Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 797 (2003) 37–49
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Drug delivery systems: polymers and drugs monitored by capillary electromigration methods

Carolina Simó [a], Alejandro Cifuentes [a,*], Alberto Gallardo [b]

[a] *Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*
[b] *Institute of Science and Technology of Polymers (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*

Abstract

In this paper, different electromigration methods used to monitor drugs and polymers released from drug delivery systems are reviewed. First, an introduction to the most typical arrangements used as drug delivery systems (e.g., polymer–drug covalent conjugates, membrane or matrix-based devices) is presented. Next, the principles of different capillary electromigration procedures are discussed, followed by a revision on the different procedures employed to monitor the release of drugs and the degradation or solubilization of the polymeric matrices from drug delivery systems during both in vitro and in vivo assays. A critical comparison between these capillary electrophoretic methods and the more common chromatographic methods employed to analyze drugs and polymers from drug delivery systems is presented. Finally, future outlooks of these electromigration procedures in the controlled release field are discussed.

Keywords: Reviews; Capillary electrophoresis; Polymers; Drugs

Contents

* Corresponding author. Tel.: +34-91-562-2900; fax: +34-91-564-4853.
E-mail address: acifuentes@ifi.csic.es (A. Cifuentes).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1570-0232(03)00430-6

1. Drug delivery systems: an introduction

During the last decades, the field of drug delivery and controlled release has seen a dramatic development, with several organizations (as the CRS, Controlled Release Society) and scientific journals (as the *Advanced Drug Delivery Reviews*) specifically devoted to it. Besides, excellent textbooks and encyclopaedias covering this topic can be found in the literature [1–4].

Theoretically, an ideal drug delivery system (DDS) should deliver a drug to a specific site, in a specific time and release pattern. In the early times, the basic deal was to get a constant (zero order kinetic) or sustained drug release in order to avoid the problems associated with a multiple conventional administration in chronic treatments. Nowadays, the challenges are much more ambitious. The current trends of new controlled release devices include the optimization on the targeting to specific sites and the fitting of the drug release to the circadian rhythm. Besides, drug delivery technology covers other specific needs such as: (1) to get a slow release of water soluble drugs; (2) to improve the bioavailability of low water soluble drugs; (3) to deliver two or more agents in the same formulation; (4) to develop carriers readily clearable; (5) to improve the biodistribution of drugs with a high rate of metabolism or rapid clearance; (6) to control the release of highly toxic drugs and; (7) to improve the targeting to the target tissues or cells.

Therefore, zero order release is not the only goal. In fact, in many cases other kinetics or requirements are much more appropriate. Insulin is a very clear example. A constant supply of this drug for the treatment of insulin-dependent diabetic patients would be a disastrous therapy. What the body needs in this case is an “intelligent” device (different approaches can be found in the literature [5–7]) able to respond to the glucose concentrations. In addition to the zero order release, single pulse, multipulse, targeted, modulated, one order or even increasing delivery can be appropriate in some specific treatments.

Although lipid-based drug delivery systems (LBDDSs), as liposomes, are currently very advanced devices, in this review we will focus on the polymer-based systems. After more than 40 years of research and development of a large quantity of systems, it is clear that the polymeric supports are decisive in the design and preparation of controlled delivery formulations. In fact, the great versatility of polymers from a structural point of view, together with the enormous possibilities to combine hydrophobic and hydrophilic components, as well as the interactions between polymer–polymer macromolecules, polymer–drug, polymer–solvent or polymer–physiological medium, offer huge possibilities to design and prepare formulations with specific properties and functions.

Besides, degradability and/or resorbability is an obvious requirement in parenteral applications, in order to avoid unwanted polymer accumulation. This characteristic can be achieved by using biodegradable supports finally converted to non toxic low-molecular-mass products, or hydrosoluble macromolecular matrices readily eliminated by renal pathway.

The DDS can be classified as a function of the structure and the release mechanism, as shown in Table 1. In this table, additional columns have been included to emphasize the importance of the degradability/resorbability and the kinetics of these systems. Some commercial examples (or in advanced clinical trials) have been quoted in the last column of Table 1. A more detailed description on the different DDSs is next presented.

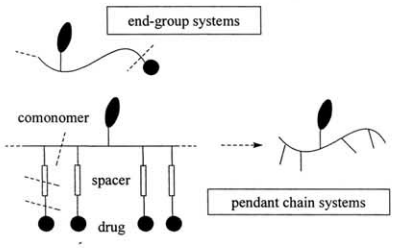
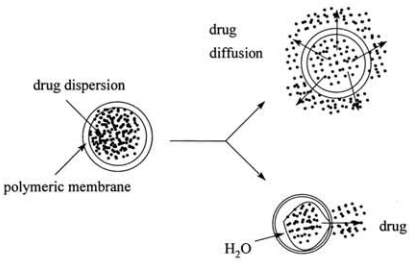
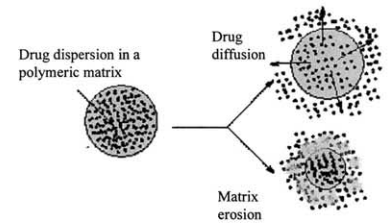
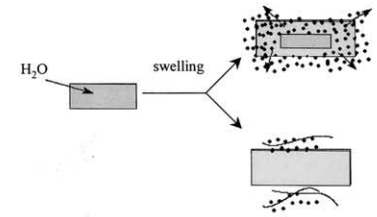
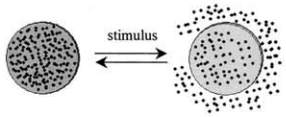
1.1. Polymer–drug conjugates

In this kind of DDS, the active compound is covalently bound to the macromolecular backbone through a labile bond. These DDSs are probably the most attractive devices because they are designed on a molecular basis. However, in despite of the high versatility of this approach, it is difficult to find marketed products mainly because these conjugates are viewed as new drug molecules and the path needed to be approved is arduous and expensive. The conjugation sites may be at one (or both) end of the macromolecule or they may be pendant to the main chain, with the sites repeating along the backbone (see Table 1) [8].

1.1.1. Pendant chain systems

The scheme shown in Table 1 for the pendant chain systems is based on the famous cartoon published by Helmut Ringsdorf in 1975 [9]. In this pendant-type conjugates, spacer linkages may sometimes be incorporated between the drug and the main chain to sterically facil-

Table 1
Characteristics of the main types of drug delivery systems (DDSs)

Type of DDS	Structure and release mechanism	Type of control on the release	Degradability Resorbability	Release kinetics	Clinical examples
Polymer-drug conjugates		chemical	hydrosoluble (and/or biodegradable)		protein pegylation
			hydrosoluble residue (and/or biodegradable)		SMANCS® HPMA conjugates
Membrane-based systems		drug diffusion	membrane can be biodegradable	zero order	Ocusert® Progestasert® Transdermal systems
		osmotic	membrane non biodegradable		Oros® systems
Matrix-based systems		drug diffusion	non degradable polymeric matrix	Fick's law (one order)	some oral DDS
		[diffusion/erosion]	degradable polymeric matrix		PLGA systems (LHRH analogues)
		erosion	degradable polymeric matrix	zero order (hydrophobic matrices)	Gliadel®
Hydrophilic matrices		swelling controlled [water penetration]	hydrogel, can be biodegradable		Geomatrix®
		dissolution controlled	hydrosoluble		
Stimuli-responsive systems		Stimuli-responsive		multipulse delivery	

itate the hydrolysis and the release or to provide groups that allow an enzymatic specific recognition (a targeting). The Ringsdorf model includes other comonomers that may regulate the physico-chemical properties of the carrier (as the solubility) or may lead to a targeting by means of specific molecular recognition. The original polymeric carrier, or the final residue, must be in general water-soluble and clearable. It is now accepted that chains with molecular masses below 30–50 000 (depending on the nature of the polymer) are amenable of renal elimi-

nation [10]. The support must also be non toxic and non immunogenic.

Numerous conjugated systems have been designed and investigated [11–14]. Particularly relevant are two conjugates, based on the work of Duncan and Kopecek [15] and Maeda et al. [16], currently used in clinics. These two systems are based in hydroxypropyl methacrylamide (HPMA) and styrene–co-maleic anhydride (SMA) copolymer, respectively.

1.1.2. End-group systems

This approach finds an important application in the so-called protein pegylation, that is, the conjugation of polyethylene glycol (PEG) to protein drugs to protect them from recognition by the body's immune system and to prolong their circulation time in the body, as it was originally described by Abuchowski et al. in 1977 [17]. There are several companies marketing different PEG–protein or PEG–drug conjugates for the treatment of cancer, hepatitis, etc.

1.2. Membrane-based systems

In this case, the DDS consists of a drug-dispersion reservoir contained in a membrane device. There are two main types of systems: (1) devices where the release is achieved by diffusion of the drug across the polymeric membrane, and (2) osmotic systems, which present a semi-permeable membrane that allows the entrance of water by osmotic pressure and the drug delivery by a small orifice.

1.2.1. Diffusion controlled membrane-based systems

The drug release in this case is controlled by the transport rate of the active compound across the membrane, that is, by the diffusion coefficient and the thickness of the layer (according to the Fick's law). These systems exhibit a very interesting zero order kinetic, a constant release rate independent of time. Membranes can be porous or non-porous, and biodegradable or not biodegradable. If it is biodegradable, the polymer degradation must take place after the total release of the drug in order to avoid premature membrane disruption and overdoses.

Several commercial products can be included in this type of DDSs, as Ocusert to deliver pilocarpine in ocular therapies, Progestasert for the deliver of the contraceptive progesterone (both made of ethylene–vinyl acetate copolymer), and specially the transdermal devices for the delivery of different drugs as nitroglycerine or nicotine. Fig. 1 shows a schematic diagram of a transdermal drug delivery system. These DDSs are very attractive because are not invasive and the drugs reach the bloodstream directly via the intact skin, but they have an important limitation: there are few drugs that can cross the skin efficiently enough to achieve a therapeutic concentration. Although great improvements have been made on this topic, mainly based on iontophoresis, it is still an important limitation.

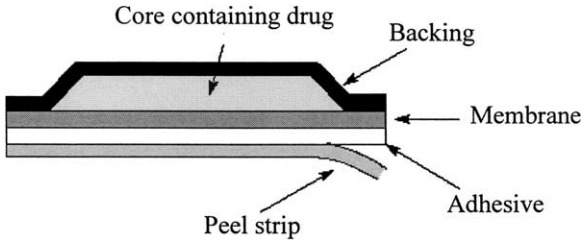


Fig. 1. Cross section of a transdermal DDS.

1.2.2. Osmotic systems

The most representative devices are the OROS and DUROS products, very well-known oral DDSs for the delivery of different active compounds. Fig. 2 shows a cross-section of the elementary osmotic pump OROS. The device is a reservoir made of a polymeric membrane permeable to the water but not to the drug. This reservoir presents a small delivery orifice. As the core is a concentrated solution of the drug, water crosses the semi-permeable membrane due to the osmotic pressure, leading to the exit of the drug solution thought the orifice. These systems present several advantages: the release rates are independent of the agent properties, they can deliver macromolecules and ionic species and it may give rise to relatively high fluxes. A disadvantage is the potential danger associated to an overdose due to the breakdown of the membrane.

It is also possible to use the osmotic phenomenon to get one-pulse systems. This is achieved with a closed membrane-based system that undergoes the burst of this external layer when the osmotic pressure is high enough [18].

1.3. Matrix-based systems

In this case, the drug is dispersed in a monolithic polymeric matrix. If the polymer is not biodegradable, the release is controlled by the diffusion of the active molecule through the matrix (giving rise to a theoretical first order kinetic). If the matrix is biodegradable, different situations may take place. If the polymer is highly

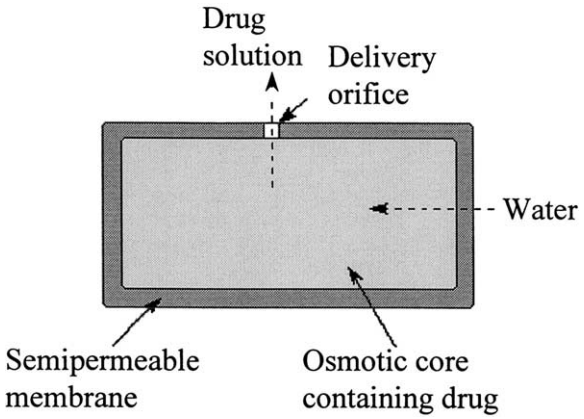


Fig. 2. Cross section of the OROS technology.

hydrophobic, as it happens with the polyanhydrides or the polyorthoesters, the systems undergoes a surface erosion and the release is controlled by this degradation mechanism, giving rise to a zero order kinetic (constant release) [19]. A clinical example is the Gliadel device, which consists of polyanhydride discs and it is implanted at the site where a brain tumor was surgically removed as a palliative treatment.

The family of biodegradable polymers most exhaustively studied in this field, is the PLGA (poly-lactic–co-glycolic acid), very attractive because its degradation gives rise to the formation of lactic and glycolic acid, two well-known metabolites usually present in the human body. In this case, the release is controlled by both the diffusion of the drug and the degradation of the matrix, because not surface but bulk erosion takes place [20]. Different commercial products for the delivery of LHRH analogues have been already launched in the market.

1.4. Hydrophilic matrices

In this category, we can include those hydrophilic systems in which the release is controlled by the entrance of the water (excluding the osmotic devices). The water penetration leads to the swelling or to the dissolution of the matrix and the drug release is associated to this phenomena.

1.4.1. Swelling controlled systems

The matrix is in this case an originally glassy polymer that swells in water or physiological fluids. The macromolecular system undergoes a glass/rubber transition due to the water, forming a hydrogel-like material. The previously incorporated drug can diffuse through the swollen polymer layer, and the solvent penetration front controls the drug release. There is a commercial device, called Geomatrix, based on this mechanism, which is a simple swellable polymer partially covered with an impermeable coating. The release is controlled by the uncoated area and probably by molecular changes during swelling [21].

1.4.2. Dissolution controlled systems

In this case, the water swelling controls the dissolution of the carrier. Drugs with poor water solubility, or macromolecular active compounds, can be released in a controlled way by this mechanism [22,23]. The solubilization of such a macromolecular structure may be a very slow process because the matrix must undergoes an initial unfolding (if semicrystaline) and after that, a disentanglement of the chains [24].

1.5. Stimuli-responsive systems

As most of organ functions follow circadian rhythms, constant release is sometimes invalid and the current trend is to treat patients based on chronopharmacokinetics and chronopharmacodynamics [25]. Moreover, the ideal DDS should be able to supply drugs on demand. These points have impelled researchers to investigate responsive drug delivery systems from which the drug release can be controlled by different internal or external stimuli [26]. Usually the stimulus makes the polymer to collapse or to expand, controlling in this way the release of the active compound.

These systems can be classified in physically modulated (stimulus can be the temperature, an electrical signal or light), chemically modulated (stimulus can be the pH or glucose) and those modulated by a combination of stimuli. One of the most investigated sensitive polymers is the poly(*N*-isopropylacrylamide), PNIPAM, since this polymer presents a low critical solution temperature (LCST) and, therefore, it can show a reversible transition close to the physiological temperature [27]. The pH-dependent systems are also very interesting because of the differences in pH along the gastrointestinal tract, from acidic in the stomach to slightly basic in some parts of the intestine. Some attractive examples have been proposed to target drugs to the colon [28]. Most of these systems are polyelectrolytes because the pH affects their ionization degree and, therefore, their swelling. Finally, it has to be stressed the research done in glucose-sensitive systems [5–7], very relevant for the self-regulated insulin delivery systems in the treatment of insulin-dependent diabetic patients. However, in spite of these very attractive developments, few systems have yet emerged to clinical trials.

From the huge variety of DDSs showed above, it can be understood the importance of developing new analytical procedures able to monitor the diverse polymers and drugs that are expected to come from the release, degradation or solubilization of these different DDSs. In this sense, capillary electromigration procedures have demonstrated to be a good alternative for this type of analysis.

2. Capillary electromigration procedures: principles

Capillary electrophoresis (CE) is a combination of separation techniques based on the different electrophoretic mobilities of the dissolved substances under the action of an electric field [29–31]. It could be said that this technique combines the power of separation of conventional electrophoresis [32] with the concept of automation of high-performance liquid chromatography (HPLC). CE presents a great variety of applications, from metallic ions to particles including peptides, proteins, fragments of DNA, pharmaceuticals, cells, etc.

There are different forms of CE, mainly based on the nature of the separation media that is introduced in the capillary and also in the characteristics of the analytes that will be separated with this technique. Interestingly, the instrumentation is practically the same for all of them. In the following sections, a short description of all the currently existing CE modes can be found.

2.1. Free solution capillary electrophoresis (FSCE)

It was the first electrophoresis mode developed, and, nowadays, it is the most frequently used [33–35]. Inside the capillary there is only the separation buffer, therefore, it is possible to simultaneously separate positively and negatively charged substances when the magnitudes of the electrophoretic and electroosmotic mobilities are suitable.

Following these criteria, compounds with higher positive charge density and smaller radius, will be eluted in a shorter time. When the electric field increases, the migration times of the compounds will decrease. There is an optimum for this field giving the shortest analysis time with a suitable efficiency. Over this value, phenomena related to heat generated by Joule effect appear and separation efficiency decreases.

FSCE presents several limitations (frequently some of them can be overcome by using other different CE modes) that can be summarized as follows: (a) generally, separation of uncharged species or with the same charge-to-mass ratio [as for example, DNA fragments or protein–sodium dodecyl sulfate (SDS) complexes] cannot be accomplished by using FSCE; (b) compounds bearing a high positive electrical charge density can be adsorbed onto the capillary wall (this adsorption will influence negatively on the separation process); (c) RSDs for peak areas are in the range from 2 to 5% in real samples analysis and; (d) the sensitivity of the technique does not permit the trace analysis. The points (c) and (d) are common for all the CE modes.

2.2. Micellar electrokinetic chromatography (MEKC)

This CE mode was initially developed to solve the limitation related to the separation of non charged compounds [36,37], although it can also be applied to the separation of charged substances.

MEKC involves the addition to the separation buffer of a surfactant at a concentration level at which micelles form. Detergents or surfactants are molecules that have a hydrophilic/ionic moiety on one end of the molecule and a hydrophobic moiety on the other. Micelles, aggregation of individual detergent molecules, form in aqueous solution when a detergent is present at a concentration higher than its critical micelle concentration (CMC). Micelles are generally spherical in shape being the hydrophilic groups of the detergent on the outside of the micelle, toward the aqueous buffer. The hydrophobic hydrocarbon molecules are in the center of the micelle. Detergents can be anionic, cationic, zwitterionic, or non ionic and their external charge will determine their migration towards the anode or the cathode or whether they are just moved by the electroosmotic flow.

Micelles constitute a stable second phase, that, in chromatographic terms, act as a pseudo-stationary phase that moves into the capillary [36,37]. Neutral analytes will interact with the micelles depending on their specific partition coefficient, which depends on their chemical characteristics. The time spent by the analyte inside the micelle will retard it in relation to the other neutral substances (they will interact with the micelles specifically too). Therefore, the mechanism of separation depends on differences in distribution coefficients of the analytes between aqueous and the hydrocarbon pseudo-stationary phase.

2.3. Capillary gel electrophoresis (CGE)

In this type of CE, the capillary is filled with a gel that will act as a molecular sieving. The most important application of this technique will be the separation of compounds with the same charge/mass ratio, but with different molecular mass [38,39], as for example DNA fragments, polysaccharides, SDS–protein complex or ionic polymers. In CGE, the molecules with smaller molecular size are able to pass through the pores and migrate first, whereas larger molecules are retarded by the gel and migrate later.

The first gels to be used in the latter 1980s were made of crosslinked polyacrylamide covalently linked to the capillary wall. However, they showed problems related to low reproducibility, resistance and stability. Nowadays, they have been substituted by the polymeric networks. They are hydrophilic non-crosslinked polymers that are dissolved in the buffer solution in a concentration usually higher than the so-called entanglement concentration, over which a net that acts like a molecular sieve is formed (although according to Barron et al. [40] it is not necessary to reach that concentration to obtain the effect of a molecular sieve). The most frequently used polymers are: linear polyacrylamide, PEG, polyvinyl alcohol, methylcellulose, etc.

2.4. Capillary isotacophoresis (cITP)

The sample is injected between the frontal buffer, with higher mobility than the fastest compound in the sample, and the terminal buffer, with lower mobility than the slowest component in the sample. In cITP, the substances are separated based on their electrophoretic mobility (μ_I). With the application of a difference of voltage (or electric field, E) the analytes get distributed in bands that move between the frontal and terminal buffer inside the capillary [41], all of them with the same velocity ν_{ITP} (hence the prefix isotaco, which means equal velocity), according to the equation:

$$\nu_{ITP} = E_1\mu_1 = E_2\mu_2 = E_i\mu_i$$

Equilibrium is achieved between the effective mobility of each analyte and the electric field in the corresponding electrophoretic band. Before reaching a stationary state, the substances are found outside their electrophoretic bands, and therefore, with an electric field different from that in equilibrium. As a result, the velocity of the analytes changes until they arrive at a zone in the capillary where there is a potential that makes them obtain the equilibrium velocity.

This electrophoresis mode, in spite of being less used, finds its main application in on-column sample pre-concentration protocols used together with other CE

modes. When a diluted sample is injected and the voltage is applied, the zone is concentrated in the interface with the separation buffer. The pre-concentration depends on the characteristics (e.g., concentration and ionic strength, electrophoretic mobilities) of the analytes, sample matrix and separation buffer.

2.5. Capillary isoelectricfocusing (cIEF)

cIEF is an important tool in analytical biochemistry. It has been mostly applied to the separation of peptides and proteins, as shown in the pioneer work published by Hjertén and Zhu [42].

Usually, a mixture of ampholytes with different pH values is introduced in the capillary together with the sample (the peptides and proteins to be separated). When an electric field is applied, a pH gradient inside the capillary is firstly stabilized due to the ampholytes, which are distributed from the anode (with low pH values) to the cathode (with high pH values) [43,44]. Peptides or proteins with positive or negative charge, under the influence of the electric field, move through the capillary to the anode or cathode until they reach the zone of the capillary in which the pH of the buffer is the same as their isoelectric point, that is to say, they get a pH value in which the number of their positive and negative charges is the same. At this pH value, analyte migration stops, as its global electrical charge is zero. When all the compounds have achieved their isoelectric point within the capillary, elution is generally performed by applying a low pressure (keeping on the run voltage) in the anodic end, moving in that way the focused bands towards the detection point. The capillaries used in this mode usually have an internal coating that decreases or eliminates the electroosmotic flow, because that flow would prevent in most cases the formation of the pH gradient.

As already commented, cIEF is fundamentally applied to the separation of proteins and peptides (i.e., amphoteric compounds) that present isoelectric point [45].

2.6. Capillary electrochromatography (CEC)

This type of CE has a great similarity with liquid chromatography. In CEC, the capillary is filled with silica particles (3 to 10 μm of diameter and derivatized or not) that act as a stationary phase. The buffer acts as a mobile phase that moves when an electric field is applied. Its velocity is proportional to the electroosmotic flow (i.e., $\nu_{eo}=\mu_{eo}E$). Neutral compounds are carried by the electroosmotic flow and they interact specifically with the stationary phase (in the same way as in HPLC) which originates their separation.

As happened with the MEKC technique, CEC was developed mainly due to the research works directed to the separation of non charged compounds in CE [46]. This technique is currently under development, one of its main limitations being the short life of the filled capillaries. These capillaries, apart from being time consuming to prepare and/or expensive, frequently cause the formation of bubbles in the interior as a result of the application of the electric field. This makes the capillaries useless for further applications. Moreover, the application of CEC to "real life" samples has still to be proved.

2.7. Electrically-driven size-exclusion chromatography

Recently, a new separation technique called electrically-driven size-exclusion chromatography (ED-SEC) or size-exclusion electrochromatography (SEEC) [47,48] for the analysis of large macromolecular compounds has been developed. ED-SEC is based on CEC and it is, therefore, of potential interest to study drug delivery systems. This technique employs capillary columns (30–100 μm I.D.) packed with bare silica particles (typically 3–10 μm), together with high dielectric constant solvents such as water, acetonitrile or dimethylformamide (DMF). Under these conditions, after applying the high voltage, a strong electroosmotic flow is generated and with it the macromolecules move within the capillary. Polymers are separated based on their different size due to the differential exclusion from different fractions of the mobile phase in the column. According to the authors, plate numbers in SEEC can be 2–3-times higher than in standard, pressure-driven SEC [47–49].

Separation of polystyrenes by SEEC in packed capillaries using DMF as solvent was demonstrated in Ref. [47]. In that work [47], an improvement of the efficiency obtained for polystyrenes polymers was found compared to that obtained for standard pressure-driven SEC analysis of the same solutes. Unfortunately, with SEEC the retention window is smaller than under pressure conditions and, moreover, appeared to depend strongly on the ionic strength of the mobile phase. This phenomenon was attributed to the occurrence of pore flow that was further studied in Ref. [48]. To do this, the applicability of SEEC for the separation of polystyrenes was investigated in capillary columns packed with 5 μm particles with different pore sizes using DMF as the mobile phase. It was found that under SEEC conditions, a significant intraparticle pore flow was generated. Besides, the relative intraparticle velocity with respect to the average interparticle velocity increased with the pore size and ionic strength. It was also observed that with increasing pore-flow the plate height of polymers decreased considerably. On the other hand, the intraparticle velocity impaired the selectivity of the separation. These effects could be described well with a theory that was also developed in that work [48].

Recently, the use of rigid polymer monolithic capillary columns for the separation of polystyrenes in CEC was reported [50]. However, the reported chromatogram shows an extremely low selectivity and only polymers with a very large difference in molecular mass could be separated on these columns.

2.8. Affinity capillary electrophoresis (ACE)

ACE allows studying noncovalent molecular interactions between a given analyte (i.e., the drug in the DDS) and a ligand (usually the polymer used to control the drug delivery from the DDS) [51,52]. Derived from the methods and principles of AGE, ACE combines the advantages of AGE (e.g., high resolution) with the benefits of CE (e.g., high speed, precise quantification, etc.). ACE is frequently used to study short-life-time analyte–ligand interactions. The basis for the ACE methods lies in the dissolving of one of the components of the analyte–ligand pair in the running buffer and measuring the change in the mobility of the substrate as a function of concentration. In this way, it is possible to estimate binding constants between the analyte and the ligand.

3. Monitoring DDSs by capillary electromigration procedures

ACE has been used for a better understanding of the polymer, used as controlled release system, and drug affinity properties. Thus, PLGA copolymer, a synthetic polymer employed as biodegradable matrix-based system (as described in Section 1), has been studied by ACE for drug delivery of therapeutic peptides (triptorelin, angiopeptin, thyrotropin, with similar physicochemical properties and a new therapeutic peptide (named peptide 1), with different physicochemical properties, see Fig. 3) [53]. The potential of ACE was used to assess binding constants between PLGA as DDS and the mentioned therapeutic peptides. The binding constants were calculated through the measurement of mobility variations of the peptides after addition of increasing concentrations of PLGA to the running buffer, using nonlinear regression fits and different mathematical models. The ACE peptides separation was achieved in an acidic running buffer (60 m*M* ammonium formiate at pH 3 in water–acetonitrile, 90:10, v/v) in order to minimize adsorption of peptides to the capillary wall. In Fig. 3, the separation of the peptides with different concentrations of polymer added to the running buffer is shown. As can be seen, the migration order of the peptides is modified with increasing amounts of polymer. Besides, as polymer concentration increases, different behaviors are observed during ACE analysis of the peptides due to different velocity interaction kinetics. In this way, ACE was demonstrated to be a very helpful method to simultaneously compare the affinity of therapeutic peptides with polymers used as delivery systems.

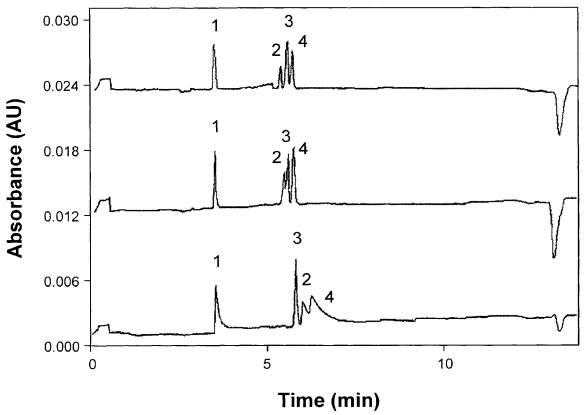


Fig. 3. Influence of PLGA concentrations, added to the running buffer on the separation of thyrotropin releasing hormone (TRH) (1), angiopeptin (2), peptide 1 (3) and triptorelin (4). Running buffer: 60 m*M* ammonium formiate at pH 3, water–acetonitrile (90:10, v/v) and PLGA added at the indicated concentrations. Uncoated fused-silica capillary: 57 cm (50 cm to the detector)×75 μm I.D. Capillary temperature: 25 °C. Run voltage: 30 kV. Injection at 20 p.s.i. for 5 s. UV detection at 200 nm. Redrawn from Ref. [53].

Kimakée et al. [54] used free solution capillary electrophoresis to monitor the release profile of the polypeptidic antibiotic polymixin B (PMB) from a bioactive DDS, namely, calcium-deficient apatite (CDA, $[Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}]$). PMB, a thermolabile drug above 60 °C, was dynamically compacted on the ceramic material without external heating. Different compaction velocities and PMB weight ratios were tested. Monitoring of PMB by CE was accomplished using 0.1 *M* sodium acetate buffer at pH 4.5. The biological suitability of CDA as DDS using dynamic compaction of the therapeutic agent PMB was proved by this FSCE method.

MEKC has been used for monitoring cyclosporine release from soluble, uncrosslinked and high-molecular-mass copolymers of vinylpirrolidone (VP) with 2-hydroxyethyl methacrylate, HEMA, prepared by free radical copolymerization [22], which behave as dissolution controlled hydrophilic matrices. Cyclosporine is a cyclic oligopeptide used in immunosuppression therapy (medicine and surgery), however, this drug has also been associated with serious toxic side effects at medium and long term, mainly renal and hepatic. The microstructural composition and distribution of the copolymer system (formed by HEMA chains and VP blocks) controls the solubilization rate of the material in aqueous media, which modulates the in vitro and in vivo release of cyclosporine. Baseline separation of cyclosporine and VP–HEMA by MEKC was optimized using different SDS concentrations in the running buffer (50 m*M* boric acid–sodium tetraborate at pH 8-methanol, 1:1). Optimum MEKC separation conditions were obtained after adding 50 m*M* SDS to the mentioned running buffer. Under these conditions, the simultaneous separation of both the drug and polymer was achieved in less than 12 min as can be seen in Fig. 4A.

Fig. 4B shows the cyclosporine release from three different DDSs differing in the VP–HEMA composition, namely, 70:30, 50:50 and 40:60 molar percentage of VP–HEMA. The experiment was monitored using the MEKC conditions described above. In vitro release of the drug versus time was carried out using an aqueous saline solution containing 10% Tween-80 and 0.9% NaCl. In the initial phase, the cyclosporine release is associated to the solubilization of the

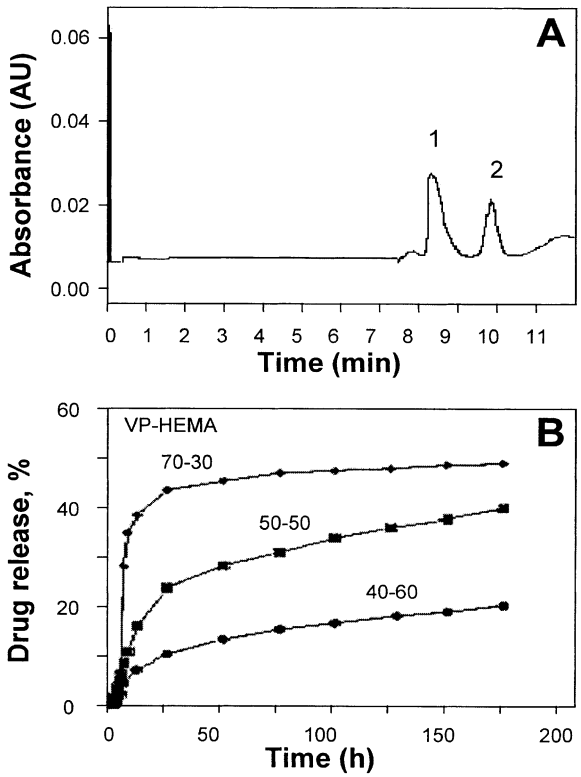


Fig. 4. (A) Electropherogram of VP–HEMA polymer (1) and cyclosporine (2). Running buffer: 25 m*M* SDS, 50 m*M* boric acid–sodium tetraborate at pH 8-methanol (1:1). Uncoated fused-silica capillary: 37 cm (30 cm to the detector) × 100 μm I.D. Capillary temperature: 20 °C. Run voltage: 13 kV. Injection at 0.5 p.s.i. for 1 s. UV detection at 214 nm. (B) Release rate of cyclosporine versus time for DDSs made of different VP–HEMA compositions (70:30, 50:50 and 40:60 as molar percentages of VP–HEMA, respectively). Redrawn from Ref. [22].

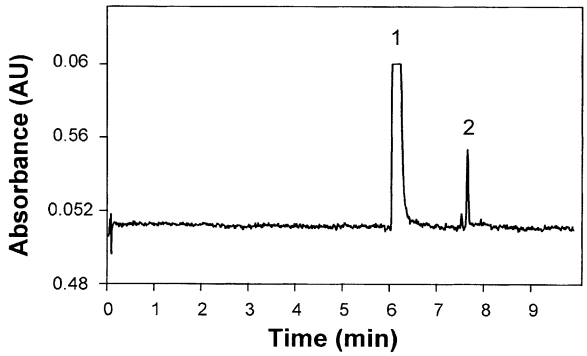


Fig. 5. Electropherogram of VP–HEMA polymer (1) and rHGH (2). Running buffer: 100 m*M* boric acid–sodium tetraborate at pH 9. Uncoated fused-silica capillary: 74 cm (54 cm to the detector) × 50 μm I.D. Capillary temperature: 30 °C. Run voltage: 20 kV. Injection of 5 mg/ml VP–HEMA (40:60) and 0.031 mg/ml of rHGH. Redrawn from Ref. [23].

VP fraction; in the second step, the drug release is controlled by the hydrophilic/hydrophobic balance of the residual HEMA-rich matrix.

In a second work, in vitro and in vivo release of cyclosporine from two VP–HEMA copolymers were compared. To do this, two implants with different hydrophilic/hydrophobic balance (VP–HEMA, 40:60, more hydrophobic, and VP–HEMA, 60:40), were used [55]. In vivo experiments were carried out with male Wistar rats that exhibited inflammation and immune response produced by an unloaded drug VP–HEMA copolymer in a cotton support (as immunogenic model) implanted over the dorsal muscle tissue. A different time response of the immunosuppression reaction of the two VP–HEMA cyclosporine systems was found. The more hydrophilic copolymer VP–HEMA (60:40) showed the immunosuppression reaction in 1–2 weeks, while the VP–HEMA (40:60) reverted the immune reaction more slowly, in 2–4 weeks. A good correlation between these in vivo experiments and the in vitro results obtained using MEKC was found.

In a later work, FSCE was used for in vitro monitoring of the controlled delivery of recombinant human growth hormone (rHGH) from a system also based in VP–HEMA copolymer [23]. A controlled delivery of rHGH might be very helpful in therapeutic treatments for tissue regeneration associated with some pathologies. In order to monitor the rHGH–VP–HEMA behavior, a new FSCE method was developed. The CE method allowed the simultaneous monitoring of both the liberation of rHGH and the relative polymer degradation by solubilization. Besides, it was demonstrated that there was no interaction (e.g., ionic or hydrophobic) between the rHGH and the VP–HEMA polymeric matrix, which could affect the availability of the drug. An rHGH and VP–HEMA baseline separation was obtained in less than 8 min using a running buffer composed by 100 m*M* boric acid–sodium tetraborate at pH 9 as shown in Fig. 5. Under these conditions, a limit of detection of 4 μg/ml for rHGH was obtained assuming a signal-to-noise ratio equal to 2.

Two different films were prepared containing the same quantity of rHGH and two different polymers of VP–HEMA (70:30) and (40:60) (w/w). During in vitro assays, it was observed that the more hydrophilic is the carrier (i.e., the higher the VP percentage), the higher the resorption and the release rate are. Therefore, according to these results, the amount of rHGH released could be controlled by adjusting the copolymer composition.

CE has been shown to be well suited for enantiomeric separations of multiple compounds [56]. In this sense, chiral CE has been used to monitor *R*-(−)- and *S*-(+)-ibuprofen enantiomers released from polymeric DDSs (polymer–drug conjugates, pendant chain type). Ibuprofen (4-isobutyl-2-phenyl-propionic acid) belongs to the arylpropionic acids family, a class of non-steroidal anti-inflammatory drugs widely used in anti-inflammatory therapy. A DDS allowing the controlled release of ibuprofen would be useful especially in high dose-dependent treatments, particularly in chronic diseases as rheumatoid arthritis. A property of ibuprofen is that its enantiomers possess

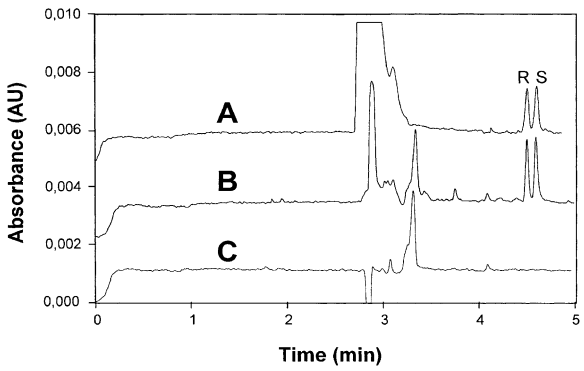


Fig. 6. Release of *R*-(−)- and *S*-(+)-ibuprofen from MAI–HEMA system in (A) 20 m*M* phosphate solution with 2% Tween at pH 7.4 for 8 days, (B) plasma for 17 days. (C) Electropherogram of a blank obtained from rat plasma extracted with acetonitrile. Running buffer: sodium tetraborate at pH 9 with 6% (w/v) Dextrin 10. Uncoated fused-silica capillary: 37 cm (30 cm to the detector)×50 μm I.D. Capillary temperature: 20 °C. Run voltage: 20 kV. Injection at 0.5 p.s.i. for 1.5 s. UV detection at 200 nm. Redrawn from Ref. [57].

different pharmacokinetic and pharmacological effects. Therefore, a possible enantioselective release of ibuprofen from these polymeric DDS was evaluated.

A new CE method was developed in Ref. [57] to monitor *R*- and *S*-ibuprofen. The chiral selector added to the running buffer for *R*- and *S*-ibuprofen enantioseparation was Dextrin 10, a linear maltodextrin. This chiral CE procedure allowed the detection of concentrations as low as 1 μg/ml and enantiomeric percentages of 0.5% of *R*-(−)-ibuprofen in the presence of 99.5% of the optical antipode. In a first study [57], the release of *R*- and *S*-ibuprofen was assessed from two slightly hydrophilic copolymer systems based on HEMA and *N*-{4-[2-(4-isobutylphenyl)propionyl]phenyl}methacrylamide (MAI) or 2-[(4-isobutylphenyl)propionyloxy]ethyl methacrylate (MEI), containing both 70% (w/w) HEMA. In vitro experiments were performed at 37 °C in plasma and in a 20 m*M* sodium phosphate solution containing 2% of Tween at pH 7.4. Fig. 6 displays the CE chiral separation of *R*- and *S*-ibuprofen delivered from MAI–HEMA in phosphate solution (Fig. 6A) and plasma (Fig. 6B). Fig. 6C shows the electropherogram of a blank obtained from rat plasma extracted with acetonitrile. From the CE results it could be concluded that liberation of ibuprofen from these DDSs is higher in plasma than in phosphate buffer. On the other hand, ibuprofen release from MAI–HEMA was higher than from MEI–HEMA. This is due to the higher hydrolytic reactivity of the labile aromatic ester between ibuprofen and MAI structure compared to the aliphatic nature of the MEI ester. In these conditions, MEI–HEMA and MAI–HEMA showed a release rate 15-times and two-times higher in plasma than in phosphate, respectively. These differences might be related to the higher flexibility of the aliphatic spacer in MEI–HEMA than MAI–HEMA where additionally the side chain is linked to the backbone by means of a rigid amide group. Although no enantioselectivity was observed in these copolymeric DDSs, in a later work the enantioselective release of ibuprofen from DDSs containing higher compositions of MAI and MEI was studied [58]. In this work [58], it was compared the ibuprofen release from MEI–HEMA, MAI–HEMA, pMAI (i.e., MAI homopolymer) and pMEI (i.e., MEI homopolymer) using different conditions, namely, 20 m*M* phosphate buffer at pH 7.4; 20 m*M* phosphate buffer at pH 10, and plasma. By using the chiral CE procedure, it was found that the release at pH 10 was higher than at pH 7.4 in both homopolymers and copolymers because of the weakness of the ester bonds in basic media. On the other hand, slight enantiomeric excess in most of the experiments was observed, which was particularly important during the in vitro experiment at pH 10. Namely, there was a tendency towards an *S*/*R* ratio of 1.05–1.10, which could be related to a higher ester reactivity of the *S* form.

Antisense nucleotides, synthetic fragments for ribo- or deoxyribonucleic acids, are novel therapeutic agents with potential interest for clinical applications due to their sequence-specific inhibition of gene expression. However, their rapid degradation in biological fluids, their low capacity for diffusion across cell membranes and their inadequate cellular compartmentalization to reach its target site into the cell, decreases their therapeutic use. In order to overcome these drawbacks, the use of nanoparticles made of proteins as potential DDS has been studied. Capillary zone electrophoresis (CZE) was used to determine the ISIS 2922 (an antisense nucleotide which has shown antiviral activity against cytomegalovirus) content in new pharmaceutical dosage forms based on the use of bovine serum albumin (BSA) nanoparticles [59]. In this work, the influence of the oligonucleotide initial concentration on the final drug content in the DDS using albumin nanoparticles was also evaluated. The ISIS 2922-loaded BSA nanoparticles were prepared by incubation of an aqueous BSA solution with a variable amount of oligonucleotide, and after adjusting pH to 5.5 with 1 *M* HCl, a coacervation process with ethanol and chemical cross-linking with glutaraldehyde took place. After ethanol elimination, ISIS 2922-loaded BSA nanoparticles were purified by centrifugation. For drug loading calculation it was necessary to measure by CZE the ISIS 2922 amount recovered in the supernatants after sample centrifugation. A new CZE method was optimized for ISIS 2922, BSA and paracetamol (as internal standard) separation. For optimization of CZE conditions, the pH and borate buffer concentration were varied. A good separation was obtained using 12.5 m*M* borate buffer at pH 9.5, as can be seen in Fig. 7.

The optimized CZE method mentioned above was used in other work [60] to assess the feasibility of BSA nanoparticles to carry a 21-mer phosphodiester oligonucleotide (PO) adsorbed onto the surface of the pre-formed nanoparticles, or entrapped into the matrix by incubation with the albumin prior the coacervation process. Also in that work [60], the pH and ionic strength of the medium on PO loading process,

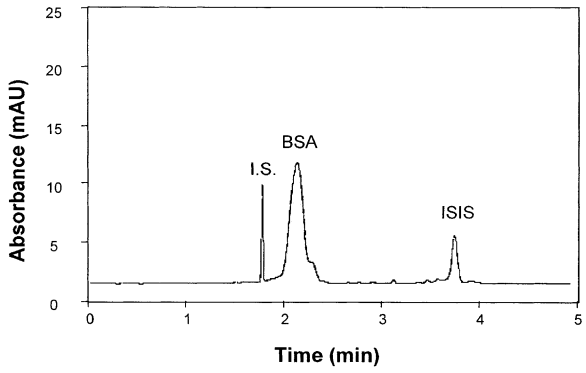


Fig. 7. Electropherogram resulting from the analysis of a supernatant obtained during the purification step of ISIS 2922 loaded-BSA nanoparticles and paracetamol (I.S.). Running buffer: 12.5 m*M* borate at pH 9.5. Uncoated fused-silica capillary: 48.5 cm (40 cm to the detector) × 50 μm I.D. Capillary temperature: 30 °C. Run voltage: 30 kV. Injection at 50 mbar for 10 s. UV detection at 270 nm. Redrawn from Ref. [59].

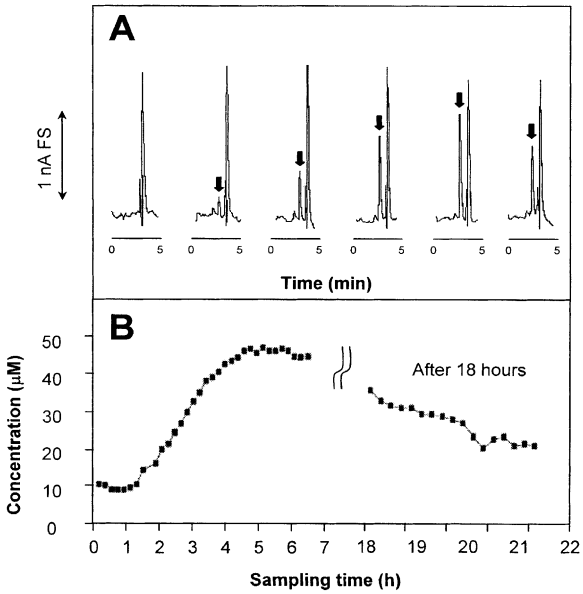


Fig. 8. (A) Electropherograms of the transdermally delivered nicotine in microdialysate. Running buffer: 50 m*M* TES at pH 7.4. Uncoated fused-silica capillary: 55 cm × 75 μm I.D. Run voltage: 15 kV. Transfer flow: 3 μl/min. (B) Time–course of nicotine in microdialysate following patch administration. Redrawn from Ref. [63].

and the protection against enzymatic degradation exerted by both systems were evaluated.

Polyion complex (PIC) micelles, formed by antisense oligonucleotides and PEG–poly(L-lysine) (PLL) block copolymers, have been used as delivery system to improve the antisense oligonucleotides in vivo efficiency [61]. These PIC complexes were prepared by a simple mixing of antisense oligonucleotide with PEG–PLL. CGE was used to quantitatively determine the resistance of the incorporated antisense oligonucleotide against deoxyribonuclease I (DNase I). For this purpose, a PIC micelle solution was incubated with DNase I for different times (5, 60 and 120 min), and CGE measurements were carried out after addition of EDTA to stop digestive reaction. The CGE separation was achieved using a polyacrylamide gel column and using 0.1 *M* Tris–borate with 7 *M* urea at pH 8.3 as running buffer. It was found that antisense oligonucleotide loaded PIC micelles exhibited a remarkable tolerance toward the nuclease attack compared to the free antisense oligonucleotide, what improves in vivo targeting.

Wagner and McGinity [62] studied the permeability of the cationic acrylic copolymer Eudragit RS 30 D [poly-(ethylacrylate – methylmethacrylate – trimethylammonio ethyl methacrylate chloride) copolymer] as function of the chloride ion exchange of the polymer. Eudragit RS 30 D has been already used as a film-coating polymer for sustained release. CZE was used with indirect detection to measure the chloride concentration from the exchanged chloride ions of Eudragit RS 30 D during dissolution testing in different media. It was demonstrated that the degree of polymer swelling and related drug release was a function of the chloride counterion interaction with the quaternary ammonium group from the polymer.

Zhou et al. [63] developed an on-line microdialysis–CE procedure with electrochemistry detection for continuous monitoring of transdermal delivery of nicotine from patches (Nicotrol) implanted in rats. These patches, commercially available, are used for smoking cessation. Cutaneous concentrations of nicotine were monitored over a 24-h period with a resolution of 10 min. The optimum electrokinetic injection, sensitivity and resolution of the nicotine and its metabolites by CE were achieved using a 50 m*M* *N*-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) buffer at pH 7.4. Fig. 8A displays some of the electropherograms used for the monitoring of nicotine delivery across the skin for 18 h. The calculated nicotine concentration versus sampling time is shown in Fig. 8B. As can be seen in that Figure, using the CE method it was possible to determine that the maximum nicotine concentration was reached at about 5 h and after 18 h the nicotine concentration decreases.

4. Monitoring DDSs: CE vs. HPLC

Up to now, monitoring of drugs release from DDSs has been mainly carried out by HPLC (see, e.g., Refs. [64–67]). However, as it has been shown above, CE seems to be a suitable analytical technique for monitoring drugs from a variety of delivery systems. The main advantages of CE with regard to HPLC are the high speed of analysis (generally under 10–20 min per run, which, given the large number of samples that have to be analyzed during any in vitro or in vivo experiment, deserves to be taken into account), high separation efficiency (usually in the interval from 10^5 to 10^6 theoretical plates per meter of column, depending on the type of analyte and the separation conditions) and the

small volumes of samples required (only a few nanoliters are injected, which becomes a very interesting property for in vivo experiments). Moreover, it is important to mention that, contrary to HPLC, CE allows the simultaneous monitoring of both the polymer used in the DDS and the released drug in a single run. This interesting property can make possible for instance the study of the control of the resorption rate of the polymeric delivery systems (and the influence of the composition percentages of the different monomeric unities) on the drug release [22,23]. However, HPLC provides better sensitivity than CE. Moreover, since HPLC and CE are complementary techniques based on different separation mechanisms, they can provide different and interesting information on the DDS under study.

5. Conclusions and future outlooks

Monitoring a drug delivery system requires frequently the development of a separation method able to follow both the drug release and the polymer degradation (or solubilization) during in vitro or in vivo assays. In this way, a more complete picture about the release (and/or degradation of the polymeric device) can be obtained. Ideally, this information can be obtained in a single analysis by using ad hoc methods that can provide information about such a different compounds (i.e., drug and polymer, whose molecular masses may in many cases differ in thousands of u and, in general, show very different physicochemical properties). This requirement has increased the need for more reliable analytical methodologies able to characterize these materials.

CE has emerged as a powerful analytical tool that can provide useful information about the chemical properties of these complex devices. Thus, it has been demonstrated that fast, reproducible and efficient separations can be obtained for both polymers and drugs in a single analysis by using CE methods. One of the main characteristics of CE is that this technique makes possible the development of uniquely tailored separation procedures to monitor DDSs of very different nature. Interestingly, such information can be in some cases complementary to that provided by other classical techniques as HPLC.

Although CE is becoming well established for monitoring DDSs as a viable alternative to HPLC and slab-gel electrophoresis (in the case of protein analysis), CE nowadays lacks the sensitivity of HPLC and the throughput capability of traditional slab-gel electrophoresis. In order to improve both sensitivity and mainly selectivity, CE can be interfaced with other techniques such as electrospray mass spectrometry (MS) to bring about a very powerful hyphenated technique [68,69]. On-line coupling of CE with electrospray-MS may solve the identification problems associated with unknown compounds arriving from the DDSs (e.g., degradation products produced during in vitro or in vivo assays). Moreover, the application of CE–MS to monitor DDS is an important and unexplored working field. Some other new and interesting developments that are nowadays being worked out in the CE domain will probably be applied for monitoring DDSs in the non-distant future. These developments include multi-capillary arrays [70,71], CE interfaced with biosensors [72,73] and chip-based separations [74,75]. The development of these systems will be an important help to overcome throughput limitations and sensitivity problems of CE.

The main effort of the current and future DDSs will be focused on the delivery of non traditional drugs or active compounds as genes, peptides, proteins as enzymes, hormones, vaccines, etc. The gene therapy field is especially remarkable, and great efforts are being directed to the development of efficient non-viral vectors, mainly based on cationic polymer systems. Improvements in chronopharmacokinetics, in the preparation of nano-DDSs, in the optimization of mucoadhesive devices, in the use of clean technologies based on supercritical fluids, and in the development of self-regulator DDSs for the delivery of insulin and other therapeutics, will probably be also very relevant issues within the DDS field.

Acknowledgements

Financial support from the grant Mat2001-1618 is gratefully acknowledged. C.S. thanks the Consejería de Educación y Cultura (Comunidad de Madrid) for a fellowship.

References

[1] E. Mathiowitz, Encyclopaedia of Controlled Drug delivery, Wiley–Interscience, New York, 1999.
[2] A. Kydonieus, Treatise on Controlled Drug Delivery, Marcel Dekker, New York, 1992.
[3] T. Okano, Biorelated Polymer and Gels, Academic Press, San Diego, CA, 1998.
[4] R. Baker, Controlled Release of Biologically Active Agents, Academic Press, New York, 1987.
[5] M. Brownlee, A. Cerami, Science 206 (1979) 1190.
[6] G. Albin, A. Horbett, S.R. Miller, N.L. Ricker, J. Control. Release 6 (1987) 267.
[7] D. Shiino, Y. Murata, K. Kataoka, Y. Koyama, M. Yokohama, T. Okano, Y. Sakurai, Biomaterials 15 (1994) 121.
[8] A.S. Hoffman, in: T. Okano (Ed.), Biorelated Polymer and Gels, Academic Press, San Diego, CA, 1998, p. 231.
[9] H. Ringsdorf, J. Polym. Sci., Polym. Symp. 51 (1975) 35.
[10] L.W. Seymour, in: S. Dimitriu (Ed.), Polymeric Biomaterials, Marcel Dekker, New York, 2002, p. 843.
[11] P.S. Steyger, D.F. Baban, M. Brereton, K. Ulbrich, L.W. Seymour, J. Control. Release 39 (1996) 35.
[12] R. Duncan, Anti-Cancer Drugs 3 (1992) 175.
[13] A. Gallardo, C. Parejo, J. San Román, J. Control. Release 71 (2001) 127.
[14] M. Akashi, K. Takemoto, Adv. Polym. Sci. 97 (1990) 107.
[15] R. Duncan, J. Kopecek, Adv. Polym. Sci. 57 (1984) 53.
[16] H. Maeda, L.W. Seymour, Y. Miyamoto, Bioconj. Chem. 3 (1992) 351.
[17] A. Abuchowski, J.R. McCoy, N.C. Palczuk, T.V. Es, F.F. Davis, J. Biol. Chem. 252 (1977) 3582.

[18] D.O. Kuethe, D. Augenstein, J.D. Gresser, D.L. Wise, J Control. Release 18 (1992) 159.
[19] J. Heller, in: B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons (Eds.), Biomaterials Science, Academic Press, San Diego, CA, 1996, p. 347.
[20] J. Kohn, R. Langer, in: B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons (Eds.), Biomaterials Science, Academic Press, San Diego, CA, 1996, p. 65.
[21] P.U. Conte, P. Giunchedi, L. Maggi, M.E. Sangalli, A. Gazzaniga, P. Colombo, A. La Manna, Eur. J. Pharm. 38 (1992) 209.
[22] A. Gallardo, F. Fernández, P. Bermejo, M. Rebuelta, A. Cifuentes, J.C. Díez-Masa, J. San Román, Biomaterials 21 (2000) 915.
[23] A. Cifuentes, J.C. Díez-Masa, C. Montenegro, M. Rebuelta, A. Gallardo, C. Elvira, J. San Román, J. Biomater. Sci. Polym. Ed. 11 (2000) 993.
[24] B. Narasimhan, N.A. Peppas, J. Pharm. Sci. 86 (1997) 297.
[25] B. Lemmer, Biomaterials 21 (1991) 915.
[26] Y.H. Bae, I.C. Kwon, in: T. Okano (Ed.), Biorelated Polymer and Gels, Academic Press, San Diego, CA, 1998, p. 93.
[27] A. Kikuchi, T. Okano, in: T. Okano (Ed.), Biorelated Polymer and Gels, Academic Press, San Diego, CA, 1998, p. 1.
[28] J. Fix, in: E. Mathiowitz (Ed.), Encyclopaedia of Controlled Drug Delivery, Wiley–Interscience, New York, 1999, p. 698.
[29] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[30] W.G. Kuhr, Anal. Chem. 62 (1990) R403.
[31] A.S. Cohen, A. Paulus, B.L. Karger, Chromatographia 24 (1987) 15.
[32] A. Tiselius, Trans. Faraday Soc. 33 (1937) 524.
[33] A.G. Ewing, R.A. Wallingford, T.M. Olefirowicz, Anal. Chem. 61 (1989) 292A.
[34] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266.
[35] A.S. Green, J.W. Jorgenson, J. High Resolut. Chromatogr. 7 (1984) 529.
[36] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[37] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, A. Teiichi, Anal. Chem. 56 (1984) 111.
[38] A.S. Cohen, B.L. Karger, J. Chromatogr. 397 (1987) 409.
[39] A. Guttman, A.S. Cohen, D.N. Heiger, B.L. Karger, Anal. Chem. 62 (1990) 137.
[40] A.E. Barron, D.S. Soane, H.W. Blanch, J. Chromatogr. 652 (1993) 3.
[41] J. Pospichal, P. Gebauer, P. Bocek, Chem. Rev. 89 (1989) 419.
[42] S. Hjertén, M. Zhu, J. Chromatogr. 346 (1985) 265.
[43] X. Liu, Z. Sosic, I.S. Krull, J. Chromatogr. A 735 (1996) 165.
[44] J.R. Mazzeo, I.S. Krull, Biochromatography 10 (1991) 638.
[45] T.J. Pritchett, Electrophoresis 17 (1996) 1195.
[46] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[47] E. Venema, J.C. Kraak, T. Tijssen, H. Poppe, Chromatographia 48 (1998) 347.
[48] E. Venema, J.C. Kraak, T. Tijssen, H. Poppe, J. Chromatogr. A 837 (1999) 3.
[49] W.Th. Kok, R. Stol, R. Tijssen, Anal. Chem. 72 (2000) 468A.
[50] E.C. Peters, M. Petro, F. Svec, J.M.J. Frechet, Anal. Chem. 70 (1998) 2296.
[51] A.L. Vergnon, Y.H. Chu, Methods 19 (1999) 270.
[52] W.L. Tseng, H.T. Chang, S.M. Hsu, R.J. Chen, S. Lin, Electrophoresis 23 (2002) 836.
[53] F. Progent, M. Taverna, I.L. Potier, F. Gopée, D. Ferrier, Electrophoresis 23 (2002) 938.
[54] S. Kimakhe, S. Bohic, C. Larrose, A. Reynaud, P. Pilet, B. Giumelli, D. Heymann, G. Daculsi, Biomed. Mater. Res. 47 (1999) 18.
[55] A. Gallardo, F. Fernández, A. Cifuentes, J. C Díez-Masa, P. Bermejo, M. Rebuelta, A. López-Bravo, J. San Román, J. Control. Release 72 (2001) 1.
[56] B. Chankvetadze (Ed.), Capillary Electrophoresis in Chiral Analysis, Wiley, Chichester, 1997.
[57] C. Simó, A. Gallardo, J. San Román, C. Barbas, A. Cifuentes, J. Chromatogr. B 767 (2002) 35.
[58] C. Simó, A. Gallardo, C. Parejo, J. San Román, C. Barbas, A. Cifuentes, Eur. J. Pharm. Sci. 16 (2002) 75.
[59] A. Arnedo, M.A. Campanero, S. Espuelas, M.J. Renedo, J.M. Irache, J. Chromatogr. A 871 (2000) 311.
[60] A. Arnedo, S. Espuelas, J.M. Irache, Int. J. Pharm. 244 (2002) 59.
[61] A. Harada, H. Tagawa, K. Kataoka, Eur. J. Pharm. Sci. 13 (2001) 35.
[62] K.G. Wagner, J.W. McGinity, J. Control. Release 82 (2002) 385.
[63] J. Zhou, D.M. Heckert, H. Zuo, C.E. Lunte, S.M. Lunte, Anal. Chim. Acta 379 (1999) 307.
[64] S.J. Corvari, R.G. Hollenbeck, J. Leslie, K.I. Plaisance, D. Young, Pharm. Res. 8 (1991) 40.
[65] A. Rogstad, B.J. Weng, J. Pharm. Sci. 82 (1993) 518.
[66] P.D. Graham, K.J. Brodbeck, A.J. McHugh, J. Control. Release 58 (1999) 233.
[67] G. Fontana, M. Licciardi, S. Mansueto, D. Schillaci, G. Giammona, Biomaterials 22 (2001) 2857.
[68] C. Simó, P. López Soto-Yárritu, A. Cifuentes, Electrophoresis 23 (2002) 2288.
[69] C. Simó, A. Cifuentes, Electrophoresis 24 (2003) 834.
[70] R. Trotha, U. Reichl, F.L. Thies, D. Sperling, W. Konig, B. Konig, Electrophoresis 23 (2002) 1070.
[71] S. Behr, M. Matzig, A. Levin, H. Eickhoff, C. Heller, Electrophoresis 20 (1999) 1492.
[72] L. Castelletti, S.A. Piletsky, A.P. Turner, P.G. Righetti, A. Bossi, Electrophoresis 23 (2002) 209.
[73] A. Bossi, S.A. Piletsky, P.G. Righetti, A.P. Turner, J. Chromatogr. A 892 (2000) 143.
[74] D.R. Reyes, D. Iossifidis, P.A. Auroux, A. Manz, Anal. Chem. 74 (2002) 2623.
[75] P.A. Auroux, D. Iossifidis, D.R. Reyes, A. Manz, Anal. Chem. 74 (2002) 2637.