Hydrogel-Based Iontotherapeutic Delivery Devices for Transdermal Delivery of Peptide/Protein Drugs

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Hydrogels were synthesized as the drug reservoir matrix for peptide-based pharmaceuticals, and the iontophoretic release and transdermal delivery of three model peptides, insulin, calcitonin, and vasopressin, from these hydrogel-based iontotherapeutic devices were investigated. The swelling behavior of polyacrylamide-type hydrogel as a function of its monomer and cross-linker concentration was studied, and a hydrogel with minimal swelling was synthesized. The release of peptides from the hydrogel matrix was found to follow a Q vs $t^{1/2}$ relationship under passive diffusion conditions, which shifted to a Q vs t relationship under iontophoresis-facilitated transport. The release flux (dQ/dt) of peptides was observed to decline when the electric current was turned off and was resumed when the current was turned on, thus allowing for modulation of drug release by varying the application parameters of iontophoresis-facilitated transport. The permeability coefficients for these peptides across the hairless rat skin were evaluated using the hydrogel formulations prepared from polyacrylamide, *p*-HEMA, and carbopol. A rank order of vasopressin > calcitonin > insulin was obtained in accordance with the order of molecular size.

KEY WORDS: transdermal; iontophoresis; insulin; calcitonin; vasopressin; hydrogel; polyacrylamide; *p*-HEMA.

INTRODUCTION

The transdermal route could permit the noninvasive delivery of peptide-based pharmaceuticals, if the delivery can be improved by chemical enhancers (1), iontophoresis (2), or other means. Hydrogels have been used as iontophoretic electrodes (3,4), although most investigators have used a simple aqueous solution of the drug. However, a hydrogel formulation can also provide an electroconductive base which will prove advantageous for clinical use.

Advantages of hydrogel formulations include ease of application, minimized skin hydration, and convective flow which often accompany iontophoretic delivery. Also, a hydrogel formulation can be designed as a unit dose-type drug-loaded hydrogel patch that permits the daily or weekly dosage replacement while reusing the same iontophoretic device. Further, drug release rates from a hydrogel can be controlled by changing the characteristics of the hydrogel formulation during synthesis. A hydrogel formulation may increase the skin compliance of a transdermal patch, since a hydrogel can absorb sweat gland secretions which, under long-term occlusion, may become irritating. Hydrogel formulations were thus developed and investigated for the iontophoretic transdermal delivery of peptide/protein drugs. *In vitro* studies were conducted first so that drug release kinetics and skin permeation profiles can be monitored without the biological variations of *in vivo* delivery studies.

MATERIALS AND METHODS

Materials

Porcine insulin, thyrocalcitonin (synthetic human calcitonin), and [Arg^8]-vasopressin (acetate salt) were procured from Sigma Chemical Company (St. Louis, MO). For radiotracer studies, ^{125}I-Tyr^{A14}-insulin (porcine) and ^{125}I-[Arg^8]-vasopressin (monoiodinated) were obtained from NEN/DuPont (Wilmington, DE), while [3-(^{125}I)iodotyrosyl-12] calcitonin, human, was obtained from Amersham International (Arlington Heights, IL). Gentamicin, sulfate, bacitracin, and aprotinin were procured from Sigma Chemical Company. Tritiated water (25.0 mCi/g) was procured from NEN (Boston, MA). Polyacrylamide hydrogel was synthesized using acrylamide and *N,N'*-methylene-bisacylamide procured from Sigma Chemical Company. For the preparation of *p*-HEMA gels, 2-hydroxyethyl methacrylate (HEMA), and ethylene glycol dimethacrylate (EGDMA) were obtained from Aldrich Chemical Company (Milwaukee, WI). 2,2'-Azo-bis(2-methyl-propionitrile) or 2,2'-azo-bis-isobutyronitrile (AIBN) was supplied by Fisher Scientific (Springfield, NJ). Carbopol 934 by B. F. Goodrich Co. was supplied by Ruger Chemical Co., Inc. (Irvington, NJ). All other chemicals used for making buffers were of reagent grade. All solutions were made with water purified by a Nanopure water purification system (Sybron, Boston, MA). The water had a resistivity of 16 MΩ or greater. Hairless rats (HRS strain) were obtained from Armed Forces Institute of Pathology (Walter Reed Army Medical Center, Washington, DC).

Analytical Techniques

For radiotracer work, the cold (unlabeled) peptide/protein was spiked with radiolabeled peptide/protein before incorporation into the hydrogel formulation and the samples taken from the receptor compartment were analyzed on a Gamma Counter (Beckman Instruments, Brea, CA). HPLC assay was performed for insulin using a Waters solvent delivery module (Model 590) and WISP (Model 712) (Waters, Milford, MA), attached to an integrator and a variable-wavelength UV detector (Spectraflow 773) (Spectra-Physics, SP 4270, San Jose, CA) using a method modified from Szepesi and Gazdag (5). Separations were performed on a μBondapak C_{18} column at a flow rate of 2.0 mL/min at a wavelength of 215 nm for detection. The mobile phase consisted of buffer:methanol:acetonitrile (at a ratio of 40:50:10). The buffer consisted of sodium sulfate (0.05 *M*) and $NaH_2PO_4 \cdot H_2O$ (0.05 *M*) (adjusted to pH 3.2 with phosphoric acid). The high degree of salt was used to control the degree of ionization on the insulin molecule.

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Synthesis of Hydrogel

Three types of hydrogels, polyacrylamide, *p*-HEMA, and Carbopol 934, were used in this investigation. Polyacrylamide and *p*-HEMA were synthesized by a simultaneous polymerization and cross-linking process, while Carbopol 934 was obtained as a cross-linked polymer which was gelled by neutralizing its aqueous solution with sodium hydroxide. The hydrogels were prepared in Teflon molds, which served as drug reservoir electrodes and were designed to fit between the receptor and the donor half-cells of the Valia-Chien (V-C) skin permeation system.

A citrate–phosphate (pH 3.6) buffer containing gentamicin sulfate and bacitracin (50 μg/mL each) was used as the medium for the synthesis of the polyacrylamide hydrogel. Urea, at a concentration of 2 mg/mL, was also used to minimize adsorption and self-aggregation of peptide/protein drugs. A solution was made in this buffer to consist of the peptide/protein, monomer, cross-linker, preservatives, urea, and all components of the catalyst system. This solution was pipetted into the Teflon molds, polymerization occurred within seconds on addition of the initiator, and uniform transparent gels were consistently obtained. The hydrogels were prepared fresh before the start of an experiment.

For the synthesis of the *p*-HEMA hydrogel, an aqueous formulation with monomer, initiator, cross-linker, and plasticizer was purged with nitrogen gas for 20 min to eliminate the entrapped oxygen. The solution was then pipetted into the Teflon molds and covered with Teflon films, and polymerization was completed by heating in a convection oven. The peptide/protein drug was then incorporated into the hydrogels by soaking the hydrogel in drug solution at 5°C overnight (before the incorporation of drug, the hydrogels were individually soaked in 1 mL distilled water for 48 hr to remove residual impurities and any unreacted monomer). The Carbopol hydrogel was prepared by neutralizing a 1% dispersion of Carbopol 934 in citrate buffer (pH 3.6) with sodium hydroxide.

Swelling Studies

Hydrogels disks synthesized in molds were removed, weighed, and then placed in amber bottles having citrate–phosphate buffer (pH 3.6). The bottles were placed in a water bath maintained at 37°C for 24 hr, and the hydrogel disks were then removed, blotted with a Kimwipe, and reweighed. The percentage weight increase due to swelling was then calculated. The dried weight of the hydrogel before and after swelling was also recorded. However, the percentage weight increase after the synthesis of hydrogel was the primary interest of this study.

In Vitro Release and Skin Permeation Studies

The Teflon device containing the drug-loaded hydrogel formulation was mounted on the V-C cell, with only the receptor half-cell filled with the buffer. Current was applied directly to the hydrogel formulation in the Teflon device through a platinum foil and a platinum wire was used as the electrode in the receptor half-cell. The current was applied from a constant-current power source, called the transdermal periodic iontotherapeutic system (TPIS) (6), with multichannel capability to perform two sets of triplicate experiments simultaneously. A pulse current, with a frequency of 2 kHz and an on/off ratio of 1:1, at a current density of 0.62 mA/cm^2, was used. In most experiments for the release studies, current was applied for 3 hr, followed by a 3-hr postapplication sampling. For skin permeation studies, a full-thickness skin specimen was freshly excised from the abdominal region of hairless rats. The Teflon device containing the hydrogel formulation was then applied onto the stratum corneum surface of the skin and the combination was then mounted between the donor and the receptor half-cells of the V-C system.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Hydrogel

Polyacrylamide and *p*-HEMA hydrogels were synthesized by simultaneous polymerization and cross-linking. Polyacrylamide could be synthesized at room temperature, and thus the peptide/protein drug was incorporated into the formulation before polymerization. An HPLC analysis indicated that the free radicals introduced by the initiator at the levels used had no deleterious effects on insulin. Differential scanning calorimetric studies confirmed the absence of any residual acrylamide monomer.

Control of Hydrogel Swelling

Hydrogels consist of cross-linked three-dimensional water-swellable networks of hydrophilic homopolymers or copolymers. Dehydrated hydrogels will swell to some equilibrium value when placed in aqueous solution. The driving force for water to enter the polymer is osmotic pressure. The swelling process is counteracted by the elastic contractility of the stretched polymer network. The swelling reaches equilibrium when these forces are balanced and the chemical potential of the water in the gel equals that in the water surrounding the gel (7).

In order to avoid complicating swelling behavior during drug release, generally the swollen hydrogels at equilibrium are used unless a time-dependent release of drug from a swelling hydrogel is desired. In the present study, the Teflon mold used for the synthesis of the hydrogel also served as the drug reservoir device for release and skin permeation studies. Thus, any swelling after the hydrogel synthesis must be avoided. Polyacrylamide hydrogel was chosen to investigate swelling behavior (Figs. 1 and 2), as it exhibited a high degree of swelling during initial experimental studies. It was found that at a fixed cross-linker concentration, swelling increases with increasing monomer concentration. As can be seen from Fig. 1, a fivefold increase in swelling was observed as the monomer concentration was increased from 5 to 20%. However, if the cross-linker concentration is based on the monomer concentration, then increasing monomer concentration did not result in increased swelling, because in a cross-linked network, the swelling process is counteracted by the elastic contractility of the stretched polymer network. Figure 1 also shows the drastic effect of variation in the cross-linker concentration at a fixed monomer concentration. At a fixed monomer concentration of 10%, the percent-

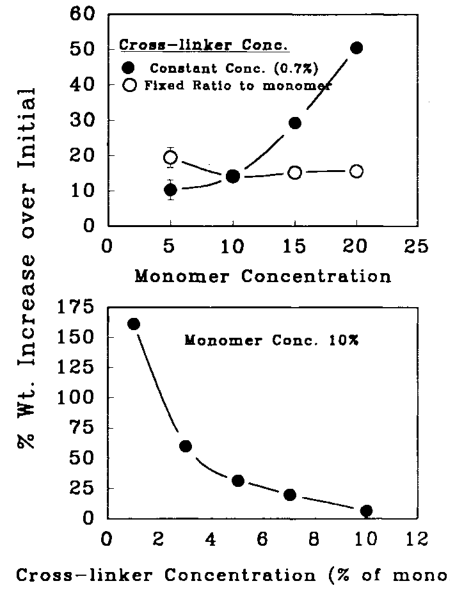

Fig. 1. Effect of increasing monomer and cross-linker concentration on the swelling of polyacrylamide hydrogel. The effect of monomer (top) was investigated at a fixed cross-linker concentration (0.7%, w/w) and at a constant cross-linker-to-monomer ratio (7.0%, w/w, of monomer). The effect of cross-linker (bottom) was investigated at a fixed monomer concentration (10%, w/w).

age weight increase decreased more than 150-fold for a 5-fold increase in cross-linker concentration.

The objective of minimizing swelling can thus be achieved by using a low monomer concentration with a high cross-linker level. However, if the monomer concentration is too low, the gels produced will lack adequate mechanical strength; on the other hand, a very high level of cross-linker leads to the formation of a translucent or opaque gel. A compromise composition which consists of 15% monomer and 1.05% cross-linker was found to be ideal in terms of the physical characteristics of the gel. This composition underwent a 12% weight increase in the swelling studies. In an effort to eliminate this weight increase without altering the composition of the gel, high molecular weight fraction dextran (MW 200,000–300,000) was incorporated in the swelling buffer to increase its osmotic pressure. The dextran molecules in this molecular weight range have a diameter of about 270 Å and are not likely to penetrate into the hydrogel (8). Dextran thus builds up osmotic pressure in the swelling medium which can be used to control the swelling of the hydrogel. A concentration of about 6% dextran was found to eliminate the swelling (Fig. 2). At higher concentrations, the hydrogel lost its water content due to osmotic dehydration, thus showing a weight loss instead of a weight increase.

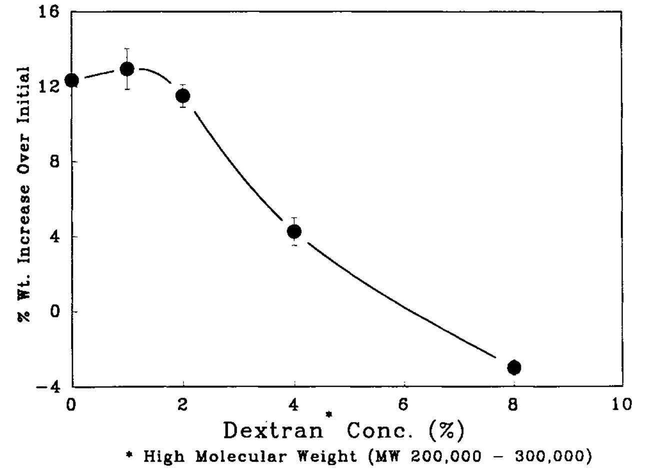

Fig. 2. Reduction in the swelling of polyacrylamide hydrogel by incorporating various concentrations of dextran in the swelling buffer.

Kinetics of Drug Loading in *p*-HEMA Hydrogel

For the polyacrylamide hydrogel, the peptide/protein drugs were included in the formulation before polymerization. This was possible because the polymerization is done at room temperature. However, for the *p*-HEMA hydrogel, the polymerization was carried out at elevated temperature and the drug was incorporated after polymerization by soaking the fabricated hydrogel in the peptide/protein solution. Loading of vasopressin and insulin occurred rapidly and most of the drug loading was completed within 4 hr. A similar profile was also seen for calcitonin but calcitonin was loaded from a solution with a concentration of 0.65 μM, which is 1000-fold lower than that for insulin and vasopressin (0.65 mM).

Release Studies on Peptide/Proteins from Hydrogel Formulations

Both the passive and the iontophoretic release of three model peptide/protein drugs—vasopressin, calcitonin, and insulin—were evaluated from two types of hydrogels—polyacrylamide and *p*-HEMA (release studies from Carbopol gel were not performed since the Carbopol gel is soluble in water, unlike the polyacrylamide and *p*-HEMA hydrogel systems). The selection of these peptide/protein drugs was based on their molecular characteristics (Table I). Insulin was used more often because of the therapeutic importance of its systemic delivery by noninvasive means. All the peptides existed as a positively charged molecule at the pH used (pH 3.6) and were delivered under the anode. Calcitonin was used at a concentration (0.65 μM) which is 1000-fold less than that for insulin and vasopressin (0.65 mM). For comparison, the diffusion coefficients were calculated. The results indicated that under iontophoresis application, vasopressin, calcitonin, and insulin are released from the polyacrylamide hydrogel system at diffusion coefficients of 5.42, 3.80, and 1.32 × 10^{-6} cm^2/sec, respectively.

Table I. Molecular Characteristics of the Peptide/Protein Drugs Used

Peptide/protein drug	No. amino acids	MW	Isoelectric point
Vasopressin	9	1084	10.9
Calcitonin (human)	32	3418	~7.9
Insulin (porcine)	51	5808	5.3

Passive vs Iontophoretic Release

Release of a drug from a hydrogel matrix by a passive diffusion process has been reported to follow the Higuchi equation for matrix release (9). Accordingly, the cumulative amount of drug released is proportional to the square root of time, t, as per the Higuchi equation:

$$Q = (2C_0C_sDt)^{1/2} \tag{1}$$

where Q is the amount of drug released from a unity surface area during time, t, D is the diffusion coefficient (cm^2/sec), C_s is the solubility of the drug in the hydrogel, and C_0 is the initial drug loading in the hydrogel.

For drugs which are dissolved in the polymer matrix. Baker and Lonsdale (10) proposed the following equation:

$$Q = 2C_0(Dt/\pi)^{1/2} \tag{2}$$

where π is a geometric constant.

Thus, a Q vs $t^{1/2}$ relationship should be followed for the release of peptide/protein molecules from the hydrogel formulation if under passive diffusion process. This Q vs $t^{1/2}$ linearity was observed experimentally for the release of vasopressin from polyacrylamide hydrogel (Fig. 3). However, the results in Fig. 3 indicate that under iontophoretic transport, the release profile of vasopressin changes from the Q vs $t^{1/2}$ relationship to a Q vs t relationship. The same behavior was also observed for the release of insulin from a polyacrylamide hydrogel. A similar pattern of drug release behavior from hydrogels under passive and iontophoretic conditions has been reported by Bannon *et al.* (3). The quantity of drug delivered iontophoretically is given as

$$Q_i = f_i \cdot i \cdot t \tag{3}$$

where Q_i is the amount of drug released from a unity surface area during time t under iontophoresis, i is the current density (mA/cm^2), and f_i is the iontophoretic constant determined from the slope of the (Q_i/t) vs (i) plots.

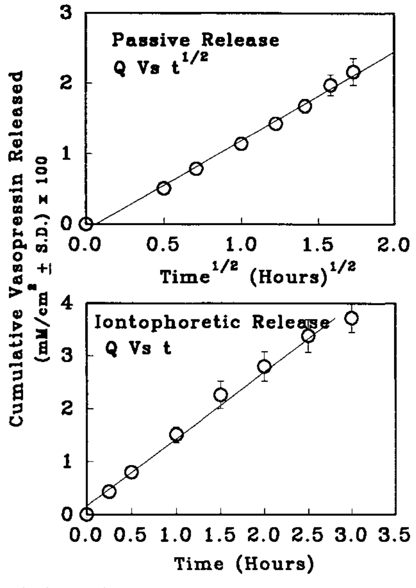

Fig. 3. Cumulative release profile of vasopressin from polyacrylamide hydrogel, which illustrates that the release by passive diffusion follows a Q vs $t^{1/2}$ relationship, while the release by iontophoretic transport follows a Q vs t relationship. Data points are connected by regression lines.

Iontophoresis-Facilitated Peptide Release Kinetic Profiles

The release kinetics of peptide/protein from hydrogel under iontophoretic transport was investigated, using insulin, under varying durations of iontophoresis application. The kinetic profiles illustrated in Fig. 4 suggest that insulin is released at a constant rate during iontophoresis application and the release rate drops as soon as the current is turned off. This behavior was observed consistently for 1- to 4-hr current application.

The effect of multiple application of iontophoresis on the release flux of insulin is seen in Fig. 5. During the first application, the delivery rate declined during current application, possibly because of a burst effect. The dQ/dt (instantaneous flux) rises as the current is applied and then declines when the current application is terminated, and this process is repeated on subsequent applications. Thus, the release of peptide/protein from the hydrogel matrix can be modulated by iontophoresis application. The higher release observed for the first application could be due to a burst effect in which the drug present on the surface layer of the hydrogel is released instantly upon contact with the receptor solution, maintained under sink conditions. This burst release does not occur in the second and third current applications, which suggests that the bulk of the drug contained in the hydrogel matrix is released under the control of iontophoresis. If the release flux profile of insulin under the first iontophoresis application is corrected for the contribution of burst release, a profile similar to that for the second and third applications is obtained. A good correlation was observed between the radiotracer measurement and HPLC assay for the release profile of insulin, which suggests that there was little or no degradation of insulin in the hydrogel formulation.

Permeation Studies on Peptide/Proteins from Hydrogel Formulations

The permeation profiles of insulin, calcitonin, and vasopressin across hairless rat skin from the hydrogels were investigated. The permeation profiles for insulin and calcitonin exhibit a depot or reservoir effect, unlike that of vasopressin. The cumulative amount of peptide/protein permeated was observed to increase continuously over the 36-hr sampling period even though the current was applied for only 2–4 hr. This observation seems to agree with earlier *in vivo* work by other investigators (11) in which blood glucose levels remained decreased for several hours following a relatively short iontophoresis treatment. Figure 6 presents the permeation flux (dQ/dt) for vasopressin, calcitonin, and insulin from polyacrylamide hydrogel across hairless rat skin for a 3-hr current application. In the case of calcitonin and insulin, the flux continues much beyond the 3-hr current application, as a result of the depot effect or skin reservoir formation. In fact, in several instances, there was a burst of radioactivity after the iontophoresis was stopped. Such a burst, seen in Fig. 6 after 3-hr current application, most likely resulted from the degradation of the peptide/protein drugs by the proteolytic enzymes of the skin and the permeation of some fragments carrying a negative charge (which

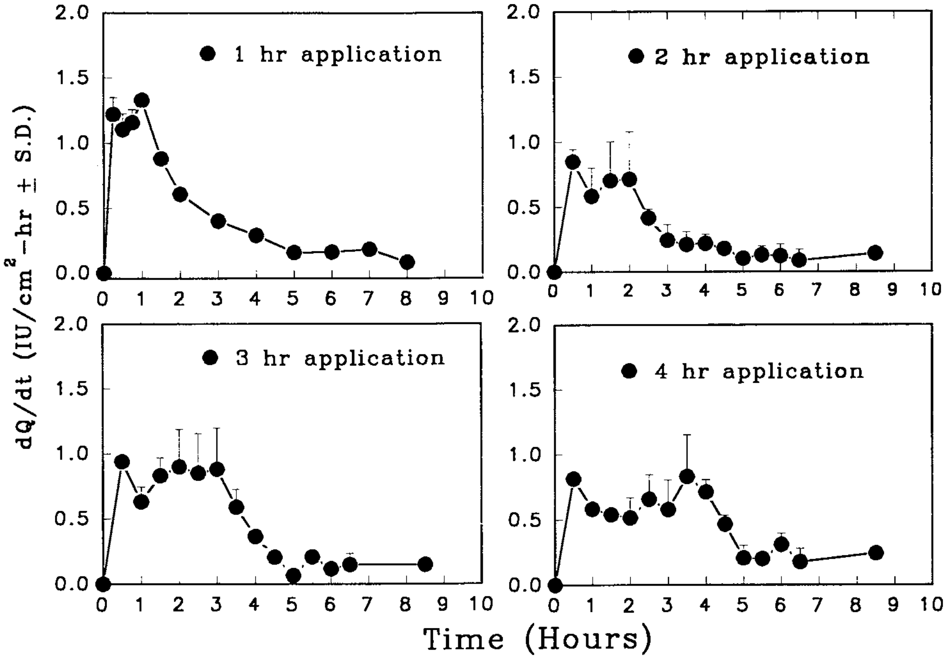

Fig. 4. The release rate (*dQ/dt*) profile of insulin from polyacrylamide hydrogel as a function of the increase in the duration of iontophoresis application with pulse current.

may have been repulsed by the cathode in the receptor solution until the termination of current application). In contrast, the skin permeation profile of vasopressin showed a decline in the permeation flux (*dQ/dt*), following the termination of current application (Fig. 6). This result suggests either that vasopressin does not form a depot in the skin or that the depot is desorbed rapidly from the skin. Such a difference in behavior between vasopressin and insulin or calcitonin could result from the lower molecular weight and higher diffusion coefficient of vasopressin than of calcitonin or insulin (Table I). However, this hypothesis does not apply to a drug such as verapamil hydrochloride (MW 491), which forms a strong depot in the skin (12). It is more likely that some functional groups in the verapamil molecule could interact with the skin tissue to form a depot. Table II compares the permeability coefficients for the iontophoresis-facilitated permeation of insulin, calcitonin, and vasopressin across the hairless rat skin from the various hydrogels used. The permeability coefficient (P_m) was calculated per the following equation:

$$P_m = 1/(C_r \cdot A_s) \cdot dQ/dt \tag{4}$$

where P_m is the permeability coefficient (cm/sec), C_r is the reservoir concentration of peptide/protein in the hydrogel formulation, A_s is the skin area available for permeation (cm^2), and dQ/dt is the skin permeation flux (mM/cm^2-sec). The skin permeation flux of vasopressin was determined over the period of current application, while the permeation flux of insulin and calcitonin was calculated over the period of postapplication sampling, because insulin and calcitonin

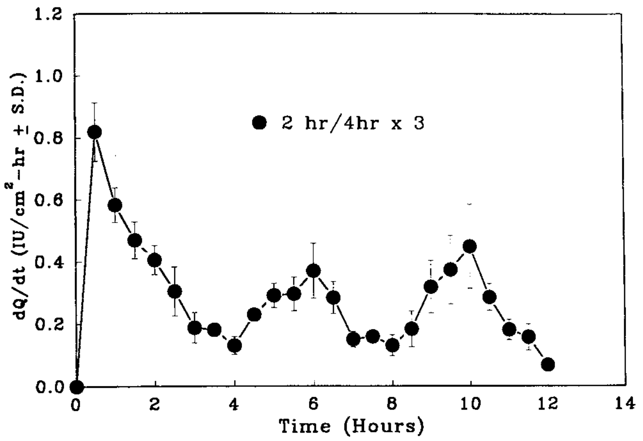

Fig. 5. Modulation of insulin release flux from the polyacrylamide hydrogel by repeated 2-hr current applications at 0, 4, and 8 hr.

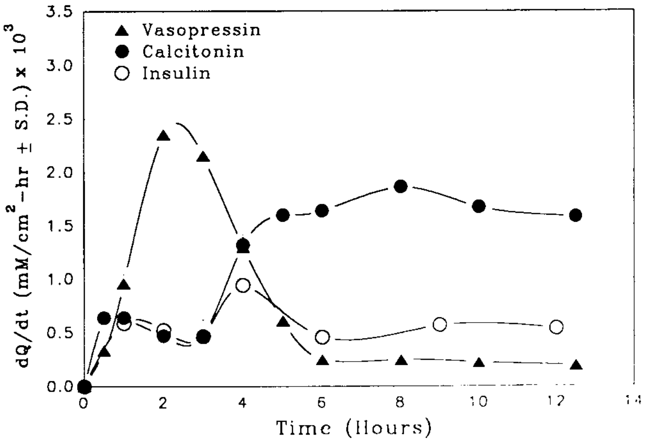

Fig. 6. Permeation rate (*dQ/dt*) profiles of vasopressin, calcitonin, and insulin, delivered from polyacrylamide hydrogel, across hairless rat skin during the 3-hr iontophoresis treatment and after the termination of treatment.

Table II. Permeability Coefficient for Transdermal Iontophoretic Transport of Peptide/Protein Drugs from Different Hydrogels

	Permeability coefficient (cm/sec) × 10^8		
Peptide/protein	Polyacrylamide	*p*-HEMA	Carbopol
Insulin	0.312	0.654	N/A[a]
Calcitonin	6.20	1.95	0.69
Vasopressin	61.6	10.6	27.4

[a] Formulation/solubility problem.

formed a depot during iontophoresis and the bulk of the permeation occurred after iontophoresis. As shown in Table II, the permeability coefficient increases from insulin to vasopressin with decreasing molecular size.

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