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Proprietary Rel-Ease™ drug delivery technology: opportunity for sustained delivery of peptides, proteins and small molecules

Ming Ye†, Gajanan Bhat, Kevin A Johnston, Heow Tan & Marc Garnick
†*Praecis Pharmaceuticals Inc., 830 Winter Street, Waltham, MA 02451, USA*

Proprietary Rel-Ease™ (Praecis Pharmaceuticals) drug delivery technology uses biocompatible polymers as carriers to incorporate a drug into a polymer matrix through opposite charge interaction or complexation. The resulting low solubility complexes can be used to prepare sustained release depot injections or potentially sustained release formulations for oral administration. As a regulatory approved and commercialised drug delivery technology, Rel-Ease is used in abarelix for injectable suspension, a monthly depot injection for the treatment of patients with advanced prostate cancer. The technology offers high drug loading and minimal-to-no initial burst effect *in vivo*. It uses aqueous processes and is compatible for complexation with many peptide and protein therapeutics; its mechanism can also be applied to many small-molecule therapeutics and offers conventional and alternative methods for sustained release delivery via an oral route.

Keywords: biocompatible polymer, depot formulation, high drug loading, low-solubility complex, oral sustained delivery, parenteral sustained delivery, peptides, proteins, small molecules, water-soluble drugs

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1. Introduction

Drug delivery has drawn a significant level of interest from pharmaceutical companies due to the increased use and important role it plays in developing pharmaceutical products. Optimising drug delivery can result in pharmaceuticals possessing more desirable characteristics, such as safer pharmacokinetic profiles and enhanced patient compliance. In addition to other routes of delivery, many drug delivery technologies are emerging for both parenteral and oral drugs [1-3]. Among those technologies, customised design and speciality techniques or equipment are often required [4-6]. Some face scale-up, transfer, quality control and/or commercialisation challenges and require extensive development effort and analytical support [5]. In the increasingly competitive world of the pharmaceutical development industry, simple and effective drug delivery systems are compelling and attractive for use in both new chemical entities and generic pharmaceutical products that are under development, especially in those drug delivery systems with performance and safety that has already been demonstrated and proven by products on the market.

This paper introduces the commercially available drug delivery technology Rel-Ease™ (Praecis Pharmaceuticals). The technology uses a fundamental principle of sequestering a drug in a polymer carrier (polymer) through charge interaction or complexation between the drug and a polymer having opposite charges. Rel-Ease was initially developed for abarelix for injectable suspension [7,101-103] to provide sustained delivery as an injectable depot when administered every 4 weeks intramuscularly or subcutaneously. Abarelix for injectable suspension, formulated with Rel-Ease technology, was approved by the FDA in 2003 and by the European Federal Institute for Drugs and

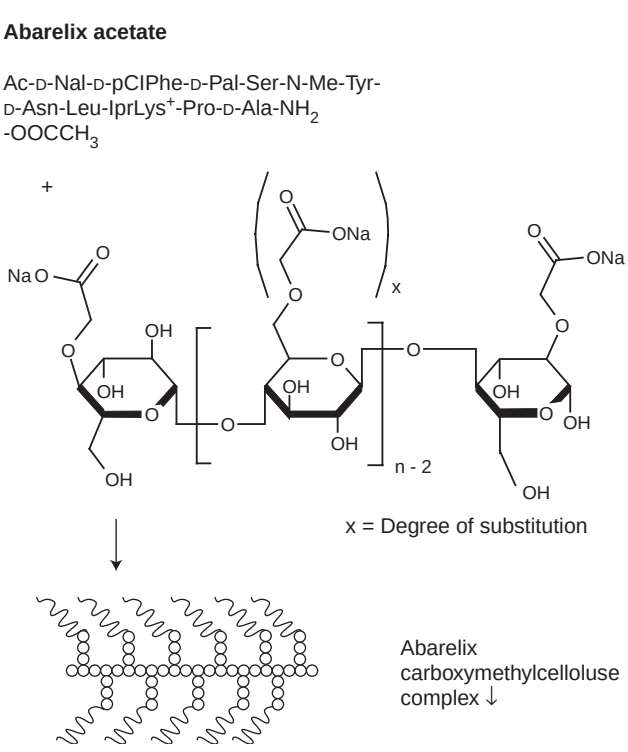

Figure 1. Abarelix carboxymethylcelloluse complex formation.

Medical Devices, Bundesinstitut für Arzneimittel und Medizinprodukte, in Germany in 2005. With the product approvals, Rel-Ease drug delivery technology became commercially available. The principles underlying this drug delivery system can now be applied to provide benefits to other therapeutics that require the use of a sustained delivery system, including many peptide and protein therapeutics.

Rel-Ease is unique in many ways. It uses biocompatible polymers, such as sodium carboxymethylcelloluse (CMC), as complexation agents; its manufacturing process is performed using only aqueous conditions, thus avoiding the use of any organic solvents; and because of its fundamental principle Rel-Ease is compatible with many different molecular types of peptide-, protein- and small-molecule therapeutics. In comparison to the commonly used polymer encapsulation drug delivery technologies for depot injection uses, such as poly(lactic acid), poly(glycolic acid) or poly(lactic-co-glycolic acid) (PLGA) depot, Rel-Ease depot provides minimal-to-no initial burst effect after administration. In addition, the drug loading of Rel-Ease (e.g., the weight percentage of drug in the complex), is considerably higher and can approximate 80% in abarelix for injectable suspension. This high drug loading capability and the aqueous manufacturing advantages may provide a very different path of sustained delivery of water-soluble peptides, proteins or small molecules, and fulfil certain unmet needs in the pharmaceutical industry worldwide for both new chemical entities and generic pharmaceuticals.

2. Rel-Ease™ drug delivery technology

Rel-Ease drug delivery technology is developed based on a simple technique of charge interactions. The opposite charge interaction between proteins and polyelectrolyes has been well understood [8-10] and has been taken into consideration in the designing of drug delivery systems for proteins with oppositely charged polymer carriers. The most recent literature reported several examples of such research. Basic proteins, such as ribonuclease, lysozyme and RNAse, were studied for complexation with polysulfonates [11] and poly(dimethyl diallyl ammonium chloride) [8,12]. In addition, acidic protein (i.e., bovine serum albumin) was studied for complexation with chitosan [13]. Chitosan was also reported to complex with small molecules and demonstrated controlled release characteristics [14].

As a commercially available drug delivery technology, Rel-Ease uses biocompatible polymers, such as CMC, to form low-solubility complexes, and provides sustained release of proteins, peptides and small molecules.

2.1 Principle of Rel-Ease™

Rel-Ease uses biocompatible polymers that include anionic or cationic cellulose-based carboxylates, phosphates, sulfates or amine derivatives. The most commonly used polymer is CMC, a 'generally recognised as safe' material that is recognised by the FDA regulatory agency and used in a number of approved parenteral products as a suspending agent, as well as in a variety of oral products on the market. Other polymers include sodium dextran sulfates (DS), chitosan, carbomers, xanthan and sodium hyaluronate. When the polymer is anionic, such as CMC, it interacts with a drug carrying a positive charge, such as abarelix. When the polymer is cationic, such as chitosan, it interacts with a drug carrying a negative charge, such as sodium ceftriaxone. The interaction neutralises the opposite charges on the polymer and the drug, resulting in the formation of a predominantly hydrophobic complex (i.e., a significant reduction of the hydrophilic character of each component). When the complexation occurs in an aqueous medium, the hydrophobic complex is then readily precipitated from the aqueous media. This unique complex-formation mechanism that is used with Rel-Ease allows the polymer to sequester the oppositely charged drug in the polymer matrix. Figure 1 illustrates the complex formation through the charge sequestering between abarelix (a decapeptide with a positive charge at the side chain of iso-propyl-lysine residue) and CMC, the negatively charged polymer.

The drug to polymer ratio can be stoichiometrically controlled based on the number of charges on the drug and the polymer so that the drug loading is uniform and consistent. Additionally, the mechanism of drug sequestration in the polymer matrix is solely by the ionic interaction between the

Table 1. Peptide and protein solubility before and after complexation.

Peptide or protein (company)	Free peptide or protein In water (mg/ml)	Solubility of carboxymethylcelloluse complex		
		In water (mg/ml)	In saline (mg/ml)	In ethanol (mg/ml)
Abarelix (Praecis Pharmaceuticals)	≥ 28.0	0.050	0.168	0.227
Octreotide (Novartis)	> 10.0	Not tested	Not tested	Not tested
Insulin (Eli Lilly)	< 0.1	0.016	0.052	0.012
GRF 1-29 amide	> 10.0	0.024	0.930	0.066
Bovine serum albumin	> 200.0	0.025	0.258	-

GRF: Growth hormone-releasing factor.

Table 2. Small-molecule solubility before and after complexation.

Small molecules (company)	% Drug loading	Free drug in water (mg/ml)	Solubility of carboxymethylcelloluse complex	
			In water (mg/ml)	In saline (mg/ml)
Fluoxetine* (Eli Lilly)	51.3	~ 15	0.5	3.9
Benzatropine* (Merck)	48.6	> 1000	1.1	2.4
Streptomycin* (Pfizer)	70.2	> 50	4.2	10.3
Doxepin* (Pfizer)	46.1	> 33	0.7	1.6
Diltiazem* (Douglas)	55.9	> 100	1.8	3.8
Tacrine (Parke-Davis)	42.8	> 100	0.0005	0.002

*Generic brands of these drugs are also on the market.

drug and the polymer; therefore, the amount of free drug is minimal in the complex (i.e., minimal free drug on the precipitation of the complex), and the free drug or the uncomplexed drug remains soluble in the aqueous media without being carried onto the polymer matrix. This lack of extraneous free drug is important in minimising the burst effect *in vivo* (see additional discussion in Sections 3 and 4).

2.2 Solubility reduction by complexation leads to the design of sustained delivery formulations

In general, therapeutic peptides and proteins are mostly water soluble and are highly charged biomolecules. Basic lysine and/or arginine residues in the peptides and proteins provide an ideal positive charge that can be used as the complexation site and are chemically compatible with CMC polymer (Figure 1). After solubilising in aqueous solutions, these peptides and proteins readily form CMC complexes upon mixing with an appropriate CMC aqueous solution. In addition to abarelix, other examples of such prepared complexes include a luteinising hormone-releasing hormone agonist and gonadotropin-releasing hormone antagonist (leuprolide and cetrorelix, respectively), octreotide, insulin, growth hormone-releasing factor (GRF 1-29 amide), bovine serum albumin and monoclonal antibodies. These complexes can be isolated by conventional centrifugation or filtration followed by water removal through vacuum drying. The isolated complexes, typically processed as powders, have different solubility properties in aqueous and organic solvents, such as water, saline and ethanol. Table 1 demonstrates the solubility information for abarelix, octreotide, insulin, GRF 1-29 amide and bovine serum albumin in water, saline and ethanol solvents before and after complexation with CMC. As shown in Table 1, the CMC complexes of these peptides and proteins exhibit significantly lower solubility when compared with the uncomplexed free forms. The solubility reduction by complexation is one of the key chemical parameters that lead to the design of depot sustained-release formulations for parenteral use for peptides and proteins.

Many of the small-molecule therapeutics are also water soluble and carry either positive or negative charges; therefore, Rel-Ease is also chemically compatible with those therapeutics. Table 2 provides a number of examples of small-molecule therapeutics that complexed with CMC. As shown in Table 2, the resulting small-molecule CMC complexes also provide significant reduction of solubility in water and saline in comparison to the parent molecules. As the majority of small-molecule therapeutics can also be administered via the oral route in addition to parenteral

routes, low-solubility Rel-Ease complexes of small molecules can also be formulated into oral dosage forms, such as capsules and tablets, providing the extended delivery of small-molecule therapeutics

In addition to the CMC polymer, other Rel-Ease polymers, such as negatively charged DS and positively charged chitosan have also demonstrated effective reduction of solubility of the oppositely charged drugs. Dextromethorphan and naltrexone complexed with DS and reduced the solubility of the parent drugs by 95%. Ceftriaxone sodium and sparfloxacin also complexed with chitosan to form low-solubility complexes.

Rel-Ease low-solubility complexes can form under the mild room temperature conditions. Complex-formation pH is primarily dependent on the chemical nature of the drug, which can vary from acidic to near neutral pH (e.g., pH range of 1.6 – 6.6). In addition, ionic strength can have an effect on the complex formation by providing competing ions, such as acetate and phosphate in the case of CMC complex formation, which can result in a low yield of the complex. Generally, polymer molecular weight has little effect on the complex formation, such as in the case of different molecular weights of CMC and DS polymers. However, the drug-to-polymer ratio determines the drug loading of the complex, which determines the drug release rate both *in vitro* and *in vivo* (see discussion in Section 5). Complexes formed from different polymers can also have different release rates. The dextromethorphan CMC complex has higher solubility and *in vitro* release rates than that of dextromethorphan DS complex.

3. Opportunity for peptide and protein sustained delivery via parenteral routes

3.1 Parenteral administration remains the primary route for peptides and proteins

Peptides and proteins are primarily administered parenterally, as bioavailability is significantly higher and less variable than the other routes of administration, such as oral, pulmonary, nasal, transdermal and buccal routes. Although breakthrough advances have improved peptide and protein delivery through pulmonary and other routes, substantial challenges still exist in order to deliver these therapeutics safely and effectively. As an example, the bioavailability of insulin via subcutaneous injection achieved 80% absorption, whereas significantly lower bioavailability was observed for oral, buccal, nasal and pulmonary routes at 0.05, 0.5, 30 and 25%, respectively. Another example, leuprolide, showed a decrease in bioavailability from 65, 8, 3 to 0.05% when administered via subcutaneous, rectal, nasal and oral routes, respectively [3]. Such significant differences in bioavailability between parenteral and other routes of administration are also reported in a recent publication [15]. In addition, it has been reported that the bioavailability of insulin via the pulmonary route can vary from 7 to 25% in non-smokers, with values up to threefold higher in smokers [15].

3.2 Rel-Ease™ is a unique drug delivery technology for parenteral sustained delivery of peptides and proteins

As few commercially available sustained release parenteral delivery systems are available on the market, Rel-Ease may present a new opportunity for biopharmaceutical companies specialising in peptide and protein therapeutic development. As previously mentioned, Rel-Ease was developed to deliver a decapeptide, abarelix, for the treatment of patients with advanced prostate cancer. The technology uses conventional manufacturing processes for abarelix-CMC complex formation. Abarelix and CMC are dissolved in appropriate aqueous solutions where the bioburdens can be controlled through the sterile filtration of each solution. On mixing the two filtered solutions, rapid charge neutralisation takes place and the abarelix-CMC complex readily precipitates from the aqueous media (Figure 1). The complex precipitate is isolated through centrifugation followed by vacuum drying. The final product, a particle size-controlled bulk powder formulation, can be produced using conventional manufacturing methods for parenteral solids. When compared with PLGA manufacturing processes, Rel-Ease provides several advantages, including the use of conventional pharmaceutical equipment and processes, few manufacturing steps and an all-aqueous system, which avoids the use of organic solvents. For proteins where their biological activities are highly dependent on their conformational structures, aqueous processes are obviously advantageous and avoid the conformational instability issues that is typically encountered in PLGA processes, such as organic solvent-induced denaturation. Because of simpler, conventional and aqueous processes, Rel-Ease is capable of producing large scales of product in a relatively short period of time.

As a polymer drug delivery system for peptide and protein parenteral use, Rel-Ease has the distinguishable advantage of providing high drug loadings. In the case of abarelix, drug loading can be achieved at ~ 80% (i.e., 80% of the weight is abarelix and 20% of the weight is CMC and water in the abarelix-CMC complex). In the case of PLGA, this ratio can be the reverse (i.e., the drug loading can be 20%, with 80% being the PLGA polymer). The typical drug loading for PLGA is ~ 10%, but not > 30%. High drug loading is very important when developing high-dose intramuscular or subcutaneous sustained-release parenteral products because a formulation with high drug loading can substantially minimise the volume of material injected, which is a major formulation constraint with high dose products. Table 3 provides examples of several peptides and proteins and demonstrates that high drug loading can be achieved for peptides and proteins with different molecular weights. Table 3 also provides additional information on the CMC complexes of selected peptides and proteins, such as CMC and water contents, peptide or protein purity before and after complexation and product particle sizes. Another advantage of Rel-Ease products is that, physically, the particles of CMC complexes are more stable than PLGA particles in terms of exposure to temperature and other physical conditions [16].

Table 3. Drug loading and additional information for CMC complexes of selected peptides and proteins.

CMC complex	Abarelix (MW 1416 Da)	Octreotide (MW 1019 Da)	GRF 1-29 amide (MW 3358 Da)	Insulin (MW 5807 Da)	BSA (MW 66.4 kDa)
Appearance	White to off-white powder	White to off-white powder	White to off-white powder	White to off-white powder	White to off-white powder
Drug loading	82.7%	70.6%	80.1	92.0%	90.9%
Peptide yield	≥ 80%	≥ 80%	≥ 80%	≥ 80%	≥ 80%
Peptide content	≥ 75%	≥ 68%	≥ 75%	≥ 80%	≥ 80%
CMC content	16.4%	28.3%	19.4%	7.5%	-
Water content	2.6%	3.9%	4.8%	1.5%	< 3
Incoming API purity	≥ 99%	≥ 99%	≥ 99%	≥ 99%	≥ 98%
Purity after complexation	98.8%	98.6%	≥ 95%	≥ 98%	-
Particle size (µm)					
D (v, 0.1)*	7	9	-	9¶	-
D (4, 3)‡	48	52	-	115¶	-
D (v, 0.9)§	102	120	-	370¶	-

*10% of the volume is composed of particules not smaller than the reported values. ‡Average diameter. §90% of the volume is composed of particules not greater than the reported values. ¶Particle sizes were measured before reduction.
CMC: Carboxymethylcelloluse; BSA: Bovine serum albumin; D: Diameter; GRF: Growth hormone-releasing factor; MW: Molecular weight.

This is because CMC complexes are formed through chemical interactions rather than physical encapsulation.

Rel-Ease products can be terminally sterilised by γ-irradiation as Rel-Ease polymers can be exposed to γ-irradiation conditions, as is the case for abarelix for injectable suspension, whereas PLGA formulations are processed solely using aseptic conditions. If the drug is less stable, such as GRF 1-29 amide, γ-irradiation may not be an option, even if formulated with Rel-Ease. If terminal sterilisation is not suitable for a peptide or protein, such as being labile under the terminal sterilisation conditions, aseptic processes can be used with Rel-Ease. In addition, it was observed that γ-irradiation did not change the stability and release characteristics of abarelix for injectable suspension.

3.3 Mechanism of Rel-Ease™ sustained delivery

By sequestering the drug in the CMC complexes, Rel-Ease can effectively preserve the water-soluble active drug at the injection site and release this drug gradually through the equilibrium solubility of the complex in the physiological fluid environment. The *in vivo* sustained release mechanism is due to the low solubility of the CMC complex. On exposure to physiological conditions, the low-solubility CMC complex forms a depot at the injection site, similar to the depot of PLGA injectables. However, in contrast to the PLGA depots, the CMC complex depot releases the drug through an ionic exchange between the drug and the cations present in the physiological fluid, such as sodium. After the cations replace the drug, it is released as the soluble form and is absorbed into the systemic circulation. This absorption drives the equilibrium solubility of the complex to release more drug and the process continues until the drug is completely released. During the process, the CMC polymer also becomes soluble and is subsequently excreted from the body. As for PLGA drug delivery, the PLGA polymer biodegrades to acidic monomers at the site of administration, whilst releasing the active drug and causing local pH excursion. For pH-sensitive drugs, such excursions may cause chemical or physical changes to the drugs, such as conformational changes or chemical degradation. The pH drop may also contribute to the local irritation at the injection sites experienced by patients.

As mentioned previously and also discussed in detail in Section 4, for parenteral use, the Rel-Ease CMC complex limits the presence of free drug, which is critical in minimising the initial burst effect and providing better overall safety profiles. Table 4 provides a summary comparison of Rel-Ease with PLGA-encapsulation technology.

4. Clinical profile and safety for parenteral use

Pharmacokinetic profiles of Rel-Ease formulation were studied in several randomised single-dose, as well as multi-dose, clinical studies for both intramuscular and subcutaneous administration. An open-label pharmacokinetic study (Study 1) was conducted to determine the relative bioavailability of abarelix for injectable suspension (with Rel-Ease) after a single intramuscular injection, compared with a single intramuscular injection of abarelix injectable solution (naked abarelix without Rel-Ease), as shown in Figure 2 [17]. Healthy volunteer subjects received a single intramuscular injection of

Table 4. Comparison of Rel-Ease™ with poly(lactic-co-glycolic acid) microsphere encapsulation.

Parameter	Rel-Ease™	Poly(lactic-co-glycolic acid)
Burst	No burst effect	> 5%, could be up to 20% and vary from batch to batch
Drug loading	High: 70% for peptide; 50% for small molecule	Low: generally no greater than 30% and typically about 10%
Process and yield	Aqueous; can approach 90%	Organic (CH_2Cl_2) or supercritical fluid; less than 50%
Application	Injectable and oral	Injectable
Patent	Unique for Praecis Pharmaceuticals	Challenging (i.e., many competitors)

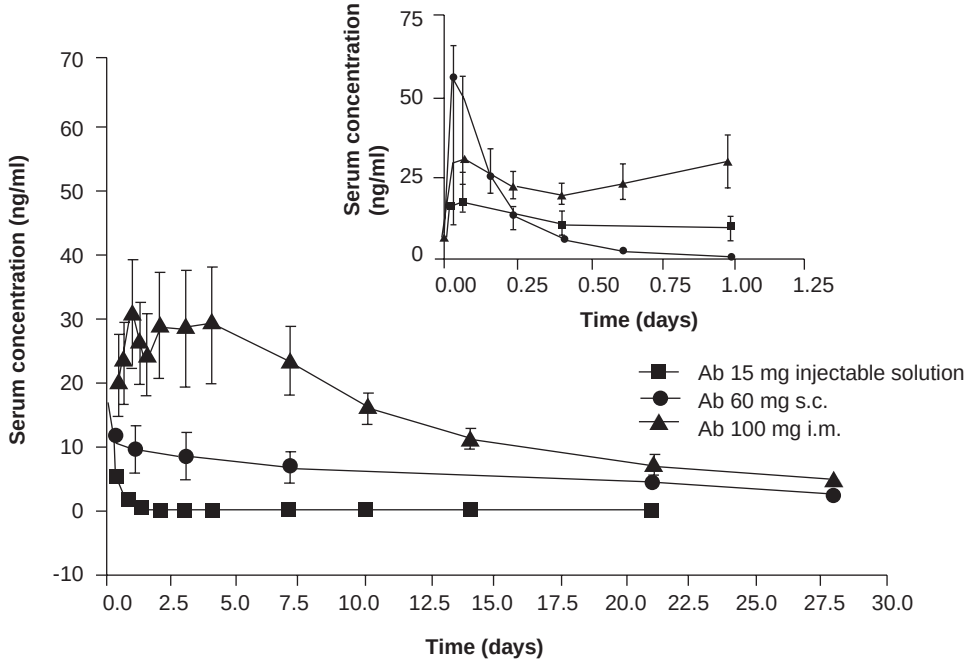

Figure 2. Study 1: mean (standard deviation) serum concentration profiles of abarelix-injectable solution, and abarelix for injectable suspension formulations (with Rel-Ease™). Insert is an enlarged section of serum concentration time profiles up to day 1 post-injection.

abarelix 15 µg/kg for injectable suspension, followed by a 21-day washout period and a subsequent intramuscular dose of abarelix 100 mg for injectable suspension on day 22. There was no abarelix detected before dosing with abarelix for the injectable suspension, indicating that the washout period was sufficiently long. Another open-label study (Study 2) was conducted to determine the pharmacokinetic profile of a single 60-mg subcutaneous injection of abarelix for injectable suspension. Mean serum abarelix concentrations are plotted in Figure 2, with an insert showing the mean serum concentration for day 1 post-dose for abarelix solution intramuscular and subcutaneous injections. Mean (standard deviation) serum concentration time profiles are plotted for the first dose of injection in each treatment. Table 5 summarises the mean results of pharmacokinetic parameters of the above studies.

For a depot sustained-release formulation (abarelix for injectable suspension), the elimination $t_{½}$ and the initial drug release (burst) are considered to be important factors. The mean observed elimination $t_{½}$ was significantly increased from 0.2 day, for the injectable solution, to 13.2 and 31.5 days for the intramuscular and subcutaneous injection of abarelix for injectable suspension, respectively; an approximate 66- and 157-fold improvement. As shown in the Figure 2 insert, in day 1 following administration of abarelix for injectable suspension, there was no, or very minimum, observed burst of the drug. Regardless of the route of administration, abarelix was gradually released from the abarelix for injection suspension. The other parameters listed in Table 5 show the dose response information.

In a long-term multi-dose study (Study 2), abarelix for injectable suspension was studied in a multi-centre, double-blind, randomised dose ranging trial in women with endometriosis-associated pain. Patients received 1 of 3 doses of abarelix for injectable suspension (abarelix 30 [n = 92], 60 [n = 92], or 120 [n = 91] mg) by subcutaneous injection.

Table 5. Pharmacokinetic profiles of abarelix for injectable solution and abarelix for injectable suspension.

Parameter		Abarelix for injectable suspension	
	(n = 14)	(n = 14)	(n = 20)
Dose	15 µg/kg	100 mg	60 mg
Route	Intramuscular injection/solution	Intramuscular injection/depot	Subcutaneous injection/depot
C_{max} (ng/ml)	57.8 (15.3)	43.4 (32.3)	18.9 (7.6)
T_{max}	1.0 (0.3) h	3.0 (2.9) days	0.1 (0.1)
$t_{1/2}$ (days)	0.22 (0.1)	13.2 (3.2)	31.5 (21.2)
AUC_{0-1} (ng.day/ml)	11.6 (2.1)	23.1 (10.1)	-
AUC_{0-28} (ng.day/ml)	Not available	399.9 (105.2)	145.0 (84)
$AUC_{0-\infty}$ (ng.day/ml)	12.0 (1.9)	500.4 (95.7)	251.0 (94)

Numbers in parentheses represent standard deviation.

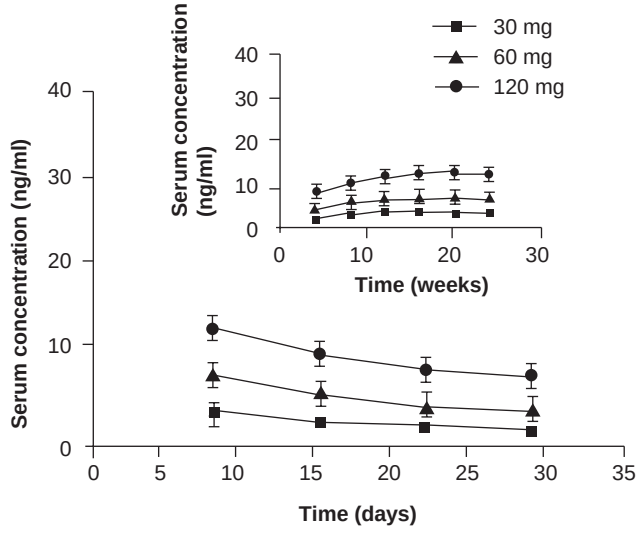

Figure 3. Study 2: dose-dependent pharmacokinetic profiles of abarelix for injectable suspension after subcutaneous administration (once every four weeks) for up to 6 months. Insert presents trough level serum concentration between treatments up to 24 weeks.

The study drug was administered once every 4 weeks for a 24-week treatment period. In Figure 3, each dose level exhibits a dose response–drug release without any burst observed; and the insert in Figure 3 shows abarelix trough levels, just before the next dosing. Very little drug was carried over into the next dosing period, indicating the suitability of subcutaneous abarelix depot administration over multiple months.

Abarelix for injectable suspension, using the Rel-Ease technology, has been administered to ~ 3000 patients, including ~ 2700 patients receiving intramuscular injections and > 300 patients receiving subcutaneous injections. In general, the administration has been well tolerated with no significant specific local tissue reactions observed in either clinical studies or with subsequent commercial usage. In the clinical studies, rare allergic reactions occurred but were later confirmed to be caused by the active peptide moiety only, as described below. As there were several case reports of allergic reactions to CMC noted in the literature (when used with other pharmaceuticals) [18], investigations were conducted to evaluate whether any patients who developed an allergic reaction developed any immunological response to the CMC portion of the abarelix CMC complex. In the studies, no IgG or IgM antibody specific to CMC were found in those patients who received abarelix for injectable suspension using the Rel-Ease technology. Thus, the overall safety profile associated with the administration of the abarelix CMC, as formulated in Rel-Ease, is acceptable.

5. Potential applications for orally administered small-molecule drugs

Although there are small-molecule therapeutics administered via parenteral routes, the majority of small-molecule therapeutics are administered orally. As Rel-Ease polymers are also common excipients that are used for oral dosage forms (e.g., as binders and disintegrants used in capsules and tablets), Rel-Ease is well suited for use in many oral drugs. Table 6 lists a number of small molecules in various therapeutic areas that form complexes with a CMC or DS polymer. As these polymers are used as complexation agents, rather than binders or disintegrants, small-molecule therapeutics using Rel-Ease exhibit very different solubility characteristics from those of the parent molecules (Table 2). With the significantly lower solubility of the complexes, small-molecule therapeutics can be potentially designed using Rel-Ease in order to prolong the dissolution rates and provide extended release. Several studies demonstrated that Rel-Ease complexes showed prolonged *in vitro* dissolution rates in simulated gastric and intestinal fluids for benzatropine, doxpine and diltiazem when compared with the parent drugs, as shown in Figures 4 and 5.

Table 6. Rel-Ease™ compatibility with small molecules in different therapeutic areas.

Therapeutic area	Small molecules CMC or DS complex*
Alzheimer or alzheimer type	Tacrine (Parke-Davis), rivastigmine (Novartis)
Antiallergic	Promethazine (Rhône Poulenc), phenyltoloxaime
Anticholinergic and/or antihistaminic	Benzatropine (Merck)
Antidepressive	Amitriptyline (AstraZeneca), doxepin (Pfizer), fluoxetine (Eli Lilly), tetracycline (AVENTIS), trazodone (Bristol-Myers Squibb)
Antihypertensive	Diltiazem (Douglas), propranolol (Wyeth-Ayerst)
Anti-infective	Ciprofloxacin (Bayer), acyclovir (GlaxoSmithKline), levofloxain and ofloxacin (Ortho-McNeil), norfloxacin (Merck), lomefloxacin (Searle), gentamycin (Teva-Sicor), tobramycin (Eli Lilly, Sicor), streptomycin (Pfizer)
Antiobsessional	Clomipramine (Novartis)
Antipsychotic	Fluphenazine (Teva-Sicor), loxapine (Watson), chlorpromazine (SmithKline Beecham)
Tuberculosis	Rifampicin (Aventis)
Pain management	Cyclobenzaprine (Merck)
Parkinson's disease	Apomorphine (Mylan)
Combination cold medicine	Dextromethorphan‡
Others	Amiodarone (Wyeth-Ayerst), hydroxyzine (Pifzer, Watson), prochlorperazine (SmithKline Beecham), naltrexone (DuPont)‡

*Generics of most of these drugs are also on the market, ‡DS complexes.
CMC: Carboxymethylcelloluse; DS: Dextran sulphate.

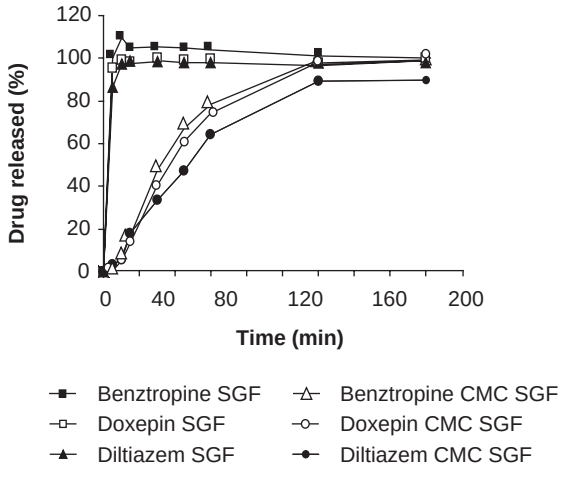

Figure 4. *In vitro* dissolution with and without Rel-Ease™ in the SGF, pH 1.0. An active drug of 10 mg, with and without complexation with CMC, was used. Experimental conditions: 37°C and 50 rpm for the first hour, followed by increasing agitation to 250 rpm.
CMC: Carboxymethylcelloluse; SGF: Simulated gastric fluid.

More interestingly, with Rel-Ease, the *in vitro* release profiles can be modified or designed through the variation of the drug loading and/or polymer selection. As shown in Figure 6, ciprofloxacin CMC complexes with different drug loading exhibit different *in vitro* release profiles, in comparison to the three marketed ciprofloxacin dosage forms of immediate-release Cipro® (ciprofloxacin HCl; Bayer), extended-release Cipro XR (ciprofloxacin HCl and ciprofloxacin; Bayer) and Proquin® XR (ciprofloxacin HCl; Depomed). In Figure 6, ciprofloxacin CMC complexes can be engineered to not only mimic the profile of Cipro XR, but also to further extend the release profiles beyond that of Proquin XR.

In addition, *in vitro* studies also showed that Rel-Ease formulations are resistant to the influence of ethanol, that is the dissolution profiles were not altered with and without ethanol for small molecules formulated with Rel-Ease, such as dextromethorphan and naltrexone. Both low solubility and resistance to the influence of alcohol have been observed in *in vitro* solubility and dissolution studies for dextromethorphan and naltrexone DS complexes. These characteristics may underscore important applications of Rel-Ease in the following two areas: i) abuse mitigation of opioid pharmaceuticals; and ii) prevention of overdose; for example, in some sustained release formulations where concomitant alcoholic beverage consumption may break down the sustained-release mechanism of the dosage form, causing an unintended, immediate release of the full dosage strength and a subsequent overdose of the medication.

Although *in vivo* evaluations of Rel-Ease for oral use are under investigation, the *in vitro* study results to date, as compared with the three marketed dosage forms of ciprofloxacin are encouraging. Rel-Ease, therefore, has many areas in oral extended delivery arena that are available for further exploration.

Despite its potential advantages for oral delivery applications, there exist limitations for Rel-Ease broad applications for oral drugs. As mentioned, the drug must carry a minimum one

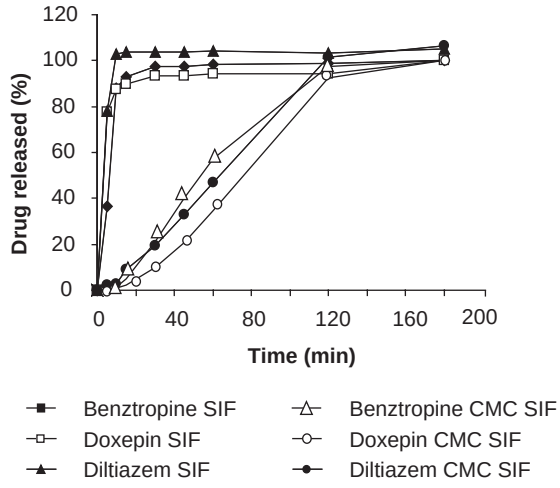

Figure 5. *In vitro* dissolution with and without Rel-Ease™ in the SIF, pH 6.8. An active drug of 10 mg, with and without complexation with CMC, was used. Experimental conditions: 37°C and 50 rpm for the first hour, followed by acidifying media to pH 1.0 and increasing agitation to 250 rpm.
CMC: Carboxymethylcelloluse; SIF: Simulated intestinal fluid.

positive charge and is soluble in water. As many oral drugs are also insoluble, Rel-Ease is limited for its applications for those drugs. In addition, for high-dose oral drugs, such as doses as high as one gram, Rel-Ease is limited by its drug loading to ~ 50% in the molecular weight range of 200 – 300 Da for small-molecule oral drugs.

6. Manufacturing processes

In the previous sections, a substantial amount of information is provided to present Rel-Ease as a unique drug delivery technology for its simplicity and broad compatibility with peptides, proteins and small molecules for parenteral and oral sustained-release applications. In this section and in Figure 7, a systematic description of the Rel-Ease manufacturing processes is provided. Using conventional pharmaceutical equipment and processes, Rel-Ease products can be manufactured in different ways based on the nature of, and the requirements for, the product(s). Figure 7 highlights the typical manufacturing steps of abarelix for injectable suspension, which is a commercial process that can be directly transferred to other peptides. Rel-Ease processes are flexible and can be adapted for different products. For example, spray-drying, the industrial technique that is predominantly used for oral product preparations due to the large material volume requirements, has been developed at the laboratory scale for Rel-Ease complexes. Direct vial/syringe filling and/or lyophilisation could be employed for products requiring aseptic manufacturing; those processes are also under development at the laboratory scales.

7. Proprietary patent positions

Rel-Ease is protected by strong patent positions worldwide. Praecis Pharmaceuticals owns the exclusive rights to several patents and patent applications in the US and worldwide, covering the Rel-Ease technology for peptide-, protein- and small-molecule therapeutics, including 3 issued US patents [101-103] and 14 granted international patents.

8. Other commercially available drug delivery technologies

Drug delivery technologies have achieved significant technical advancement in the past decades. One of the most successful polymeric drug delivery systems is the biodegradable microsphere encapsulation of poly(lactic acid), poly(glycolic acid) or PLGA copolymers. The marketed peptide and protein products that have used this delivery system include Lupron Depot® (leuprolide acetate for depot suspension; Takeda), Sandostatin LAR® (octreotide acetate for injectable suspension; Novartis), Trelstar® (triptorelin pamoate; Debiopharm) and Nutropin Depot® (somatropin [rDNA] for injectable suspension; Genentech). One of the examples of PLGA microsphere formulations for small molecules is Risperdal Consta® (risperidone; Johnson & Johnson).

Pegylation is another important drug delivery technique for delivering protein therapeutics. Commercialised products using pegylation include Adagen® (pegademase bovine; Enzon), Aranesp™ (darbepoetin alpha; Amgen), PEG-Intron® (PEG-IFN-α_{2b}; Schering-Plough), Pegasys® (PEG-IFN-α_{2a}; Roche), Neulasta® (pegfilgrastim; Amgen) and Somavert® (pegvisomant; Pfizer).

There are other emerging polymeric drug delivery technologies, such as PolyActive™ (OctoPlus), an IFN-α biodegradable polymeric drug delivery system for the controlled release of proteins and lipophilic small molecules that demonstrated positive Phase I results for Locteron™ (IFN-α for the treatment of chronic hepatitis C) [201]. A thorough review of different polymeric drug delivery technologies has been published [19].

8.1 Poly(lactic-co-glycolic acid) microsphere encapsulation

Although major breakthroughs have been achieved for the parenteral sustained delivery of peptides and proteins [20-24], the unmet needs for the sustained delivery of water-soluble biomolecules and small molecules remain substantially large. Although the PLGA microsphere-encapsulation technology has proved to be a technological advance in many areas, limitations still exist in the incorporation of hydrophilic, water-soluble biomolecules into the hydrophobic microsphere environment, as the polymer itself is highly hydrophobic and water insoluble. Such limitations also introduce additional processing challenges. The encapsulation processes typically need to be developed on a case-by-case basis for the proper selection of multiple solvent systems, including surfactants,

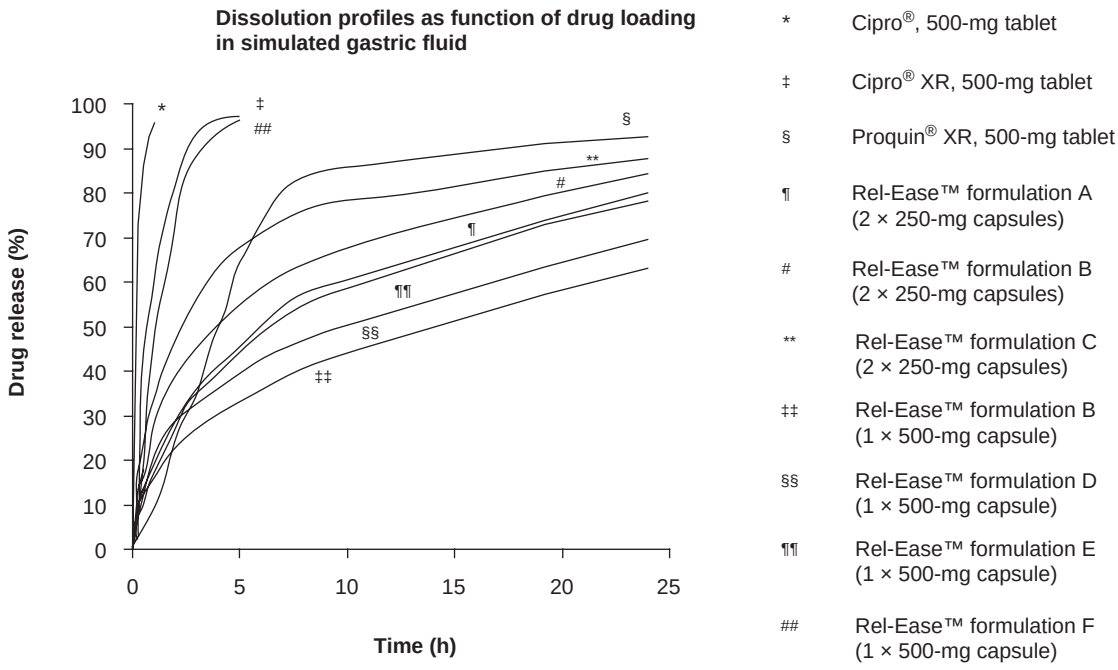

Figure 6. Ciprofloxacin CMC of different drug-loading *in vitro* dissolution profiles in simulated gastric fluid, pH 1.0 and comparison with Cipro®, Cipro® XR and Proquin® XR. A to F are Rel-Ease™ ciprofloxacin CMC complex formulations with different drug loadings: A, 33.1%; B, 42.1%; C, 50.4%; D, 47.3%; E, 51.9%; F, 57.5%. Experimental conditions: 37°C and 50 rpm.
CMC: Carboxymethylcelloluse.

the development of complex fabrication techniques, as well as the need for customised equipment [4,6].

PLGA processes may also involve conditions of high-shear forces, organic solvents and/or non-ambient temperatures. These conditions can cause biomolecule instability during encapsulation, such as protein denaturation, resulting in a the loss of bioactivity in the encapsulated form. In addition, the free drug concentrations on the surfaces of microspheres can be more difficult to control for water-soluble drugs, due to incompatible hydrophilicity or hydrophobicity of the drug and polymer. It is this incompatibility that makes the drug more difficult to encapsulate, resulting in a product that contains free drug residing on the microsphere surfaces after solvent removal. Consequently, due to the above-mentioned factors that need to be controlled, including efficiency and quality of encapsulation, the manufacturing costs of PLGA-encapsulated products for water-soluble peptides and proteins can be very high.

8.2 Pegylation

Another example of a commercially available, sustained delivery system for water-soluble biomolecules is the chemical conjugation of proteins with PEG water-soluble polymers, known as pegylation. The pegylation process requires either non-specific conjugation with multiple attachments of PEG chains to a target protein or site-specific conjugation with a high molecular weight PEG chain [25,26]. The chemical process of pegylation can require different technical considerations. First, PEG is required to be activated through the endgroup chemical modification. However, due to the lack of commercially available or ready-to-use endgroup modified PEG polymers on the market, PEG polymers with specific end groups and a defined average molecular weight must be custom prepared. Second, if site-specific pegylation of the protein is desired, which is often critical in maintaining the biological activity of the protein after pegylation, then site-directed mutagenesis modification of the protein itself may also be required, such as the incorporation of a cysteine residue at a specific site.

Pegylation may also involve the use of toxic chemicals. For instance, sodium cyanoborohydride is used as a reducing agent during pegylation through the amino groups of a protein. In addition, lengthy chromatographic purification, with subsequent solvent removal and analysis of the pegylated protein, is required to ensure the quality of the pegylated product. Other issues may limit PEG applications, including the quality and stability of end groups in the modified PEG polymer, as well as the precise control of pegylation conditions, such as pH and temperatures. The PEG manufacturing time can also be lengthy, resulting in a higher cost of goods.

9. Expert opinion and conclusions

As the majority of drug candidates requiring parenteral delivery are water-soluble molecules, such as peptides and proteins, the

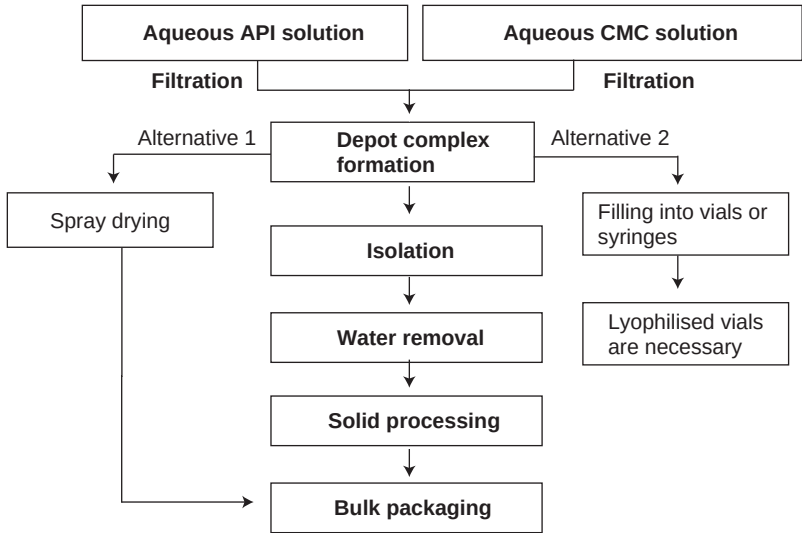

Figure 7. Rel-Ease™ manufacturing processes. The commercial process is shown in bold. This process was used for abarelix for injectable suspension. Alternative 1 is a conventional process for small-molecule drug applications using Rel-Ease. Alternative 2 can be used for parenteral products under aseptic conditions.
API: Active pharmaceutical ingredient; CMC: Carboxymethylcelloluse.

market demand for alternative sustained-release drug delivery systems is high for several reasons. Many companies need different approaches other than the existing technology platforms to overcome technical difficulties inherent with their project or to achieve certain clinical objectives, such as the requirement of a large (both in volume and dosage) sustained-release dose. Regarding this largely unmet need for parenteral drug delivery technologies, Rel-Ease, as a unique drug delivery system, may fill some of this market demand by offering many advantages in addition to sustained delivery. Rel-Ease uses commercially available, water-soluble polymers, such as CMC, among others. CMC, which was used in the initial development and commercialisation of Rel-Ease, is a compendial material listed in US, European and Japanese Pharmacopoeias, and has been used in other parenteral products as a suspending agent. It is inexpensive and is readily available in specific polymeric grades, and offers the requisite negative-charge attributes for complexation, ensuring compatibility with the basic lysine and/or arginine residues of most peptides and proteins. Through the charge neutralisation with CMC, the solubility of peptides and proteins can be significantly reduced, forming a low-solubility complex and, subsequently, a depot at the injection site providing a sustained release of the active drug over a period of time. Another technological breakthrough is the potential for the high-drug loading of peptides and proteins, making Rel-Ease a viable delivery option for therapeutics requiring a high administration dose.

Clinically, Rel-Ease has no or minimal burst effect in abarelix for injectable suspension when administrated intramuscularly or subcutaneously, which is distinguished from, and advantageous over, the conventional PLGA microsphere-encapsulation technologies. For many biopharmaceuticals, especially for those of high potency or with narrow therapeutic window, the burst effect can be a critical consideration in selecting an appropriate delivery system. An initial drug burst may cause undesirable side effects, resulting in safety concerns for certain patient populations, such as in the case of Lupron, which causes a significant testosterone surge prior to achieving the treatment benefit of testosterone suppression.

Considering the process requirements, flexibility, scalability, duration of manufacture, quality control and overall cost requirements, which contribute to technology selection, Rel-Ease is one of the few viable, commercially tested drug delivery options for biopharmaceutical development on the market that could potentially meet formulation-product goals. Rel-Ease uses readily available conventional process equipment, without customisation requirements, so that multiple products can be adapted into a single manufacturing facility producing multiple Rel-Ease products. Rel-Ease can be integrated with the development of new chemical entities, incorporated into life-cycle management, or used to produce generic product formulations as specialty pharmaceutical products. The Rel-Ease development time is significantly shorter than other available technologies, such as microsphere encapsulation or pegylation, due to its simplicity and use of readily available polymer materials. The product quality can be tightly controlled through the precise stoichiometric charge ratios of the drug and the polymer under the complexation conditions. Compared with the other technologies, the requirements for sensitive reaction controls are significantly less. In addition, Rel-Ease may use terminal sterilisation for the finished product, such as in the case of abarelix for injectable suspension that uses γ-irradiation. If

terminal sterilisation is not an option or not desired, aseptic processing may also be employed. The quality of the product can be consistently managed from scale-up to commercial quantities; due to its simplicity, the overall manufacturing cost is also relatively low.

There exists a strong medical need for commercially available drug delivery technologies to deliver peptides, proteins and small molecules effectively and safely, and Rel-Ease is now a viable option. As with the pharmaceutical field itself, the drug delivery landscape is likely to continue to be altered by new technologies and ideas. It is probable that many of these future innovations will be for niche applications; however, the continued demand for broad, proven platform technologies is likely to be a constant.

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Affiliation

Ming Ye[†1] PhD, Gajanan Bhat[2] MS, PhD, Kevin A Johnston[3], Heow Tan[4] & Marc Garnick[5] MD
[†]Author for correspondence
[1]Associate Director of Pharmaceutics and Drug Delivery Technology,
Praecis Pharmaceuticals Inc., 830 Winter Street, Waltham, MA 02451, USA
Tel: +1 781 795 4100;
Fax: +1 781 890 7015;
E-mail: mingye02@hotmail.com
[2]Director of Biometrics,
Praecis Pharmaceuticals Inc., 830 Winter Street, Waltham, MA 02451, USA
[3]Associate Director of Pharmaceutical Development and Analytical Technology,
Praecis Pharmaceuticals Inc., 830 Winter Street, Waltham, MA 02451, USA
[4]Vice President of Development and Industrial Operations, Praecis Pharmaceuticals Inc.,
830 Winter Street, Waltham, MA 02451, USA
[5]Chief Medical Officer,
Praecis Pharmaceuticals Inc., 830 Winter Street, Waltham, MA 02451, USA