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Review

Applications of the Ion-Pair Concept to Hydrophilic Substances with Special Emphasis on Peptides

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Previous investigations have shown that ionic drugs with high aqueous solubilities can be lipophilized by ion-pair formation, with appropriate counter-ions. This type of association may prove promising for several biopharmaceutical, analytical and technological applications. This review examines the ion-pair concept with special emphasis on its application to peptides. The conditions for ion-pair formation of different molecules, as well as their transfer to organic solvents, are described. The use of ion-pairs to increase the permeation of various therapeutic agents, including peptidic drugs, is also discussed. Recent uses of ion-pairs in micro- and nanoencapsulation of peptides are also commented upon.

KEY WORDS: ion-pair; peptides; counter ions; ion-pair extraction; ion-pair enhanced absorption.

INTRODUCTION

Peptides and their analogues are becoming a significant new class of therapeutic agents. This is due to the structural elucidation of numerous natural peptides and the understanding of their role in several physiological processes, the development of systematic methods to produce therapeutic peptides and also to the rapidly expanding field of recombinant DNA technology. Accordingly, the commercial production of peptides for pharmaceutical purposes is now well established and the list of peptides available as therapeutic agents is growing rapidly (1). Unfortunately, peptide drugs can possess chemical and physical properties, including high molecular weight, low partition coefficient, short biological half life, immunogenicity and denaturation, which make them unsuitable for delivery using the conventional absorption routes (2). Among these drawbacks, low lipophilicity is probably the most important factor to overcome. Although the chemical modification of peptides, using natural fatty acids and macromolecules, seems to be appropriate for increasing their lipophilicity, the pharmacological effect of these derivatives is questionable and their enzymatic bioconversion must be confirmed in each case. Furthermore, their formulation and stability have not been studied and like other drug derivatives, they are considered as new drugs by the regulatory agencies and have to be submitted to extended toxicological studies. It is clear that lipophilization of peptides without modification of their chemical structures would be ideal (3,4), not only from the biopharmaceutical point of view, but also from the analytical (e.g. separation and determination in biological samples) and technological (e.g. protein synthesis and incorporation into carriers) standpoints.

Experimental evidence to date suggests that ion-pairing effectively increases lipophilicity of charged drug molecules, both *in vitro* and *in vivo*. The concept has also been applied to peptides in order to obtain more lipophilic drugs that are stabilized via electrostatic interactions (5–7).

This review will focus on the physicochemical parameters involved in ion pair formation with special emphasis on peptidic drugs and will focus on solvent extraction of drugs, and on the enhanced absorption as ion-pairs. Results from experiments carried out in our laboratory involving extraction, permeation and nanoencapsulation of aminoacids and dipeptides as ion-pairs, will also be presented. Analytical aspects such as high-performance ion-pair chromatography for the separation and quantitation of peptides and ion-selective liquid membrane electrodes, as well as the pharmacokinetic and toxicologic features of ion-pair transport across membranes, will not be discussed, since excellent reviews are already available (5,8).

THE ION-PAIR CONCEPT

One of the main problems of the proposed theories for electrolyte solutions has been to account for ion-ion and ion-

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ABBREVIATIONS: a, radius of spherically charged molecule; A^+, cation; A^+B^-, ion-pair; α, side-reaction coefficient; A(OH), conjugated base of A; B^-, anion; B'^-, anion of a fatty acid; C_L, drug concentration in the luminal phase; C_M, drug concentration in the membrane; C_B, drug concentration in the blood; D, distribution ratio; d, distance; e, electron charge; E_A, degree of extraction of A^+; E, Born energy of charging; ϵ, dielectric constant; k, Boltzmann's constant; K, partition ratio; K_{12}; K_{21}; K_{23}, drug-absorption rate constants for a three-compartment model; $K_{A,Q}$, extraction constant; $K'_{A,Q}$, conditional extraction constant; IPM, isopropyl myristate; q, phase volume ratio; R^+ or R^-, ionizable side chain; S, solvent; T, absolute temperature; V_{org}; V_{aq}, organic and aqueous phase volume; X, adduct; Z_+ and Z_-, charge of the ions.

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solvent interactions. The first satisfactory theory of ionic solutions was proposed by Arrhenius in 1887 (9). He made the bold assumption that electrolytes were completely dissociated into their ions when the solution was infinitely dilute. Arrhenius ascribed the decrease of equivalent conductance, with increasing concentration, to association of the ions into neutral molecules. Arrhenius' basic principles of electrolytic solutions marked a great advance in the electrolyte field and some of them are still valid today. However the application of these principles was limited to solutions of weak acids and bases in water. They failed completely to explain the behaviour of strong electrolytes such as ordinary salts and strong acids and bases where interionic attractive forces cause deviations from the ideal behaviour.

It was not until 1923 that the solution to the electrolyte problem was found by Debye and Hückel (10). Their theory accounted for the thermodynamic properties of electrolyte solutions. They assumed that strong electrolytes were completely dissociated into their ions in aqueous solution and that the deviations of electrolytic solutions, expressed in terms of activities, activity coefficients, and ionic strengths, were due to the electrostatic effects of the oppositely charged ions. However, the Debye-Hückel theory of complete dissociation did not explain the low conductance of strong electrolytes in non-aqueous solutions and it did not extend to include all solvents. In 1926 Bjerrum developed a theory that took into account the interaction of ions at short range (11). He introduced the ion-pair concept and showed how the mass action constant of the equilibrium between ions and ion-pairs was dependent on the dielectric constant of the solvent as well as on the temperature and the size of the ions. The Bjerrum theory was strongly supported by the work of Kraus in the late Thirties and Forties (10,12,13).

Atherton and Weismann (14) investigated the existence of ion-pairs, using electron spin resonance spectroscopy, and demonstrated beyond doubt the association of a sodium cation with a naphthalene radical anion. Ion-pair formation is now an accepted fact, at least in non-aqueous media.

Although ion-pairing phenomena were initially investigated in the field of physical chemistry, the concept was rapidly adopted in the pharmaceutical sciences. In particular, Higuchi and Schill and their co-workers made large contributions and established the basis for its application to molecules of pharmaceutical interest (4,15).

Definition and Nature of Ion-Pairs

Ion-pairs may be defined as neutral species formed by electrostatic attraction between oppositely charged ions in solution, which are often sufficiently lipophilic to dissolve in non-aqueous solvents (7,16). It should be emphasized that the formation of an ion-pair is due only to the so-called outer-sphere interaction and even though this molecular interaction can be written according to the mass action law, no chemical bond of any kind is formed. The general notation A^+, B^- is used to describe an ion-pair product which exists as a stable, thermodynamically distinct species and not as a transient, continuously exchanging association (7,13,15). It is clear therefore that any charged molecule in solution, under certain conditions, can form an ion-pair, with an ion of opposite charge. Thus, as peptides present multiple ionizable sites, depending on their primary structure and the pH of the solution, they are capable of interacting *in vitro* or *in vivo* with appropriate counter-ions. The formation of a peptidic ion-pair results in the "burying" of the charges involved and the alteration of physical properties, for example, lipophilicity (17,18).

Forms of Ion-Pairs

The work of Sadek and Fuoss (19) and that of Winstein *et al.* (20), later confirmed by Roberts and Szwarc (21), showed that an ion-pair can exist in two forms: as a tight or intimate ion pair, or as a loose or solvent separated ion-pair, depending on the nature of the solvent-ion interaction. These authors established that free ions in solution are surrounded by solvent molecules polarized by the electric fields generated by the ionic charges. A sufficiently strong polarization and solvent-ion interaction result in the formation, around each ion, of a tight solvation shell. The presence of such a solvation shell is reflected in the fact that the Stokes radius of the solvated ion is substantially greater than that predicted for the bare ion. An ion possessing a tight solvation shell may approach a counter-ion without hindrance until its solvation shell contacts the partner. Thereafter, either the associate maintains its structure as a loose, solvent-separated ion-pair, or the solvent molecules separating the partners are squeezed out and a tight contact ion-pair is formed. This implies that solvent-separated ion pairs may exist only in those media in which the free ions acquire tight solvation shells; otherwise, only tight contact ion-pairs are produced. It is important to mention that Bjerrum's original concept of a pair of solvated ions that are held together by coulombic attraction, in a solvent of a low dielectric constant, remains valid without modification despite the presence of a solvation shell. For example, if the solvated ion is paired with a bulky counter-ion, the gain in coulombic energy arising from the approach of the partners into close proximity may not be sufficient to accomplish the destruction of the solvation shell. Therefore, such pairs exist only in the loose form.

Solvation of Ion-Pairs

As indicated above, the formation of ion-pairs is only possible if the ions approach each other and reach a critical separation distance (d) given by the Bjerrum's equation:

$$d = |Z+Z-|e^2/2\epsilon kT \qquad (1)$$

where $Z+$ and $Z-$ are the ionic charges, e is the electron charge, ϵ is the dielectric constant, k is Boltzmann's constant and T is the absolute temperature. The equation shows the importance of the dielectric constant (ϵ) in ion pair formation; accordingly a solvent with a high dielectric constant such as water ($\epsilon = 78.5$) will be unfavourable for ion pair formation, but this does not mean that it is impossible (as we will see later). On the other hand, the interaction becomes increasingly important in solvents with $\epsilon < 40$. Although this rule is applicable to a large number of ion-pair extraction systems (4,15,22), some authors have shown that other non-coulombic contributions can be involved in the ion-association, for example hydrogen bonding, lipophilicity of the ions, and other factors such as the solubility parameter would explain more satisfactorily the solvation of the ion-pair (19,23).

The behaviour of the solvating agent and its affinity for the ion-pair can be explained by the solvation theory proposed

by Higuchi (24). Ion-pairs can be classified, according to the degree of charge accessibility, into three different categories (Fig. 1). In the first case, it is assumed that the cation is large and lipophilic except for the positively charged center. The small external surface would be expected to carry a relatively negative charge per unit area (shown by the external shadow in Fig. 1). This type of system may be effectively solvated by lipophilic molecules having a positively charged surface, e.g. dipolar molecules with acidic protons such as chloroform, phenols and alcohols. Since the bonded solvating molecules would have their polar end buried adjacent to the anion, the appearance presented to the surrounding solvent by the solvated ion-pair would be that of a relatively nonpolar aggregate.

In the second case, the situation is reversed, the ion pair having its cationic charge largely exposed. Solvating species containing nucleophilic sites may be expected to be particularly effective for this type of ion-pair, e.g. ethers, ketones, amides and phosphate esters. The third case is that of an ion-pair with deeply buried charges. Having no exposed electrically unbalanced surface, it would be expected neither to require solvation in order to be readily extractated by nonpolar solvents.

On the other hand, Higuchi attributes ion-pair solubility to the formation of complexes involving association with a discrete number of solvent molecules, which take part in the formation of the ion pair in the organic phase. This solvation can be written as an equilibrium:

$$A^+,B^- + nS \rightleftarrows A^+,B^- \cdot nS \qquad (2)$$

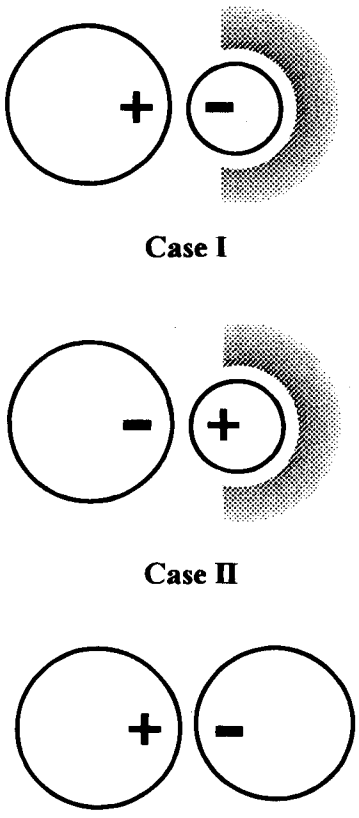

Fig. 1. Classification of ion-pairs according to Higuchi (24). See text.

where A^+ and B^- are oppositely charged ions in solution and nS is the discrete number of solvent molecules assumed to be complexed with the ion-pair. The concept of a specific solvation for ion-pairs has been confirmed using infrared techniques, and nuclear magnetic resonance and electron spin resonance spectroscopies (13).

Ion-Pair in Aqueous Systems

Although ion-pair formation has been considered only for solvents with a low dielectric constant (Bjerrum's ion-pair), the existence of an ion-pair in water or in other highly structured (bonded) solvents is possible when the ions involved are largely hydrophobic. In this case, ion-pairing is due to a solvent mediated effect rather than to an electrostatic interaction (4,7). The term "water structure enforced" ion-pairing was introduced by Diamond (25) in order to explain the existence of ion-pairs in aqueous systems. If both the cation and anion, are large hydrophobic species, the hydrogen-bonded water structure forces them together to maximize the water-water interactions and to minimize the structural perturbation. Water structure enforced ion-pairing involves both electrostatic and hydrophobic interactions, the relative contribution of which is dependent upon both ions' structures and on their immediate environment.

Despite the possibility to form ion-pairs in aqueous solution, the usefulness of this phenomenon is very limited due to the low association constant; furthermore, the ion-pairs exist only at very low concentrations, because of the poor solubility of the ions (4,12,23). An interesting phase diagram for the interactions between large ions in aqueous solution was proposed by Tomlinson (6) for a dianionic drug and a cationic surfactant. This diagram, shown in Fig. 2 reveals that the ion-

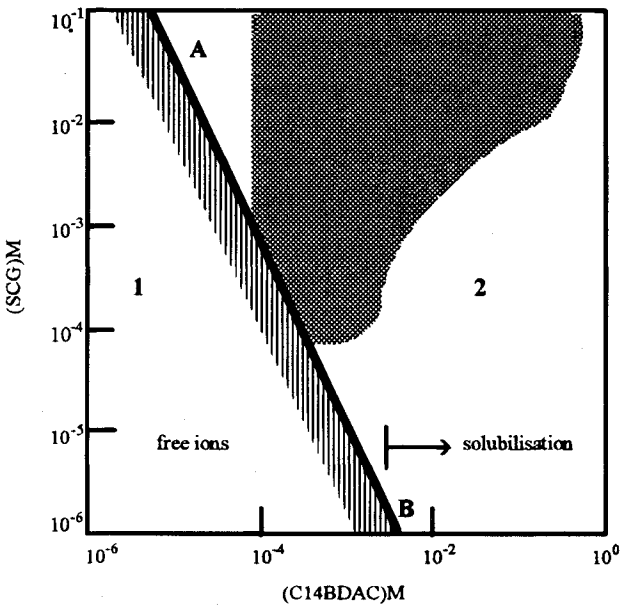

Fig. 2. Phase diagram for the interaction of the dianionic drug sodium cromoglycate (SCG) and the cationic surfactant tetradecyldimethylammonium chloride (C14BDAC). Region 1 has no coacervation and has as its boundary the solubility product line A-B. The shaded area of the visual turbidity region 2 is an area of solubilized coacervate. The dots represent an area of ion pair formation. Adapted from 6.

pair exists only in a narrow range of low concentrations; above this range, colloidal aggregates will be observed.

ION-PAIR EXTRACTION

Probably the most important applications of the ion-pair concept in the pharmaceutical field is the isolation and determination of drug molecules. Ion pair extraction can be described as the extraction of a complex of two oppositely charged ions in an aqueous phase (subscript aq) by a solvent that is not miscible with water (subscript org). This equilibrium can be written as (15):

$$A^+_{aq} + B^-_{aq} \rightleftarrows A^+,B^-_{org} \quad (3)$$

The equilibrium constant of the reaction is normally called the extraction constant of the ion pair, $K_{A,B}$:

$$K_{A,B} = [A^+,B^-]_{org}/[A^+]_{aq}[B^-]_{aq} \quad (4)$$

The magnitude of this constant is dependent upon the nature of the components of the ion-pair and on the properties of the organic solvent.

The distribution ratio, D, of an ion (e.g. for A^+) is defined as:

$$D_A = [A^+,B^-]_{org}/[A^+]_{aq} = K_{A,B} \cdot [B^-]_{aq} \quad (5)$$

The distribution of an ion-pair between an aqueous and an organic phase depends on the equilibrium constant of the ion-pair and on the equilibrium constants of chemical processes (side-reactions) in both phases. Some types of side-reactions are shown in Fig. 3. The side-reactions of A^+,B^- in the organic phase, e.g. dissociation of the ion pair (A^+,B^- to A^+ and B^-) polymerization ($(A^+,B^-)_n$) and adduct-formation with an agent X ($A^+,B^- \cdot X_n$) result in an increase of $K_{A,B}$. On the other hand protolysis of A and B in the aqueous phase and partition result in decrease of $K_{A,B}$. When side-reactions occur, correction coefficients (α) must be introduced to the conditional constant $K'_{A,B}$:

$$K'_{A,B} = K_{A,B}\alpha_{A,B}/\alpha_A \cdot \alpha_B \quad (6)$$

In such cases the extraction constants are calculated by graphical methods (26). It must be emphasized that these side-reactions are not to be considered entirely in a negative light since they can improve both selectivity and the degree of extraction.

The degree of extraction (E) of A^+ (as A^+,B^-) into the organic phase with a single extraction can be calculated from:

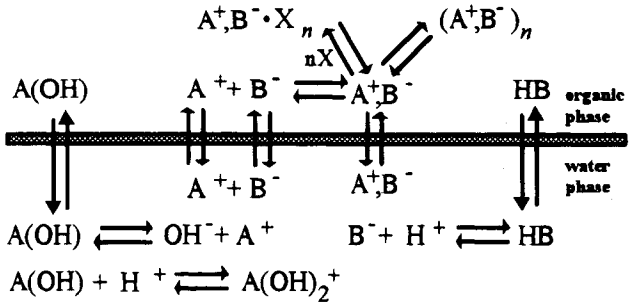

Fig. 3. Schematic representation of side-reactions involved in the extraction of an ion-pair in the two-phase system. Adapted from (15).

$$E_A(\%) = 100D_A \cdot q(1 + qD_A)^{-1} \quad (7)$$

where q is the phase volume ratio V_{org}/V_{aq}.

The extraction of a drug can be regulated by the nature and the concentration of the counter-ion as well as by the nature of the organic solvent. It should be noted that the solvent is a component which takes part in the formation of the ion-pair, and that its selection will influence the degree of extraction. In most studies chlorinated solvents such as chloroform and dichloromethane are used as the organic phase. These solvents exhibit low dielectric constants and are hydrogen donors, which makes them particularly suitable for the extraction of several ion-pairs. Furthermore, their tendency to extract anions as acids is limited, and the incidence of other side-reactions such as dissociation and polymerization of the ion-pairs is low. When poor extraction is observed with chlorinated solvents, due to the high hydrophilicity of ion-pair components, the addition of more polar solvents (e.g., alcohols) which form adducts with the ion-pair in the organic phase is recommended (27).

Extraction of Peptides as Ion-Pairs

Considering that peptides are ampholytes with very low partition ratios, it is to be expected that their extraction as ion-pairs, with solvents of low dielectric constants, will be limited; however, more polar solvents should give better extractions. Experiments made in our laboratory seem to confirm this idea. Tables 1 and 2 show some extraction constants with different solvents for tryptophan (Trp) and tryptophan-leucine (Trp-Leu), respectively. The ion-pair partition was made with the species in cationic form, due to the low extraction achieved in previous tests at the isoelectric point. Chlorides, nitrates and sulphates were used as counter-ions, using the corresponding acids at 0.1 N in aqueous solution. It is clear that an increase in solvent dielectric constant (ϵ) significantly improves the extraction. For propylene carbonate and butanol, the log K value clearly increases with the hydrophobicity of the counter-ion in the aqueous phase at pH 1, $NO_3^- > Cl^- > SO_4^{-2}$ (28,29). The dif-

Table 1. Effects of Inorganic Counter-Anions on the Logarithm of the Partition Ratio (log K) of Tryptophan Using Different Solvents at pH 1

Solvent (dielectric constant; hydrogen bonding capability)	log K (±CV[a])		
	SO_4^{2-}[b]	Cl^-[b,c]	NO_3^-[b,c]
Chloroform (4.8; poor)	b. d. l.[d]	b. d. l.	b. d. l.
Dichloromethane (9.1; poor)	−3.46 (0.42)	−1.66 (0.93)	−0.77 (0.65)
Ethyl acetate (6.0; moderate)	−0.04 (1.46)	−1.57 (0.47)	−1.46 (0.40)
1-Butanol (17.1; strong)	−0.68 (1.46)	0.01 (0.39)	0.26 (2.32)
Propylene carbonate (65.1; moderate)	−0.18 (0.73)	−0.12 (2.0)	0.47 (0.49)

[a] CV: Coefficient of variation (%) (n=4).
[b] The hydrophobicity of anions decreased as $NO_3^- > Cl^- > SO_4^{2-}$ (28).
[c] The log of distribution constant of HNO_3 and HCl between propylene carbonate and water is −1.05 and −1.60, respectively (29).
[d] b. d. l. : below detection limit.

Table 2. Effects of Inorganic Counter-Anions on the Logarithm of the Partition Ratio (log K) of Tryptophan-Leucine Using Different Solvents at pH 1

Solvent (dielectric constant; hydrogen bonding capability)	log K (±CV[a])		
	SO_4^{2-}[b,c]	Cl^-[b,c]	NO_3^-[b,c]
Chloroform (4.8; poor)	b. d. l.[d]	b. d. l.	b. d. l.
Dichloromethane (9.1; poor)	−2.03 (0.43)	−1.64 (0.63)	−2.23 (0.93)
Ethyl acetate (6.0; moderate)	−1.05 (1.01)	−0.92 (0.65)	−0.94 (3.12)
1-Butanol (17.1; strong)	0.25 (2.31)	0.88 (2.33)	1.13 (3.21)
Propylene carbonate (65.1; moderate)	0.48 (2.15)	0.58 (2.11)	1.07 (1.2)

[a] CV: Coefficient of variation (%) (n=4).
[b] The hydrophobicity of anions decreased as $NO_3^->Cl^->SO_4^{2-}$ (28).
[c] The log of distribution constant of HNO_3 and HCl between propylene carbonate and water is −1.05 and −1.60, respectively (29).
[d] b. d. l. : below detection limit.

ferences in the extracted quantity of Trp and Trp-Leu in butanol and propylene carbonate are attributed to their hydrogen-bonding capacity.

Akamatsu *et al.* (17), studying the partition of peptides in octanol/water systems (Table 3), found somewhat surprisingly that a considerable increase in the partition coefficient was obtained using inorganic ions in the aqueous phase at pH 1; in the same way, these authors observed that the degree of extraction depended on the hydrophobicity of the counter ions. These data suggest that for the same counter-ion, the degree of extraction of Trp dipeptides depended on the relative hydrophobicity of the attached amino acid: Trp>Phe>Leu>Ala. Accordingly, the hydrophobicity scales for amino acid side-chains (30) could be useful for the prediction of the degree of extraction.

A recent interesting contribution dealing with the ion-pair extraction of a therapeutic peptide has been made by Adjei *et al.* (18). They evaluated the partition of leuprolide acetate, a nanopeptide with multiple ionizable sites, in octanol/water systems at different pH values and using different counter-ions. The results showed that partition is increased by the presence of alkyl sulfonic acids, depending on the length of the alkyl chain and that the increase in lipophilicity of leuprolide ion-pairs may be proportional to the extent of ionization of the imidazolyl nitrogen of histidine.

Table 3. Effects of Counter-Anions on the Logarithm of the 1-Octanol/water Partition Ratio (log K) for Some Dipeptides at pH 1 (from reference 17)

Peptide	log K (pH 1)		
	ClO_4^-[a]	NO_3^-[a]	Cl^-[a]
Trp-Trp[b]	1.10	0.50	0.21
Trp-Phe[b]	0.77	0.17	0.02
Trp-Leu[b]	0.68	0.08	−0.18
Trp-Ala[b]	−0.68	−1.22	−1.42
Phe-Phe	0.37	0.17	0.43
Leu-Phe	0.14	−0.44	−0.66

[a] The hydrophobicity of anions decreases as $ClO_4^->NO_3^->Cl^-$. While the free energy of ClO_4^-, NO_3^-, Cl^- ions from water to nitrobenzene is 8.7, 24.4 and 30.5 kJ/mol; the log of the distribution constant of $HClO_4$, HNO_3 and HCl between propylene carbonate and water is −0.05, −1.05 and −1.60, respectively (29).
[b] Relative hydrophobicity of amino acid side chains.

An illustrative analytical development using ion-pair extraction for peptide isolation and analysis was presented by Uvashkiv (31), who developed a spectrophotometric method for the determination of cyclic octapeptidic antibiotics during fermentation. The method is based on the extraction of antibiotic from alkaline broth with butanol. Ion-pairs formed between the octapeptides and bromothymol blue were extracted into chloroform from a solution buffered to pH 7.5. It is important to point out that the extraction of these peptides by chloroform as ion-pairs was possible because of their exceptionally low water solubility.

Although there are only a few publications concerning ion-pair extraction of peptides, two common themes seem to be evident: the transfer of peptides from aqueous to organic solution is improved by using polar solvents and by decreasing, or avoiding, the presence of ionized groups which do not take part in ion-pair formation (ion suppression). The possibility of transferring peptide intermediates and protected peptides to organic solvents, could have interesting implications for peptide and protein synthesis (32,33).

ION-PAIRING AS A SYSTEM FOR ABSORPTION ENHANCEMENT

Biological membranes can be considered as lipoidal barriers, and thus ionized molecules cannot generally cross them due to unfavourable partitioning (7,34,35). However, ionized solutes like paraquat, suxamethonium, phenothiazines and some quaternary ammonium compounds, are readily absorbed from the gut. Shanker (35) proposed that one of the probable mechanisms for the penetration of organic ions through the gastrointestinal barrier was by formation of a less polar complex with some material normally present in the lumen. Passage of an ionized drug through a membrane (ionophoresis) must involve either the existence of an aqueous pore, or the transport of the ion from an aqueous environment to a lipid environment, then across the lipid (lipoprotein) and finally transfer into the aqueous phase on the other side of the membrane. This hypothesis proposes that the ion-ionophore interaction provides a mechanism for drug absorption. One way of visualizing ion-pair transport is in terms of the difference in the level of specific resistance encountered by ions and ion-pairs for permeation through membranes. Thus, an ionized drug could be considered as a spherically charged molecule of radius with an energy of charging determined by the dielectric constant of the medium (ϵ), according to the expression $E = e^2/2\epsilon a$, where E represents the Born energy of charging. Due to the inverse dependence of energy on ϵ, large energy differences exist between low and high dielectric media. This produces a great energy barrier for ion partitioning and flux into hydrophobic membranes. The formation of ion-pairs might lower E by "burial" of the ion charge by the counter-ion and thus the transport of ionic drugs through hydrophobic membranes would be enhanced (4).

Irwin *et al.* (16) were the first to test the ion-pair hypothesis for the lipophilization of an ionic drug (isopropamide) using

an exogenous counter-ion (trichloroacetate). The results of this study indicated that the rate and efficiency of gastrointestinal absorption of isopropamide were increased by ion-pair formation with trichloroacetate. The authors stated that it might be possible through selection of appropriate ion-pair formers to improve the efficiency and uniformity of absorption of highly ionized drugs from the gastrointestinal tract. This hypothesis received support from subsequent work investigating the permeation of different hydrophilic ionizable drugs through artificial and biological membranes *in vitro*, *in situ* and *in vivo*, *ex vivo* experiments (4,16,34,36,37). It is important to point out that absorption experiments with ion-pairs have not always pointed at clear-cut mechanisms. In those cases, an increase of drug absorption was not evident in the presence of a counter-ion, or the increase of the absorbed amount was not attributed to ion-pair formation, but to a direct effect of the counter-ion: mucosal binding, mucosal erosion, interfacial tension lowering effect on the gut wall, increased surface activity of the drug, etc. (38,39). Thus, additional experiments using neutral molecules such as caffeine, which is unable to form ion-pairs, should be carried out in order to distinguish whether the enhancement is due to ion-pair formation or to the disruptive effect of the counter ion on membrane integrity (40).

The increase of drug permeability by ion-pair formation can be generally attributed to an increase in the partition coefficient factor. Suzuki *et al.* (36) studied the partition of quinine for its absorption across rat rectal mucosa, using a three compartment model of drug absorption:

$$C_l \underset{K_{21}}{\overset{K_{12}}{\rightleftarrows}} C_m \xrightarrow{K_{23}} C_b \qquad (8)$$

where C_l, C_m and C_b represent the drug concentration in the luminal phase, membrane and blood, respectively, and K_{12}, K_{21}, and K_{23} are rate constants. The results showed an evident increase of K_{12} caused by the presence of the counter-ion. The authors stated that this may be caused by the increase of drug concentration in the absorptive membrane surface which is related not only to the physicochemical properties of the ion-pair systems in the medium, but also to the chemical nature of the surface of the absorptive site. This could explain why the permeation of certain drugs was unaffected by ion-pair transport. However, the physicochemical basis of the failure of these drugs to undergo ion-pair transport remained unclear and ion-pair enhancement of transport *in vivo* remains controversial (5).

In the Seventies *in vitro* studies used artificial membranes (37) as models of biological membranes for evaluating the transport of drugs as ion-pairs. The model membranes provided improved control and interpretation of the variables involved and made interpretation of results easier (4,34). In general, those studies were performed using a two-compartment diffusion cell, with a lipophilic membrane, porous or not, that could be impregnated with a liquid representing the biological barrier (41). The donor compartment was a buffer solution with a pH which favoured the formation of the ion-pair, or a non-toxic non-aqueous medium (i.e. propylene glycol) in which the ion-pair was formed (4).

An interesting variation on these studies, was the incorporation into the membrane of a counter-ion as a carrier. Hadgraft *et al.* (42) showed that ionic drug transport across an artificial lipid membrane impregnated with isopropyl myristate (IPM) could be increased by incorporation of a counter-ion capable of forming ion-pair into the membrane. A pH gradient was used to facilitate the transport of the ions from an aqueous compartment across a non-polar organic phase to an aqueous receptor against the ion concentration gradient. This principle was developed in an attempt to facilitate the transport of ionic drugs across the stratum corneum. Green *et al.* (34) showed that a pH-gradient made it possible to increase the *in vitro* flux of naphazoline across human cadaver skin previously pretreated with fatty acids (Fig. 4).

In general, the mechanism of penetration through synthetic membranes is assumed to involve a partitioning between the aqueous phases and, further, a diffusion through the film. Ågren *et al.* (37), studying the permeation of a quaternary compound through nylon membranes with perchlorate as the counter-ion, suggested that transfer was taking place mainly by partitioning and diffusion and, to a much lesser extent by penetration through pores. On the other hand, Lee and Kim (4) proposed that the permeation of ion-paired drugs in non-aqueous systems through 2-hydroxyethyl methacrylate/styrene membrane could proceed via both the partition and the pore mechanisms. This conclusion was founded on permeation experiments based on the free volume theory (22,42) to identify the mechanism of solute diffusion through membranes, the permeation being thus dependent on the molecular radius of the drug.

Ion-Pairing to Enhance Peptide Absorption

The use of absorption enhancers as a means of making peptide administration more facile has been extensively studied (2,42) The results have shown that these can indeed promote peptide absorption. One of the first papers, by Nishihata *et al.* (43), studied the disappearance of phenylalanine and its analogues by perfusion across rat rectal tissue, enhanced by the presence of salicylate or 5-methoxysalicylate. The effect of these adjuvants was attributed to the alteration of the membrane barrier, increasing its permeability; however, the simultaneous absorption of the adjuvant was also required. Data from Okada

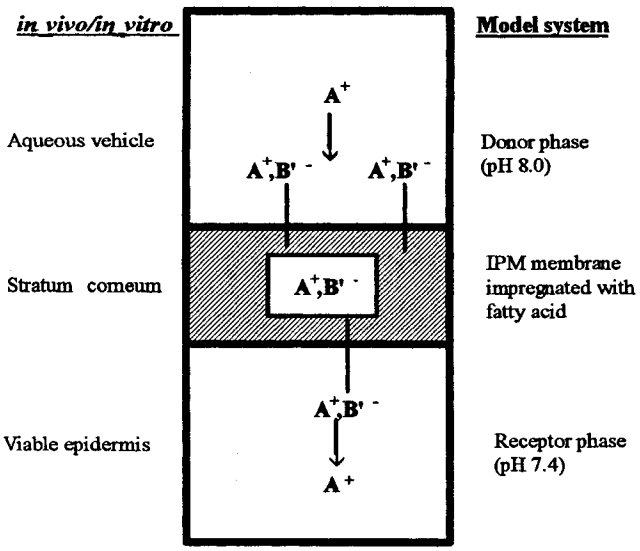

Fig. 4. Conditions required to facilitate the transport of a cation (A^+) from an aqueous donor phase across an IPM membrane impregnated with fatty acid (B'^-) to an aqueous receptor phase. Adapted from (32).

et al. (44) showed that vaginal absorption of leuprolide and insulin could be increased by the use of organic acids. The mechanism was attributed to the acidification and chelating ability of the acids. Studies on the nasal absorption of insulin in the presence of surfactants (45) have suggested that the promoting effect of bile acid salts was due to their direct effect on the nasal mucosa and their inhibitory effect on proteolytic enzymes. It is evident from these investigations that the mechanisms of action of the peptide absorption enhancers are not clearly understood and different hypotheses can be made to explain them. Lee (46) established that ion-pair formation between the enhancer and the peptide drug was a probable way to affect the thermodynamic activity of the peptide and its permeability. Zhou and Po (47) have produced arguments in favour of this idea. They reevaluated the effect of cholate and its analogues on the nasal absorption of insulin, stating that these compounds might enhance the absorption of peptide and protein drugs by binding to insulin. This binding may protect against hydrolysis too, because this would prevent the formation of an enzyme-insulin complex which aligns the catalytic site on the protease. Other facts that would support this assumption are the tendency of cholate and its analogues to form ion-pairs and their low activity as aminopeptidase inhibitors (38).

Green *et al.* (48) showed that it is possible to enhance the flux of peptides across the skin using conventional enhancers for ion-pairing peptides, in order to mask their ionic characteristics thereby increasing their solubility in the stratum corneum. [D-Ala^2]-methionine-enkephalinamide was examined as a model peptide since it possessed only one NH_2 group which was significantly ionized over the pH range of interest and therefore had the potential to pair with oleate anions. The carboxyl residue was blocked by amide formation. Results showed an evident increase of the *in vitro* permeation of peptide across human skin, due to the presence of oleic acid.

Ganem *et al.* (49) in our laboratory found that the *in vitro* permeation of a dipeptide (Trp-Leu) through the palatal mucosa of pig was increased using saline hydroalcoholic solutions. In the absence of sodium chloride, lower permeation was observed. The results suggested that the effects of conformational changes or possible denaturation of keratin in the stratum corneum or the lipid extraction, caused by ethanolic systems, were insufficient to allow the passage of the peptide. The increased permeability was attributed to the probable ion-pair formation between sodium chloride and the peptide.

APPLICATION OF ION-PAIR FORMATION TO THE MICROENCAPSULATION OF PEPTIDES

Micro- and nanospheres are promising candidates as carriers for oral, parenteral and other routes of administration of peptidic drugs. However, one of the main problems of these techniques is the poor entrapment of water-soluble drugs, principally with those that involve an o/w emulsification, due to partition of the drug from the organic phase into the continuous aqueous phase. Yamakawa *et al.* (50,51) studied the influence of different fatty acid salts contained in the external phase on the encapsulated amount of an hexapeptide (neurotensin) in poly(D,L-lactic acid) microspheres, by an o/w solvent evaporation technique. The results indicated a considerable increase of the entrapment ratio in the presence of fatty acid salts. The authors stated that the effect of fatty acid salts was due to partitioning into the oily phase at the solvent-water interface. The coexistence of neurotensin and fatty acid salts as ion pairs in the oily phase was effective in entrapping neurotensin into the microspheres.

Recently, Niwa *et al.* (52) showed that the leakage of nafarelin acetate during the preparation of nanospheres of poly(D,L-lactic-co-glycolic acid) by the nanoprecipitation method can be partially avoided by the addition of a small amount of negatively charged phospholipid such as dipalmitoyl phosphatidylglycerol or dicetyl phosphate. The presence of these additives in the organic phase (acetone) decreased the total leakage of peptide into the aqueous phase during polymer precipitation. The proposed mechanism involved ion-pair formation between the amine groups of nafarelin (histidyl or arginine residues) and the negatively charged phospholipids.

The experiments made in our laboratory to encapsulate a dipeptide (Trp-Leu) in poly(D,L-lactic acid) nanospheres by the emulsification-diffusion method (53), using the peptide in the form of ion-pair in propylene carbonate (Table 2), indicated a low encapsulation efficiency (<3%). This result was attributed to the leakage of the peptide contained in the globules into the aqueous phase, during solvent diffusion. Apparently, the velocity of ion-pair dissociation was faster than polymer precipitation in the interfacial region, where the transport of solvent to the continuous phase was effected.

CONCLUSIONS

It has been shown that ion-pair formation allows the lipophilization of hydrophilic molecules and ions. This kind of association has been widely used for the separation and determination of molecules of pharmaceutical interest. In this respect, it is important to emphasize that the routine separation of peptides with reverse-phase high-performance liquid chromatography is based on ion-pair formation essentially with trifluoroacetic acid, using increasing concentrations of acetonitrile.

Although the usefulness of ion-pair systems to promote permeation of drugs through membranes is controversial, and in some cases questionable, the data presented in this review suggest that ion-pairing should be considered as a useful method for increasing the bioavailability of drugs and enhancing permeation, mainly when the administration is made by routes where the physicochemical equilibrium is not rapidly undermined by the surrounding medium. Therefore, an understanding of the correlation between the physicochemical properties of ion-pairs and their interaction with membranes is necessary.

The application of ion-pair formation to peptides opens interesting perspectives. The concept could provide a useful approach to solve the problems of limited peptide absorption by non-parenteral routes, and a peptidic ion-pair might contribute to the development of formulations with optimized absorption. The transfer of peptides as ion-pairs into solvents could also have applications in the synthesis of proteins and in the development of methods for their micro- and nanoencapsulation. Clearly, further investigations are necessary to identify the possible applications of peptide-based ion-pairs.

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REFERENCES

1. V. H. L. Lee. *Peptide and Protein Drug Delivery.* Marcel Dekker, New York, 1991.
2. X. H. Zhou and A. L. W. Po. Peptide and protein drugs: I. Therapeutic applications, absortion and parenteral administration. *Int. J. Pharm.* **75**:97–115 (1991).
3. H. Sesaki, Y. Takakura, and M. Hashida. Chemical modification and disposition of proteins and peptides: Biopharmaceutical aspects. In D. J. A. Cromelin, and K. K. Midha, (eds.), *Topics in Pharmaceutical Sciences 1991*, Medipharm Scientific Publishers, Stuttgart, 1992, pp. 47–57.
4. S. J. Lee and S. W. Kim. Hydrophobization of ionic drugs for transport through membranes. *J. Contr. Rel.* **6**:3–13 (1987).
5. R. Neubert. Ion pair transport across membranes. *Pharm. Res.* **6**:743–747 (1989).
6. E. Tomlinson. R_x: never mix a cation with an anion? *Pharm. Int.* **Aug**:156–158 (1980).
7. A. T. Florence and D. Attwood. *Physicochemical Principles of Pharmacy*, Macmillan, London, 1988.
8. G. Schill. High performance ion-pair chromatography. *J. Bioch. Biophys. Methods.* **18**:249–270 (1989).
9. A. Martin. *Physical Pharmacy*, Lea and Febiger, Philadelphia, 1993.
10. C. A. Kraus. The ion-pair concept: its evolution and some applications. *J. Phys. Chem.* **60**:129–141 (1956).
11. N. Bjerrum. Kgl. Danske Selskab. *Matt.-fys. Medd.* **7**:No. 9 (1926).
12. H. Freiser. Ion pair partition. *Acta Pharm. Suec.* **9**:609–611 (1972).
13. M. Szwarc. Living polymers: a tool in studies of ions and ion-pairs. *Science*. **170**:23–31 (1970).
14. B. M. Atherton and S. Y. Weissman. Association between sodium and naphthalenide ions. *J. Am. Chem. Soc.* **83**:1330–1334 (1961).
15. A. Bult. Ion-pair and metal complex formation in drug analysis. In D. D. Breimerand and P. Speiser (eds.), *Topics in Pharmaceutical Sciences 1983*, Elsevier, Amsterdam, 1983, pp. 3–14.
16. G. M. Irwin, H. B. Kostenbauder, L. W. Dittert, R. Staples, A. Misher, and J. V. Swintosky. Enhancement of gastrointestinal absorption of a quaternary ammonium compound by trichloroacetate. *J. Pharm. Sci.* **58**:313–315 (1969).
17. M. Akamatsu, Y. Yoshida, H. Nakamura, M. Asao, H. Iwamura, and T. Fujita. Hydrophobicity of di and tripeptides having unionizable side chains and correlation with substituent and structural parameters. *Quant. Struct.-Act. Relat.* **8**:195–203 (1989).
18. A. Adjei, S. Rao, J. Garren, G. Menon, and M. Vadnere. Effect of ion-pairing on 1-octanol-water partitioning of peptide drugs. I: The nanopeptide leuprolide acetate. *Int. J. Pharm.* **90**:141–149 (1993).
19. H. Sadek and R. M. Fuoss. Electrolyte-solvent interaction. IV. Tetrabutylammonium bromide in methanol-carbon tetrachloride and methanol-heptane mixtures. *J. Am. Chem. Soc.* **76**:5897–5901 (1954).
20. S. Winstein, E. Clippinger, A. H. Fainberg, and G. C. Robinson. Salt effects and ion-pairs in solvolysis. *J. Am. Chem. Soc.* **76**:2597–2598 (1954).
21. R. C. Roberts and M. Szwarc. The chemistry of the radical anion of tetraphenylethylene. *J. Am. Chem. Soc.* **87**:5542–5548 (1965).
22. S. J. Lee, T. Kurihara-Bergstrom, and S. W. Kim Ion-paired drug diffusion through polymer membranes. *Int. J. Pharm.* **47**:59–73 (1987).
23. H. Freiser. Relevance of solubility parameter in ion association extraction systems. *Anal. Chem.* **41**:1354–1355 (1969).
24. T. Higuchi, A. Michaelis, and A. Hurwitz. Ion pair extraction of pharmaceutical amines. *Anal. Chem.* **39**:974–979 (1967).
25. R. M. Diamond. Aqueous solution behavior of large univalent ions. A new type of ion-pairing. *J. Phys. Chem.* **67**:2513–2517 (1963).
26. R. Modin and G. Schill. Quantitative determinations by ion-pair extraction. Part 1. Ions pairs of quaternary ammonium ions with organic anions. *Acta Pharm. Suec.* **4**:301–326 (1967).
27. R. Modin and M. Schröder-Nielsen. Quantitative determinations by ion pair extraction. Part 9. Extraction of penicillins with ion-pairing and adduct-forming agents. *Acta Pharm. Suec.* **8**:573–584 (1971).
28. K. S. Murthy, and G. Zografi. Oil-water partitioning of chlorpromazine and other phenotiazine derivatives using dodecane and n-octanol. *J. Pharm. Sci.* **59**:1281–1285 (1970).
29. J.-Y. Carbon, M. l'Her, and J. Courtot-Coupez. Salt effects on the distribution of metal ions and their complexes between water and propylene carbonate. *J. Chem. Research.* **S**:344–345 (1978).
30. B. Y. Zaslavsky, N. M. Mestechkina, L. M. Miheeva, and S. V. Rogozhin. Measurement of relative hydrophobicity of amino acid side-chains by partition in an aqueous two-phase polymeric system: Hydrophobicity scale for non-polar and ionogenic side-chains. *J. Chromat.* **240**:21–28 (1982).
31. E. Ivashkiv. Colorimetric determination of peptide antibiotics: In-process assay of cyclic octapeptidic antibiotics in fermentation broths. *J. Pharm. Sci.* **64**:1401–1403 (1975).
32. M. Narita, M. Doi, H. Sugasawa and K. Ishikawa. Syntheses and properties of oligo-L-leucines containing α-amino-isobutyric acid residues. The novel strategy for solubility improvement in helical oligopeptides based on the restriction of the values of the backbone dihedral angles ϕ and ϑ of α-aminoisobutyric acid residues. *Bull. Chem. Soc. Jpn.* **58**:1473–1479 (1985).
33. M. Narita, S. Honda, H. Umeyama and S. Obana. The solubility of peptides intermediates in organic solvents. Solubilizing potential of hexafluoro-2-propanol. *Bull. Chem. Soc. Jpn.* **61**:281–284 (1988).
34. P. G. Green, R. H. Guy and J. Hadgraft. In vitro and in vivo enhancement of skin permeation with oleic and lauric acids. *Int. J. Pharm.* **48**:103–111 (1988).
35. L. S. Schanker. On the mechanism of absorption of drugs from the gastrointestinal tract. *J. Med. Pharm. Chem.* **2**:343–359 (1960).
36. E. Susuki, M. Tsukigi, S. Muranishi, H. Sezaki, and T. L. K. Kakemi. The absorption from gut of quinine and chlorpheniramine given with various anionic agents. *J. Pharm. Pharmacol.* **24**:138–144 (1972).
37. A. Ågren, B. Nilsson, R. Sjökvist, and A. Brodin. Penetration of organic compounds and ion pairs trough nylon membranes. *Acta Pharm. Suec.* **11**:523–532 (1974).
38. T. S. Gaginella, P. Bass, J. H. Perrin, and J. J. Valliner. Effect of bile salts on partitioning behavior and GI absorption of a quaternary ammonium compound isopropamide iodide. *J. Pharm. Sci.* **62**:1121–1125 (1973).
39. W. D. Walking, A. C. Bonfilio, and H. Y. Jacoby. Possible ion-pair-mediated absorption of mixidine I: Partitioning and lethality studies. *J. Pharm. Sci.* **67**:945–948 (1978).
40. P. G. Green, J. Hadgraft and G. Ridout. Enhanced *in vitro* skin permeation of cationic drugs. *Pharm. Res.* **6**:628–632 (1989).
41. B. J. Poulsen, E. Young, V. Coquilla, and M. Katz. Effect of topical vehicle composition on the *in vitro* release of fluocinolone acetonide and its acetate ester. *J. Pharm. Sci.* **57**:928–933 (1968).
42. X.-H. Zhou, and A. L. W. Po. Peptide and protein drugs: II. Non-parenteral routes of delivery. *Int. J. Pharm.* **75**:117–130 (1991).
43. T. Nishihata, C.-S. Lee, M. Yamamoto, J. H. Rytting, and T. Higuchi. The effects of salicylate on the rectal absorption of phenylalanine and some peptides, and the effects of these peptides on the rectal absorption of cefoxitin and cefmetazole. *J. Pharm. Sci.* **73**:1326–1328 (1984).
44. H. Okada, Y. Yamazaki, Y. Ogawa, S. Hirai, T. Yashiki, and H. Mima. Vaginal absorption of a potent luteinizing hormone releasing hormone analog (leuprolide) in rats I: Absorption by various routes and absorption enhancement. *J. Pharm. Sci.* **71**:1367–1371 (1982).
45. S. Hirai, T. Yashiki, and H. Mima. Mechanism for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.* **9**:173–184 (1981).
46. V. H. L. Lee, A. Yamamoto, and U. B. Kompella. Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug Carriers Syst.*, **8**:91–192 (1991).

47. X. H. Zhou, and A. L. W. Po. Effects of cholic acid and other enhancers on the bioavailability of insulin from a subcutaneous site. *Int. J. Pharm.* **69**:29–41 (1991).
48. R. D., Green, J. Hadgraft, and K. R. Brain. Enhanced penetration of peptides through the skin. *J. Pharm. Pharmacol.* **43**:113P(1991).
49. A. Ganem, F. Falson-Rieg, and P. Buri Permeation enhancement for peptide absorption through palatal mucosa. Proc. Sixth Eur. Congr. Biopharm. Pharmacokinet., 1996, pp. 111.
50. Y. Yamakawa, Y. Tsushima R. Machida, and S. Watanabe Preparation of neurotensin analogue-containing poly(dl-lactic acid) microspheres formed by oil-in-water solvent evaporation. *J. Pharm. Sci.* **81**:899–903 (1992).
51. Y. Yamakawa, Y. Tsushima, R. Machida, and S. Watanabe. *In vitro* and *in vivo* release of poly(dl-lactic acid) microspheres containing neurotensin analogue prepared by novel oil-in-water solvent evaporation method. *J. Pharm. Sci.* **81**:808–811 (1992).
52. T. Niwa, H. Takeuchi, T. Hino, N. Kunou, and Y. Kawashima. *In vitro* drug release behavior of D,L lactide/glycolide copolymer (PLGA) nanospheres with nafarelin acetate prepared by a novel spontaneous emulsification solvent diffusion method. *J. Pharm. Sci.* **83**:727–732 (1994).
53. J.-C. Leroux, E. Allémann, E. Doelker, and R. Gurny. New approach for the preparation of nanoparticles by an emulsification-diffusion method. *Eur. J. Pharm. Biopharm.* **41**:14–18 (1995).