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Stability of hydrocortisone salts during iontophoresis

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Abstract

The effect of pH on the stability of hydrocortisone sodium succinate (HCSS) and hydrocortisone sodium phosphate (HCSP) during iontophoresis has been evaluated. It was found that the stability of the drugs are dependent on the pH shifts induced in the matrices due to the applied current. These pH shifts are the result of the migration of ions toward the appropriate electrode and due to the oxidation and reduction occurring at the negative and positive electrode, respectively. It was found that short term sampling of the entire receptor cell provided a closer laboratory estimate of the in vivo situation. The technique prevented the drug from being exposed to conditions that cause its degradation. The microenvironment of the matrix may be buffered to prevent pH shifts and protect the model compound from degradation. This was done in the case of HCSP to protect it from hydrolysis/degradation in the receptor cell and ensure an accurate analysis of its transport. It must be noted, however, that buffering of a matrix causes the introduction of ions from the buffer salts. These ions will compete with the drug for the applied current and reduce the drug delivery efficiency of the system, especially when the donor matrix is buffered.

Key words: Iontophoresis; Hydrocortisone; Stability; Transdermal delivery; Hydrocortisone sodium succinate; Hydrocortisone sodium phosphate; Hydrolysis

1. Introduction

Iontophoresis is a well documented technique for the introduction of ionized drugs into the body (Grimmes, 1984; Tyle, 1986; Banga et al., 1988). By the passage of a direct electric current of appropriate polarity through a matrix containing the drug, the compound may be forced from the matrix into the stratum corneum and the underlying tissues. However, the ionization state of compounds used for iontophoresis is dependent on the pH of the solution or matrix into which the drug candidate is incorporated. pH shifts may occur due to the migration of ions under the influence of the applied current or due to water hydrolysis caused by electrode materials such as platinum or nickel (Harpuder, 1937; Adamson, 1976; Siddiqui et al., 1985, 1987). These pH shifts have the potential of affecting the ionization state of the drug candidate and, therefore,

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its delivery efficiency under current. In the case of certain compounds (steroid esters, for example), the combination of current mediated hydrolysis and pH shifts can cause hydrolysis of the parent drug compound and, in extreme cases, their degradation. In order to ensure patient safety and to maintain efficiency during iontophoresis, the stability of the drug must be assured. To ignore this factor may translate into misinterpretation of results.

It was the objective of these studies to evaluate two salt forms of HC, HCSS and HCSP, as potential drugs for in vitro iontophoresis across a synthetic membrane.

2. Materials and methods

HCSS (29F-5010) and HCSP (107F-0948) were purchased from Sigma Chemical Co., St. Louis, MO. Sodium hydroxide (731304) and potassium phosphate dibasic (872406) were obtained from Fisher Scientific Co., Fairlawn, NJ. Agar-ultrapure (61091) was purchased from US Biochemical Corp., Cleveland, OH. Nickel wire (02005-BV), 0.5 mm diameter, 99.99% purity and nickel foil (06508-PV), 0.125 mm diameter, 99.9 + % purity, were purchased from Aldrich Chemical Co., Milwaukee, WI. Platinum wire (EV-1723JT), 0.25 mm diameter, 99.99% purity and sodium chloride (60977) were obtained from Merck and Co., Rahway, NJ. The synthetic membrane MSX-640, a microporous, polyethylene membrane with its pores filled with a hydrophilic polymer, was received as a gift from 3 M Co., St. Paul, MN. All materials were used as received.

2.1. Studies with HCSS

Transmembrane studies

The procedure consisted of placing a freshly prepared 1 mg/ml stock solution of HCSS in the donor compartment of a three-port diffusion half cell (Crown Glass Co., Inc., Somerville, NJ). The receptor cell was filled with a 0.05 M NaCl solution to induce initial conductivity and stirred at a constant rate of 600 rpm to ensure constant hydrodynamics and even distribution of the analytes. The two half-cells, 9 ml each, were separated by a synthetic membrane which had been hydrated in deionized-distilled water for at least 1 h prior to the study (Tu and Allen, 1989a,b). Platinum electrodes were used; the cathode placed in the donor and the anode in the receptor halves of the iontophoretic setup. An identical setup without any electrical connections was used to monitor the drug's behavior under passive diffusion and served as the control. A computerized, programmable power source (Scepter® (Keltronics Corp., Oklahoma City, OK), was used to deliver a constant current of 1.5 mA to the iontophoretic setup for the duration of the study (Allen et al., 1989a,b). The current was applied and samples withdrawn according to the time intervals and methods specified below. All samples were immediately frozen at −20°C until analyzed. The current was terminated after the appropriate interval and the pH of each half-cell measured using a microprocessor based pH meter (Ionalyzer, Orion Research, Cambridge, MA).

The viability of HCSS for long-term iontophoresis was examined by withdrawing a 500 μl volume of receptor cell fluid at time intervals of 1, 2, 4 and 24 h. An equivalent volume of fresh receptor cell fluid was replaced in the receptor cell to maintain sink conditions. This allowed the receptor cell contents at the sampling time to be assayed while leaving the majority of the drug in the receptor exposed to the applied current for the duration of the experiment. This was done to analyze the effects of long-term current application on the stability of HCSS.

The viability of HCSS for short-term iontophoresis was examined by withdrawing the entire receptor cell volume at time intervals of 5 min for 30 min. The entire contents of the receptor cell was replaced with fresh receptor cell fluid. By replacement of the entire receptor cell volume at each sampling interval, the HCSS was subjected to current for an extremely short interval of time. Thus, the short-term effects on HCSS stability could be studied.

HPLC analysis

The samples were analyzed by HPLC to test for HCSS degradation that could have occurred

during the experiments. The instrumentation consisted of a single piston pump (Model 110A, Beckman Instruments, Inc.) in conjunction with a UV absorbance detector set at 254 nm (Model 160, Beckman Instruments, Inc.) and a 20 μl loop injector (Model 210A, Beckman Instruments, Inc.). A Novapak C-18 column (3.9 mm $\times$ 5 μm packing, Waters Associates) was used with a mobile phase consisting of 50 : 50 v/v methanol-water at a flow rate of 1 ml/min. The retention time of HCSS was found to be approx. 1.5 min while that of its primary hydrolysis product, HC, was approx. 6.2 min. The detector output was connected to an electronic integrator (HP3390A, Hewlett Packard).

2.2. Studies with HCSP

Stability indicating assay

A freshly prepared 20 mg/ml drug solution was sealed in ampoules and placed in an oven at 120°C to forcibly degrade HCSP and obtain samples of its breakdown products. Samples were withdrawn at days 0, 1, 2 and 34. The samples were stored in a freezer at −20°C until analyzed. The instrumentation used for HPLC was the same as that used in the analysis of HCSS. A mixture of HCSP and HC was injected into a chromatograph using a variety of mobile phases. A combination of 40 : 60 v/v methanol/0.02 M phosphate buffer, gave the best separation of the two compounds and was selected for the study. The flow rate was set at 1 ml/min. The retention time of HCSP was approx. 2.7 min and that of HC 14.8 min. The following injections were made; HCSP alone, a mixture of HC and HCSP and samples from the forced degradation study. This was carried out to determine not only the presence of the primary hydrolysis or degraded product of HCSP, i.e., HC (Allen et al., 1989a,b; Tu and Allen, 1989a,b) but also the existence of any other breakdown products that might occur.

Transmembrane studies

Agar gel discs (0.7% w/w) were prepared by mixing the gelling agent in cold water in predetermined ratios by weight. The mixture was gently boiled with stirring until the solution became clear. The appropriate amount of drug dissolved in hot water (80–90°C) was added to the solution, which was then brought to the required weight by further addition of hot water. 5 g of this solution was poured into molds fashioned from the donor half of a Franz cell and allowed to cool. Gel discs weighing approx. 5 g, containing 50 mg of active drug, were obtained on cooling.

Franz diffusion cells (FDC-400, Crown Glass Co., Inc., Somerville, NJ) in a nine-unit drive console were used for the study. The cells were adapted for iontophoresis by using specially modified membrane holders (Allen et al., 1989a,b). The synthetic membrane was sandwiched between the two halves of the membrane holder. The receptor cell fluid, 0.02 M phosphate buffer, was stirred at a controlled rate to ensure constant hydrodynamics. The effective modified receptor cell volume was 20 ml. Nickel wire, as the anode, was inserted into the receptor and nickel foil, as the cathode, was placed in even contact with the gel discs in the donor compartment of the cell. The electrodes were connected to the computerized, programmable power source. The iontophoretic transport of HCSP from the agar gel discs was evaluated at 1.65 mA for up to 4 h. The drug transport under conditions of no current was used as the control. 0.5 ml receptor cell solution was sampled at 1-h intervals. The samples were stored immediately at −20°C in a freezer until analyzed by HPLC under identical conditions to those employed with the forcedly degraded samples. pH changes were monitored using an identical setup exclusively for this purpose.

3. Results

3.1. Studies with HCSS

Fig. 1 and 2 are HPLC chromatograms of the receptor cell solution sampled by withdrawing a 500 μl sample and replacing it with an equivalent volume of fresh receptor cell fluid. Fig. 1 represents HCSS transmembrane flux under passive diffusion at 1 and 24 h after the study had begun. Fig. 2 represents HCSS flux under an applied

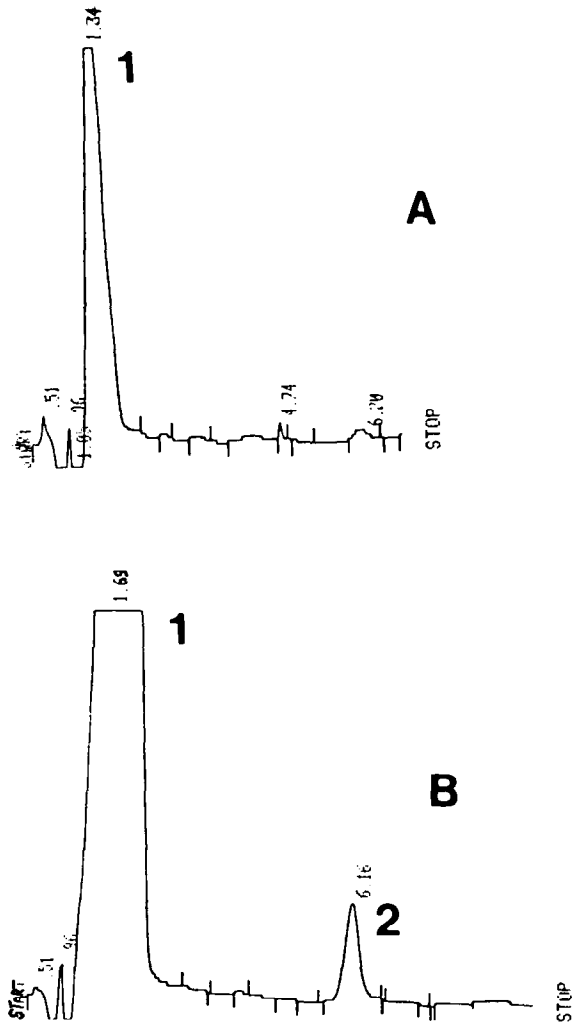

Fig. 1. (A) Typical HPLC chromatogram showing HCSS (1) after 1 h of passive diffusion (sample diluted 1:11) and (B) showing relative HCSS (1) and HC (2) concentrations after 24 h of passive diffusion (sample diluted 1:121).

current at similar time intervals. The chromatograms in Fig. 1A and 2A were obtained at a dilution of 1:11, whereas those in Fig. 1B and 2B were obtained at a dilution of 1:121. The two sets of Fig. 1 and 2 reveal the stability characteristics of HCSS in solution and upon exposure to applied current over both relatively short and long time intervals.

It may be seen from the solitary peak in Fig. 1A that HCSS is relatively stable in solution after 1 h. From Fig. 1B it is evident that only a relatively minor amount had hydrolyzed to HC. Fig. 2A shows that after 1 h of applied current the primary analyte present in the receptor cell was

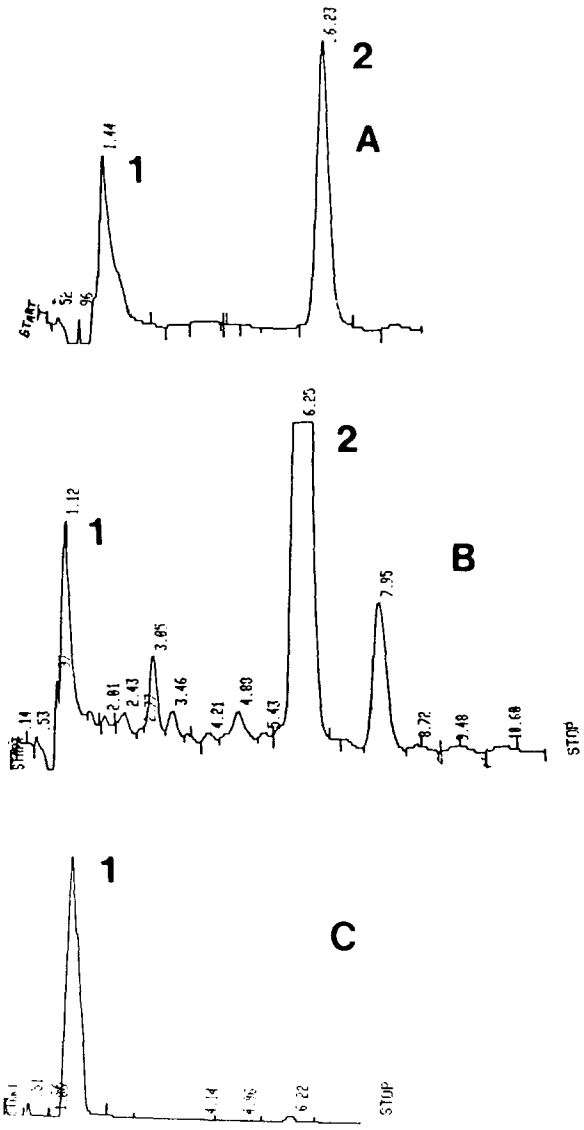

Fig. 2. (A) Typical HPLC chromatograms showing relative HCSS (1) and HC (2) receptor cell concentrations after 1 h of iontophoresis (sample diluted 1:11), (B) showing relative receptor cell concentrations of (1), (2) and other breakdown products after 24 h of iontophoresis (sample diluted 1:121) and (C) showing (1) after 5 min of applied current (sample not diluted).

HC rather than HCSS. This is evident from the larger peak at $t_R = 6.23$ min. At the end of 24 h of applied current the amount of HCSS seen in the receptor cell did not increase proportionately, as demonstrated in Fig. 2B. The presence of a number of additional peaks apart from HC show that HCSS has degraded in the receptor cell. Table 1 lists the pH changes during the course of these experiments. For passive diffusion, the pH changes in the donor and receptor cells before and after the study were minimal. In the case of iontophoresis, however, the pH changes were notably greater.

Fig. 2C shows the HPLC chromatogram of receptor cell HCSS concentration after 5 min of applied current. The receptor cell contents were injected directly without dilution. As seen from the single peak that appears in the chro-

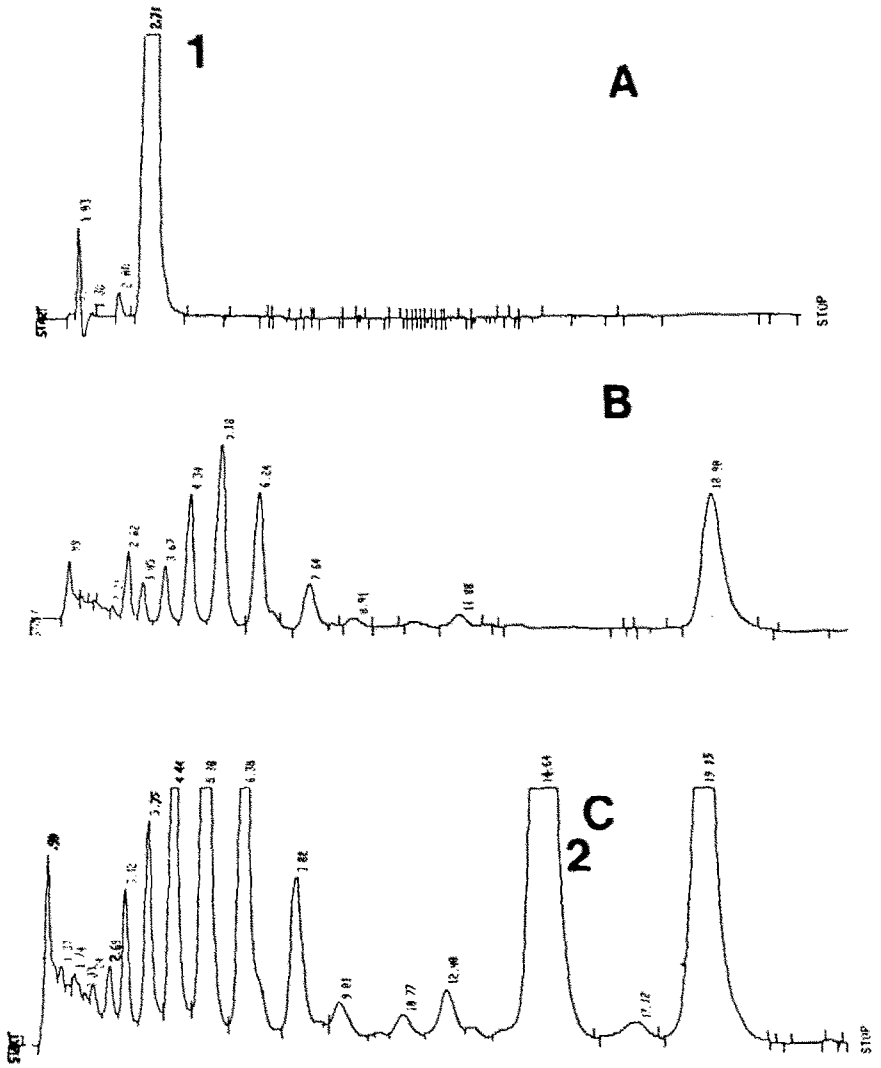

Fig. 3. HPLC chromatograms showing (A) HCSP (1) as the major component in the day zero sample, (B) the 34 day sample consisting of degradation products and (C) the 34 day sample that was spiked with HC (2).

Table 1
Mean pH changes occurring in donor and receptor cells during iontophoresis and passive diffusion of HCSS

	Time (h)	Donor pH	Receptor pH
Passive diffusion	0	7.52	6.61
	1	7.51	6.62
	24	7.31	6.75
Iontophoresis	0	7.52	6.61
	1	8.14	5.83
	24	10.65	2.65

Table 2
Mean pH changes occurring in donor and receptor cells during iontophoresis and passive diffusion of HCSP

	Time (h)	Donor pH	Receptor pH
Passive diffusion	0	8.0	8.0
	1	8.15	7.93
	4	8.07	8.12
Iontophoresis	0	8.0	8.0
	1	11.31	7.43
	4	11.75	7.11

matogram, essentially no detectable hydrolysis or degradation product of HCSS occurred for up to 5 min of applied current.

3.2. Studies with HCSP

Fig. 3A is a chromatogram of the initial sample from the forced degradation study, showing a single peak at $t_R = 2.72$ min corresponding to that of HCSP. Fig. 3B is the chromatogram of the 34 day sample and shows several peaks that correspond to the development of various degraded products. Fig. 3C is a chromatogram of the 34 day sample that was spiked with a concentrated solution of HC. This shows as the peak at $t_R = 14.64$ min, which is the retention time of pure HC.

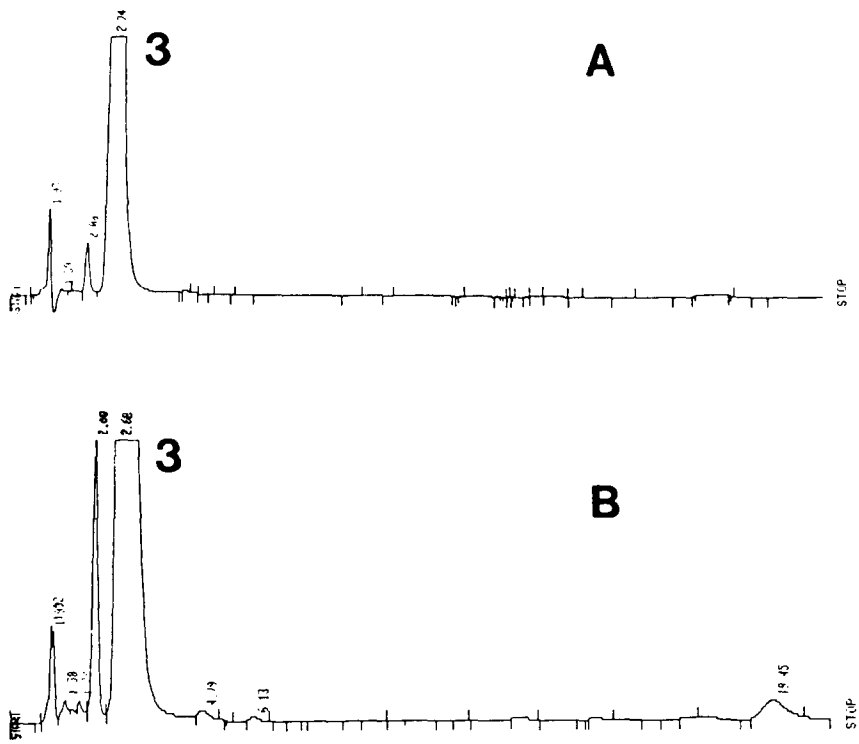

Fig. 4. Typical chromatograms of HCSP (3) after (A) 2 h of applied current and (B) 4 h of applied current. HCSP is the primary peak at 2.72 (A) or 2.63 (B) min with a few breakdown products showing up at $t_R = 2.00$, 4.79, 6.13 and 19.45 min.

Thus, Fig. 3 shows the development of various hydrolysis and degraded products of HCSP, along with their retention times, as indicated by the HPLC assay.

Fig. 4A and b are HPLC chromatograms of HCSP receptor cell concentration, when subjected to an applied current of 1.65 mA. Fig. 4A shows essentially no degradation of HCSP after current application for 2 h. Fig. 4B shows the appearance of minor peaks at t_R approx. 5, 6 and 19.5 min, at the end of 4 h of applied current. Table 2 lists the pH changes in both donor and receptor mediums that occur during iontophoresis and passive diffusion of HCSP.

4. Discussion

4.1. Studies with HCSS

A comparison of Fig. 1A and b (passive diffusion) with Fig. 2A and b (iontophoresis) yields the following information. HCSS, under conditions of passive diffusion, is relatively stable up to 24 h, with only a minor amount being hydrolyzed to HC. Under conditions of iontophoresis, the chromatograms show a significant presence of HC in the receptor cell after 1 h. At the end of 24 h of iontophoresis, significant degradation of HCSS has occurred, as is evident from the appearance of several other peaks in Fig. 2B. Table 1 shows that minimal pH changes occurred during passive diffusion, while the pH changes occurring during iontophoresis were more pronounced. This finding may be linked to Fig. 1 and 2 to provide an explanation of the observed stability of HCSS.

From the literature (Marcus, 1960; Flynn et al., 1970) there are two possible mechanisms that cause HCSS hydrolysis below pH 8 (in our case in the receptor cell). Hydrolysis may occur either by an intramolecular pathway or because of hydronium ion catalysis. Above pH 8 (the donor cell in our case), hydrolysis occurs primarily due to a base-catalyzed mechanism propagated by the hydroxyl ion. The succinic acid side chain in HCSS is a good leaving group that is highly stabilized by resonance. Hence, hydrolysis of HCSP to HC occurs easily with the drug being unstable in solution for long periods of time (Merck Index, 1983).

It is postulated that these pH shifts are the primary factor governing the hydrolysis of HCSS during iontophoresis. These pH shifts are the result of the migration of ions toward the appropriate electrode and due to the oxidation and reduction occurring at the negative and positive electrode, respectively (Adamson, 1976). Passive diffusion conditions had minimal pH shifts and at the essentially neutral pH of both donor and receptor, minimal hydrolysis occurs. Hydrolysis in the donor cell would decrease the amount of HCSS available for repulsion into the receptor cell. Consequently, the amount of HC available in the donor cell for passive diffusion would increase. Hydrolysis in the receptor cell would also decrease the amount of HCSS present, while simultaneously increasing the amount of HC. Pinpointing the nature of the exact mechanisms of hydrolysis of HCSS is beyond the scope of this study. However, the experimental evidence shows that even after one hour of continuous exposure to current, the amount of hydrolysis of HCSS to HC is considerable. Thus, it may be concluded that HCSS is not a viable candidate for long-term exposure to applied current.

The duration of applied current that HCSS is exposed to is 30 min in the donor cell and 5 min in the receptor cell. It was not possible to achieve continuous replacement of the receptor cell fluid that would simulate an in vivo situation. However, replacing the entire receptor cell solution every 5 min does provide a closer estimate to what occurs in vivo than the previous technique and results in exposure of receptor cell HCSS to current for an even shorter period of time. As seen from Fig. 2C, the HPLC chromatogram of the receptor cell solution sampled by this technique shows only one peak corresponding to HCSS. Thus, it may be concluded that this sampling technique enables HCSS to be viable as a model compound for short-term iontophoretic studies.

4.2. Studies with HCSP

Fig. 3B shows that the primary hydrolysis products appear at $t_R = 4.34$, 5.18, 6.24 and 19

min. Fig. 3C depicts the same sample spiked with HC, which appears at $t_R = 14.64$ min. This peak does not correspond with any of the primary degradation peaks in Fig. 3B. It is therefore evident that under the conditions used to forcibly degrade HCSP, the primary degradation product is not HC but some other compound.

The stability of HCSP may also be linked to the pH of the matrix (Marcus, 1960; Flynn et al., 1970). HCSP contains two potential sites for negative charges and, depending on the pH, may exist either as an anion or a dianion. As it has a $pK_{a1} = 2$ and a $pK_{a2} = 6.2$, it exists in its highest concentrations as a monoanion between pH 3 and 6 and as a dianion above pH 7.5. The stability of HCSP depends on the nature of its charge, it being the most stable when it exists solely as a dianion. This may be understood on purely electrostatic grounds, as the dianion cannot be easily approached by a hydroxyl group. Consequently, its maximum rate of hydrolysis occurs when it exists as a monoanion.

The experimental conditions were designed so that minimum hydrolysis would occur in both donor and receptor during current application. Table 2 lists the pH changes that occurred in the setups used for the study. The pH changes occurring during passive diffusion remain essentially the same. During iontophoresis, the donor cell pH rises to 11.31 after 1 h and to 11.75 after 4 h. As HCSP exists almost solely as a dianion at such high pH values, the potential for hydrolysis is almost nonexistent (Marcus, 1960). In the receptor cell, the pH drops to 7.4 after 2 h. This pH is well over 1 unit above the pK_{a2} value for HCSP and more than 90% of HCSP exists as the dianion. Fig. 4A shows good agreement with the literature, displaying only one peak corresponding to that of HCSP (Marcus, 1960).

After 4 h of iontophoresis, the receptor cell pH is 7.11. At this pH value, HCSP will exist primarily as a dianion. However, some portion of the monoanion which is susceptible to hydrolysis also exists. Fig. 4B shows the emergence of minor peaks at t_R approx. 4.8, 6.1 and 19.5 min. These peaks correspond to the major degradation products of HCSP (Fig. 3C). Thus, it may be deduced that the current-induced pH drop has caused some of the monoanion to degrade. However, even at this time period, more than 98% of the peak area belongs to HCSP, indicating minimal degradation (Fig. 4B).

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