevent adverse effects [2]. Previous work in vitro and in vivo has shown that lithium could be quickly and efficiently extracted via iontophoresis [3,4]. Furthermore, this research has suggested that pharmacokinetic parameters of selected drugs, such as half-life, may be estimated from the iontophoretically extracted flux profiles. The use of sodium as an internal standard to normalize lithium iontophoretic extraction flux has also been investigated [3,4].

To date, simple aqueous solutions have been used to fill the electrode chambers on the skin surface into which the analyte of interest is extracted by iontophoresis. While this allows easy analytical chemistry, with little or no sample preparation, such vehicles are obviously not well adapted for practical purposes. In fact, the commercial development of iontophoretic drug delivery or sampling devices favours the use of gel or polymer formulations for the electrode compartments [5]. A suitable vehicle for in vivo studies should combine a consistency that allows easy application and removal with appropriate conductive properties, as well as facilitating the subsequent analytical steps. It should be noted that an 'off-line' assay of the drug is probably

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sufficient for drug monitoring and pharmacokinetic applications (i.e. an 'on-board' biosensor as, for example, in the GlucoWatch Biographer® is not obligatory [6]).

The specific aim of this work was to formulate a gel or polymer-type collecting vehicle convenient for lithium reverse iontophoretic monitoring. As thermoreversible gels have been proposed as vehicles for iontophoretic drug delivery [7], it was decided to explore the possibility of using them as collection media for reverse iontophoresis. Pluronic F-127, a copolymer of polyoxyethylene and polyoxypropylene, (70/30 w/w; M.W. 11500), forms a gel at room temperature thereby allowing easy topical application and removal [7,8]. Subsequently, on cooling to 4 °C, the gel becomes fluid allowing the potential for facile recovery of the analyte(s) accumulated therein during reverse iontophoresis.

The first step was to formulate a conductive gel stable throughout the iontophoretic extraction period. Next, a dialysis procedure was developed to recover lithium and sodium from the collection gel. Finally, reverse iontophoresis of lithium (and sodium) was performed in vitro to compare the performance of the gel with that of a standard buffer solution.

2. Materials and methods

Materials. 8 M LiCl solution and H_2SO_4 were purchased from Fluka Chemie AG (Buchs, Switzerland). Pluronic F-127, Tris, Tris HCl, MOPS, NaCl, Ag wire 99.9%, AgCl 99%, Pt 99.9% were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).

Gel formulation. Pluronic F-127 gel was prepared by the cold method [9,10]. The required amount of Pluronic F-127 was dispersed in a cold pH 7.4 (Tris 32 mM, MOPS 34 mM) buffer. The solution was continuously stirred at 4 °C until it was clear. For the dialysis experiments (see below) appropriate amounts of lithium and sodium chloride were added to the fluid preparation at 4 °C.

Skin. Porcine ears were obtained fresh from the local slaughterhouse and were cleaned under cold running water. The tissue from the inner face of the ear was then dermatomed (Air Dermatome, Zimmer TM, France Medica, Illkirch, France) to a thickness of approximately 750 m. The skin was either used at once or stored at −20 °C for no longer than 1 month before use [11].

Reverse iontophoresis experiments. The skin was clamped between the two halves of vertical Franz diffusion cells (2.63 cm^2). The lower half was maintained at 37 °C in a water bath and represented the anodal subdermal compartment, which was filled with a pH 7.4 buffer (133 mM NaCl, 32 mM Tris, 34 mM MOPS). LiCl was added to this buffer at one of three concentrations (0.5; 0.9; and 1.4 mM) spanning the therapeutic range of the drug. The upper half of the cell acted as the cathodal collecting chamber and was filled with 3 ml of either pH 7.4 buffer (32 mM Tris, 34 mM MOPS) or 20% (w/w) Pluronic F-127 in the same buffer. The iontophoretic extraction consisted of a single 90 min application of direct constant current (1 mA) delivered via Ag/AgCl electrodes connected to a power supply (Kepco 1000 M, Flushing, NY). At the end of the iontophoretic procedure, the cells were placed for exactly 6 min at −20 °C to provoke the vehicle transition into the fluid phase. This time was fixed in order to ensure the same contribution of passive diffusion to the total transport in all replicates. The fluid receiver solutions were then homogenized and removed for analysis.

Dialysis. A dialysis method was used to recover sodium and lithium from the Pluronic F-127 vehicles. Side-by-side Teflon cells (1 ml compartment volume and 0.79 cm^2 dialysis surface) were employed. A cellulose dialysis membrane (Spectra/Por® 6, MWCO 2000, Spectrum® Laboratories, Inc., Rancho Dominguez, California, USA) was clamped between the two compartments with the assistance of two silicone rings. 1 ml of a fluid Pluronic receptor solution and 1 ml of a pH 7.4 buffer solution (32 mM Tris and 34 mM MOPS) were placed into the donor and receiver compartments, respectively. The ensemble was kept at 4 °C and continuously stirred for 12 h. Other dialysis times were also studied (3, 6 and 24 h).

Assay. Lithium and sodium analysis were quantified by ion chromatography. A Dionex DX-600 (Dionex, Voisins le Bretonneux, France) equipped with a GP-50 pump (1 ml/min), a AS-50 thermal compartment (25 °C), a ED-50 detector (61 mA), a CS-16 column and a 6 mM H_2SO_4 mobile phase were used. Peak integration was performed with a Chromeleon TM v.6.4. software (Dionex Corporation, Sunnyvale, CA). Retention times of lithium and sodium were 6.8 and 11.8 min, respectively.

Statistics. Linear regressions were performed using Software Prism 4 (GraphPad Software, San Diego, CA). All regressions shown in this work were significant ($P < 0.01$). Data are presented as the mean and standard deviation of at least four replicates.

3. Results and discussion

The effectiveness of the 20% w/w Pluronic F-127 buffer solution to act as an iontophoretic vehicle was first investigated. As well as a sufficient electrical conductivity, the formulation must permit the appropriate electrode reactions to proceed throughout the period of iontophoresis. Silver is reduced at the cathode, a reaction which requires only that the AgCl coating of the electrode is sufficient. Thus, the electrolytes added to the Pluronic solution (Tris and MOPS) served only for buffering and conductivity purposes. Overall, the Pluronic F-127 solution worked very efficiently as a cathodal formulation into which lithium and sodium (and, presumably, other cationic, neutral and zwitterionic analytes) could be extracted.

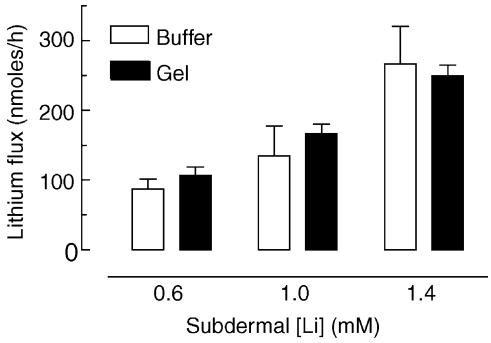

Fig. 1. Reverse iontophoretic extraction fluxes of lithium into either an aqueous or a gel collection medium. The data represent the mean ± SD of four replicates.

Ion chromatography requires aqueous samples and it was therefore necessary to perform a simple dialysis procedure to separate the two cations from the polymeric vehicle. First of all, the linearity of the recovery process was verified. Reference gels were prepared to which different concentrations of lithium (20–100 μM) and sodium (2–10 mM) had been added. The ranges of concentrations were based upon the iontophoretic extraction efficiencies observed for the cations in previous work [3,4]. After dialysis for 12 h, the cationic content of the aqueous solution was quantified. Both sodium and lithium recoveries were linear ($r^2 > 0.99$ for both cations). This simple procedure could be used, therefore, to process the samples. While other dialysis times (3, 6 and 24 h) yielded equally good results, a 12 h duration was preferred for practical reasons. The principal limitation of the approach was dilution of the analytes by the dialysis, a possibly critical issue for substances less efficiently extracted than lithium and sodium (in which case a method allowing direct quantification in the gel would be desirable).

Reverse iontophoretic extraction of lithium and sodium demonstrated the performance of the gel as a cathodal receptor media. Control experiments used the same buffer but without the polymer. Figs. 1 and 2 show the lithium extraction fluxes and the extraction flux ratio, J_{Li}/J_{Na}, for the gel and solution formulations as a function of the subdermal lithium concentration. A two-way ANOVA demonstrated, for both J_{Li} and J_{Li}/J_{Na} a significant dependence on

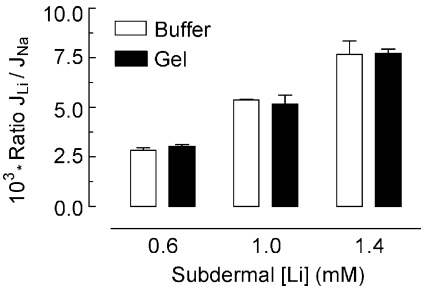

Fig. 2. Ratio of lithium to sodium extraction fluxes into either an aqueous or a gel collection medium. The data represent the mean ± SD of four replicates.

Table 1
Linear regressions of the data shown in Figs. 1 and 2 according to the equation: $Y = K \cdot$concentration + intercept, where Y is either the lithium extraction flux in nmol h^{-1}, or the ratio ($\times 10^3$) of fluxes J_{Li}/J_{Na}, and concentration refers to the subdermal level of the drug in mM

Y	Formulation	K	Intercept	r^2
Lithium flux	Gel	161 ± 12[a]	12 ± 13	0.94
	Buffer	200 ± 36[a]	−38 ± 39	0.75
$10^3 \times J_{Li}/J_{Na}$	Gel	5.3 ± 0.3[b]	$-3.10^{-3} \pm 0.3$	0.97
	Buffer	5.5 ± 0.3[b]	−0.2 ± 0.3	0.97

[a] Units are (flux)/(concentration).
[b] Units are (concentration)$^{-1}$.

concentration ($p < 0.001$). There was no significant difference, however, between the extraction fluxes of either lithium or sodium into the gel and the solution. Linear regressions between both J_{Li} and J_{Li}/J_{Na} and the subdermal concentration of the drug are shown in Table 1. The results are very similar and no statistical differences were found between the slopes and Y-intercepts in any case. This shows that the F-127 gels can be successfully employed as vehicles in reverse iontophoresis and that the analytes were proportionally recovered by equilibrium dialysis before quantification by ion chromatography. It was also noted that the use of sodium as an internal standard resulted in reduced variability, suggesting that some of the experimental error had been eliminated by this procedure.

In summary, this study demonstrates the potential value of thermoreversible gel formulations as collection media for reverse iontophoresis applications. A simple technique, such equilibrium dialysis, can (if necessary) be used to separate the compounds of interest from the polymer to facilitate the subsequent analytical procedures.

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