

January 01, 2010

## Controlling drug delivery for the application of extended or sustained-release drug products for parenteral administration

Jacklyn O'Neil  
*Northeastern University*

---

### Recommended Citation

O'Neil, Jacklyn, "Controlling drug delivery for the application of extended or sustained-release drug products for parenteral administration" (2010). *Chemistry Master's Theses*. Paper 14. <http://hdl.handle.net/2047/d20000132>

Controlling Drug Delivery for the Application of Extended or Sustained-Release  
Drug Products for Parenteral Administration

A thesis presented

by

Jacklyn O'Neil

To

The Department of Chemistry and Chemical Biology

In partial fulfillment of the requirements for the degree of

Master of Science

in the field of

Chemistry

Northeastern University  
Boston, Massachusetts  
May, 2010

Controlling Drug Delivery for the Application of Extended or Sustained-Release  
Drug Products for Parenteral Administration

by

Jacklyn O'Neil

ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science in Chemistry  
in the Graduate School of Arts and Sciences of  
Northeastern University, May, 2010

## ABSTRACT

New therapeutic agents are constantly being developed in order to treat new and current disease states. Many of these new compounds display a narrower therapeutic range, where the difference in concentrations between effective treatment and toxicity becomes smaller<sup>1</sup>. As a result, controlling drug release to stay in these tightening ranges becomes more and more important to effectively treat patients. The proper management of some diseases has been shown to be dependent upon achieving consistent pharmacokinetic profiles<sup>2</sup>, which is reliant on the timing of dosages, paired with the total compliance of the patient. For many specific patient populations compliance with daily oral dosing presents inherent complications; e.g with schizophrenic and depressive patients. However, patients receiving continuous (ie, extended release) therapies have been shown to have lower rates of relapse, because of the constant dosing of medication<sup>3,4</sup>. Increasing the treatment length of a dose given by injection<sup>5</sup> and finding preferential ways to deliver extended-release therapies are the basis for continued research in the formulation of extended or sustained-release injection products.

In many cases, trying to avoid a parenteral (injectable) route of administration through oral dosing is not feasible or even desirable<sup>5</sup>. Therefore, the development of many sustained-release drug products results in injection-based products. Extending the release of a drug may be achieved through the manipulation of physiochemical properties, the use of formulation technologies such as microspheres and nanospheres<sup>6</sup>, and balancing the *in vivo* properties of the compound (such as half-life)<sup>7</sup>. This thesis will cover current options for developing extended-release pharmaceutical parenteral (injection) delivery systems including prodrugs,

microspheres, traditional depots, and injectable implants that are currently approved or in-process of approval by the United States Food and Drug Administration or approved in Canada.

## DEDICATION

This work is dedicated to my sister, JuliAnn O'Neil.

## ACKNOWLEDGEMENTS

I would like to acknowledge my advisor, Graham Jones, for all of his helpful advice on matters covering every facet of my education at Northeastern in addition to all of his support and guidance.

Additionally, to the Formulation Development group at Alkermes, Inc for giving me the chance to learn so much about scientific discovery and allowing me the freedom to grow as a scientist.

## TABLE OF CONTENTS

	Page
ABSTRACT.....	3
DEDICATION.....	5
ACKNOWLEDGEMENTS.....	6
TABLE OF CONTENTS.....	7
GLOSSARY OF ABBREVIATIONS.....	8
LIST OF FIGURES.....	9
LIST OF TABLES.....	11
INTRODUCTION.....	12
PHARMACOKINETICS.....	15
DEVELOPING AN EXTENDED-RELEASE INJECTABLE.....	19
PRODRUGS.....	23
MICROSPHERES.....	29
DIFFUSION.....	41
DEPOTS.....	41
SALTS.....	44
INJECTABLE IMPLANTS.....	46
DISCUSSION.....	49
PREVENTING BURST RELEASE.....	49
SELECTION OF INJECTION VEHICLE.....	50
ADDITION OF EXCIPIENTS.....	52
FORMULATING A REASONABLE INJECTION PRODUCT.....	53
BIOLOGICS.....	54
CUSTOMIZING RELEASE RATES.....	55
CONCLUSIONS.....	61
REFERENCES.....	63

## GLOSSARY OF ABBREVIATIONS

CNS.....	Central Nervous System
GI.....	Gastrointestinal
GnRH.....	Gonadotropin releasing hormone
IM.....	Intramuscular
Log P.....	Partition coefficient
PD.....	Pharmacodynamic
PK.....	Pharmacokinetic
pKa.....	log Ka (acidity coefficient)
PGA.....	Polyglycolic acid
PLA.....	Polylactic acid
PLG.....	Poly-lactide-co-glycolide
PLGA.....	Poly-lactic-co-glycolic acid
SC.....	Subcutaneous
SEM.....	Scanning Electron Microscope
SR.....	Sustained release
XR.....	Extended release

## LIST OF FIGURES

	Page
Figure 1: Pharmacokinetic Profiles of Daily and XR Dosing.....	16
Figure 2: Zero-Order Release.....	18
Figure 3: First-Order Release.....	18
Figure 4: Hydrolysis of a Paliperidone Ester Prodrug to Active Alcohol.....	24
Figure 5: Fluphenazine Decanoate.....	25
Figure 6: Flupentixol Decanoate.....	25
Figure 7: Paliperidone Palmitate.....	26
Figure 8: Haloperidol Decanoate.....	27
Figure 9: Testosterone Cypionate.....	28
Figure 10: Effect of Varying Copolymer Ratios in Risperidone Microspheres.....	31
Figure 11: PLGA Molecule.....	31
Figure 12: SEM Image of Microspheres.....	34
Figure 13: Polylactic Acid.....	34
Figure 14: Polyglycolic Acid.....	34
Figure 15: Polymer Microsphere Degradation.....	35
Figure 16: Risperidone.....	35
Figure 17: Naltrexone.....	36
Figure 18: Leuprolide Acetate.....	37
Figure 19: Octreotide Acetate.....	38
Figure 20: Exenatide.....	39
Figure 21: Medroxyprogesterone Acetate.....	42

Figure 22: Lanreotide Acetate.....	43
Figure 23: Medroxyprogesterone Acetate.....	44
Figure 24: Olanzapine Pamoate Monohydrate.....	46
Figure 25: Goserelin Acetate.....	47

## LIST OF TABLES

	Page
Table 1: Extended-Release Injectable Drug Products.....	21
Table 2: Formulation Details of Extended-Release Products.....	22
Table 3: Summary of Dosing Changes with Prodrug Products.....	28
Table 4: Polymers Used in Microsphere Formulations.....	32
Table 5: Summary of Dosing Changes with Microsphere Products.....	40
Table 6: Summary of Dosing Changes with Diffusion Products.....	48
Table 7: Oils Used for Parenteral Injection Vehicles.....	52

## INTRODUCTION

From the patient's perspective, an oral dosage form is certainly more desirable than a product for injection. Since oral dosages are delivered through gastrointestinal (GI) interactions, the formulation of the dose must take into account the environments that the dosage will encounter in the GI after administration. This is mainly accomplished through the addition and manipulation of excipients to control factors such as degradation pathways, disintegration of the dosage, and release rates. Increased convenience to the patient can be gained through the development of extended or sustained-release formulations of certain therapeutics. However, the complexities of gastroretention and chemical reactivity present as serious obstacles for doses that require efficacy for longer than the total physiological digestion time of roughly 48 hours<sup>8</sup>. Loss of activity during transit along the gastrointestinal tract primarily occurs through enzyme activity, low solubility through the GI membranes<sup>5</sup>, and poor retention of a drug delivery system (tablet or other device) describe three current challenges of truly extended-release oral therapies. Therefore, the best current alternative lies within depot or implant-based products that release the desired compound over a specified range of days with a controllable release rate. This gives the patient the benefit of a more controlled plasma profile that may help control their disease more effectively when compared to traditional daily oral therapies.

In many cases, the proper managing of a disease is dependent on achieving consistent pharmacokinetic profiles of a therapeutic drug. This depends on the constant compliance of the patient, ensuring that the medication is taken exactly as prescribed. For many patient populations, daily oral dosing inherently presents complications, as is observed with

schizophrenic and depressive patients. Patients receiving continuous (ie, extended release) therapies have been shown to have lower rates of relapse, because of the constant dosing of medicine<sup>3,4</sup>, allowing a consistent and stable pharmacodynamic profile to be achieved. In schizoaffective patient populations, the incidence of relapse has been repeatedly linked to an overall poorer prognosis<sup>3,4</sup>, illustrating the importance of patient compliance for the management of diseases.

Schizophrenia and other mental disorders are diseases in which an extended-release therapy is visibly needed. Yet, there are a number of disease states in which the frequent administration of hormones are prescribed as treatments—such as acromegaly, endometriosis or hormone-responsive cancers. Disease states such as these can require constant hormone therapy in order to control or mitigate the symptoms caused by these diseases. Extended-release formulations for these therapeutics can be dosed as infrequently as three times a year in the case of Lupron® Depot, and would otherwise require daily or weekly hormone injections.

Because extended-release formulations can allow for the infrequent administration of medications to patients experiencing chronic diseases, there is an increase in the demand for these drugs, and a corresponding increase in the number of drugs being developed for extended-release use. In order to facilitate this type of drug development, formulators of extended-release drug products must manipulate and exploit all the physiochemical properties known and balance the drug's physiochemical properties with the desired product properties in order to achieve the desired release characteristics and profile.

As such, the primary purpose of this thesis will be to comprehensively review a number of extended or sustained-release pharmaceuticals for injection that are currently

approved, or under review by the United States Food and Drug Administration or approved in Canada by the Therapeutic Products Directorate. This class of therapeutics is largely limited to agents that target the Central Nervous System (CNS) and to derivatives of human hormones. Reflecting this, this review will focus on only these agents and will summarize the physiochemical properties of these compounds and the mechanisms used in their formulations to obtain an extended-release profile in order to draw conclusions for the formulation of future chemical entities and the reformulations of current compounds.

## PHARMACOKINETICS

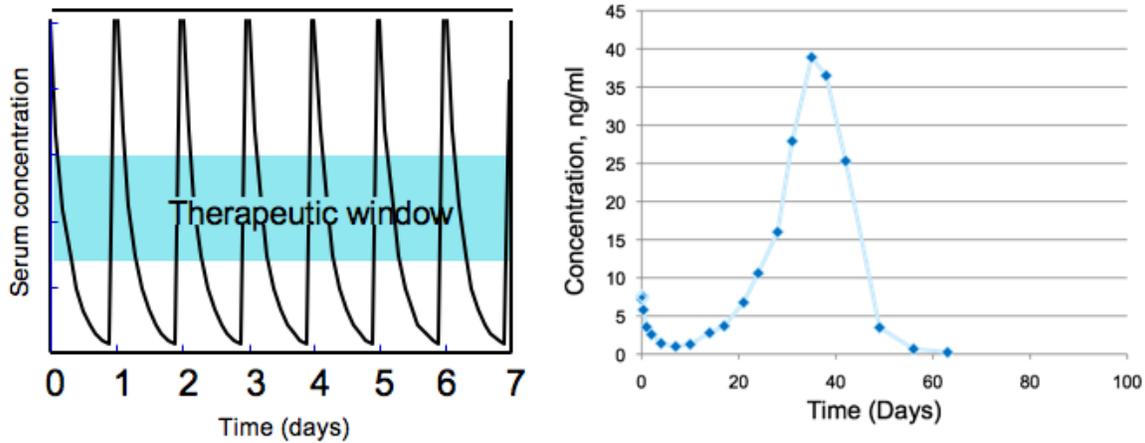
Following a parenteral route of drug administration, the drug compound is present as molecule in a solution (i.e. blood). It is systemically distributed and is accumulated in the body according to its physiochemical properties, most pronouncedly, the compound's partition coefficient (log P). The presence of drug molecules in solution makes them susceptible to factors that may increase degradation rates such as water and enzymes<sup>9</sup>. It is known that both small molecules and larger, more complex molecules (such as carbohydrates) that are sufficiently water-soluble are rapidly absorbed by the body upon intramuscular administration<sup>10</sup>, facilitating a fast release profile. This solubility presents a challenge when trying to work towards a sustained-release application.

Most drug products (for immediate release) display first order kinetics, where there is a high initial "burst" of drug in the blood concentration, followed by a constant decline in the drug plasma concentration followed by subsequent dosing (see Figure 1). This model of pharmacokinetics results in a greater number of peaks and troughs—which is usually not the ideal therapeutic state<sup>2,11</sup>. For any pharmaceutical injectable formulation, there lies two ideal goals: to create a drug release profile that displays a zero-order kinetics and to develop a reasonable injection product that delivers the desired dose in an acceptable injectable product.

Because of the constant exposure to the drug that is associated with a sustained or extended-release, this type of therapy lends itself to yield a profile resembling a zero-order release (see Figure 2), whether it is sustained over a period of days or weeks. This limits the number of peaks and troughs occurring in the plasma concentration. The final drug product must also be carefully balanced with the active compound and excipients' properties in order

ensure the delivery of a therapeutic that is easy to administer and possesses minimal side effects.

Figure 1: Pharmacokinetic Profiles of Daily and XR Dosing



When a compound is absorbed into the bloodstream, dissolution is occurring. This phenomenon is controlled by a number of different factors, as described by the Noyes-Whitney equation depicted below<sup>12</sup>:

$$\frac{dm}{dt} = \frac{DS}{Vh} (C_s - C_t)$$

where 'dm' is the rate of dissolution,

'D' is the diffusion coefficient of the compound,

'S' is the surface area of the drug product,

'C<sub>s</sub>' is the concentration of the solid in the diffusion layer that surrounds the solid

'C<sub>t</sub>' is the concentration of the solid in the bulk dissolution medium,

'V' is the volume of the dissolution media, and

'h' is the thickness of the diffusing film adjacent to the surface being dissolved.

Purely by observing the number of variables in this relationship, it is clear that a number of controlling factors may be manipulated in order to selectively influence the dissolution of a compound into the dissolution media—the bloodstream.

Another important consideration useful to this review is the concept of elimination, or biological half-life, which is the rate at which a compound is eliminated from the body. This complex process is modeled by the following relationship<sup>13</sup>:

$$t_{\frac{1}{2}} = \frac{V \ln 2}{Cl_s}$$

where ‘ $t_{\frac{1}{2}}$ ’ is the time it takes for half the compound to be eliminated

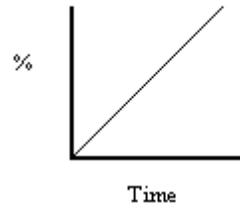
‘V’ is the distribution volume that the compound will occupy and

‘ $Cl_s$ ’ is the body clearance, which measures the capacity of the compound to be excreted.

Zero-order kinetics are observed when the plasma concentrations of a compound are above the threshold absorption constant. This is characterized by the independence of the blood concentration of the drug administered with respect to the amount that is administered, illustrated by an exponential curve that is observed when drug concentration is plotted against time from the when the dose was administered. A zero-order relationship does not follow linear kinetics<sup>14</sup> and instead, displays an inversely related systemic clearance of the drug substance. Mathematically, the depiction of zero-order release is depicted as:

Figure 2: Zero-Order Release

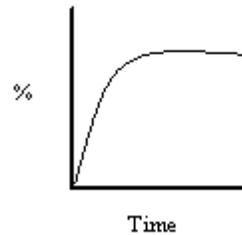
$$-\frac{dC_s(t)}{dt} = kC_s(t)$$



The more common first-order kinetics (exemplified in Figure 3) are seen when the drug concentration fall below the absorption constant for the system. This is when the plasma concentration of the drug is directly proportional to the concentration of the drug administered, obeying linear kinetics<sup>14</sup>. First-order release describes a constant systemic clearance of the drug following classic Michaelis-Menten kinetics, illustrated by the model:

Figure 3: First-Order Release

$$-\frac{dC_s(t)}{dt} = V_{\max}$$



## DEVELOPING AN EXTENDED-RELEASE INJECTABLE

For the development of injectable products, there are specific goals that are identified, highlighted by Chaubal *et al*:

1. To exert control over the pharmacokinetic profile and parameters such  $t_{max}$ ,  $C_{max}$ , and the rate of elimination.
2. To guarantee dosage and total drug compliance of the patient.
3. To have a delivery route that is appropriate for patients with nonfunctioning guts, or who are unconscious and unable to receive an oral dosage.
4. To obtain a localized effect.

When developing an injectable for extended-release, the two following goals should also be incorporated:

5. To display zero order kinetics.
6. To sustain the drug release over therapeutically appropriate period of time.

In the process of realizing any of these specific goals, a number of developmental challenges will be encountered, particularly when trying to develop a product that has a defined extended-release profile. Many different physical and chemical methodologies have been utilized to accomplish the task of developing an extended-release drug product and bringing it to the pharmaceutical market. The content of a final drug product, however, is rarely just the drug compound itself. Most products have a number of excipients that are

added to the formulation for a number of reasons: stabilizing a suspension, preventing microbial growth, modifying the viscosity, delaying the release, just to name a few.

Exploitation of the physiochemical properties such as solubility and partition coefficients of a drug may serve to completely control the release of some compounds (as seen with Somatuline® Depot), while others will need additional methods or excipients in order to develop a product for an extended-release injection. A number of products for extended-release have been developed and have experienced commercial success (or are under review) and are highlighted in Table 1. Each product relies on a particular mechanism of release to ensure an extended-release profile. In this review, three common tools will be covered: prodrugs, salts, microspheres, depots and injectable implants, as well as the mechanisms which facilitate the drug release.

Table 1: Extended-Release Injectable Drug Products

<u>Active Pharmaceutical Ingredient</u>	<u>Active Moiety</u>	<u>Release Mechanism</u>	<u>Formulation</u>
Olanzapine Pamoate Monohydrate	olanzapine	Diffusion	Suspension
Fluphenazine Decanoate	fluphenazine	Prodrug	Sesame oil solution
Paliperidone Palmitate	paliperidone	Prodrug	Nano-suspension
Flupentixol Decanoate	flupentixol	Prodrug	Vegetable oil solution
Haloperidol Decanoate	haloperidol	Prodrug	Sesame oil solution
Risperidone	risperidone	Microsphere	Suspension
Naltrexone	naltrexone	Microsphere	Suspension
Leuprolide Acetate	leuprolide	Microsphere	Suspension
Octreotide Acetate	somatostatin analogue	Microsphere	Suspension
Lanreotide Acetate	somatostatin analogue	Diffusion	Supersaturated Solution
Methylprednisolone Acetate	methylprednisolone acetate	Diffusion	Suspension
Medroxyprogesterone Acetate	medroxyprogesterone acetate	Diffusion	Suspension
Testosterone Cypionate	testosterone	Prodrug	Cottonseed Oil Solution
Goserelin Acetate	goserelin	Diffusion	Polymer Implant
Exenatide	exenatide	Microsphere	Suspension

Table 2: Formulation Details of Extended-Release Products

<u>Product</u>	<u>Dose</u> <sup>15</sup>	<u>Injection Volume</u> <sup>16</sup>	<u>Release Mechanism</u>	<u>Approval Date</u> <sup>15</sup>
Olanzapine Pamoate Monohydrate	eq. 150 mg eq 210 mg eq 300 mg eq 405 mg	1.0 mL 1.4 mL 2.0 mL 2.7 mL	Diffusion	US- December 2009
Fluphenazine Decanoate	25 mg/mL	<5 mL	Prodrug	US- June 1972
Paliperidone Palmitate	39 mg 78 mg 117 mg 156 mg 234 mg	0.25 0.5 0.75 1.0 1.5	Prodrug	US- July 2009
Flupentixol Decanoate	20 mg/mL	1 or 2 mL	Prodrug	Canada-1976
Haloperidol Decanoate	50 mg/mL 100 mg/mL	1or 5 mL 1 or 5 mL	Diffusion	US- January 1986
Risperidone	12.5 mg 25 mg 37.5 mg 50 mg	2.0 mL	Microspheres	US- October 2003
Naltrexone	380 mg	4.0 mL	Microspheres	US- April 2006
Leuprolide Acetate	3.75 mg 7.5 mg 11.25 mg 15 mg 22.5 mg 30 mg	<2 mL	Microspheres	US- January 1989
Octreotide Acetate	eq 10 mg eq 20 mg eq 30 mg	5 mL	Microspheres	US- November 1998
Lanreotide Acetate	eq 60 mg eq 90 mg eq 120 mg	0.2-0.5 mL	Diffusion	US- August 2007
Methylprednisolone Acetate	20 mg/mL 40 mg/mL 80 mg/mL	5 mL 1, 5, 10 mL 1 or 5 mL	Diffusion	US-Before January 1982
Medroxyprogesterone Acetate	150 mg/mL 400 mg/mL	1 mL 2.5 mL	Diffusion	US- Before January 1982
Testosterone Cypionate	100 mg/mL 200 mg/mL	10 mL 1 or 10 mL	Prodrug	US- Before January 1982
Goserelin Acetate	eq 3.6 mg eq 10.8 mg	-	Diffusion	US- January 1996
Exenatide	Undefined	Undefined	Microspheres	US- Pending Approval

## PRODRUGS

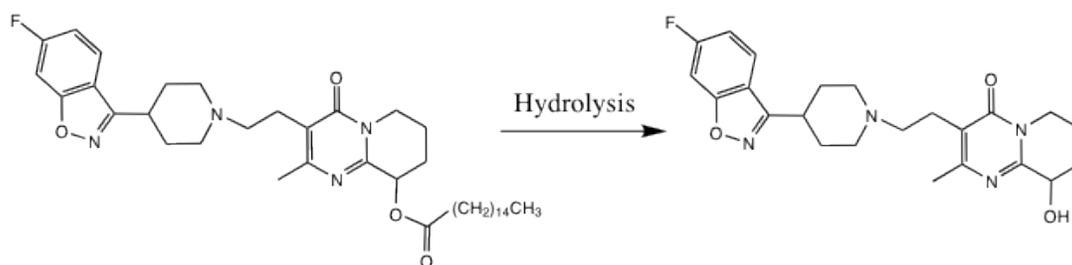
From Table 1, it is apparent that the derivatization of active compounds with cleavable prodrug moieties is a common tool used by drug formulators in order to prolong the release of the active drug. Perhaps the most widely cited indication for prodrug utilization lies in the number of steroid prodrugs for immediate release for parenteral delivery<sup>17</sup>. Many of the steroids used for clinical applications display very low solubility in aqueous media, and subsequent derivatization with short chain esters offers a hydrophilic substituent which is a large enough factor relative to the molecule's size in order to facilitate an increase in solubility that allows for a normal dosing regimen.

As covered in this review, all prodrugs appear as ester derivatives, which are easily hydrolyzed *in vivo* into the active alcohol and the linking tail. Conversely, the oxidation of an alcohol group to an ester, a reaction that was first developed by Emil Fischer in the late 1800's is a classic organic reaction<sup>18</sup> that has been exploited in order to produce potential drug analogues. A low aqueous solubility *in vivo* translates to low uptake into the bloodstream. Ester derivatives are common choices for both immediate and extended-release prodrugs analogues— they easily modify aqueous solubility, slowing uptake and subsequent, hydrolysis, as depicted in Figure 4. Based on this mechanism, the prodrug compound would not have to have a particularly low solubility, if the rate of conversion to the active form was appropriate for the desired release rate.

Although prodrug moieties have traditionally seen much utilization for the improvement of aqueous solubility and partition coefficients for water insoluble drugs, they

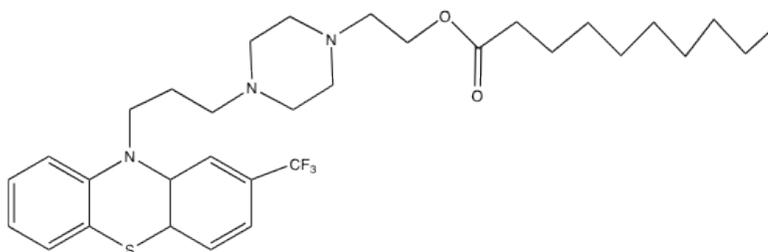
may also be effectively used in order to slow the release of the active compound, assuming the rate of hydrolysis by the appropriate enzyme is adequately slow<sup>11</sup>. When used as a release modifier, esters remain as one of the commonly used groups, especially for CNS drug derivatization<sup>19</sup>. Addition of a long-chain aliphatic ester makes the drug compound more lipophilic, which increases its solubility in oils for injection<sup>19</sup>.

*Figure 4: Hydrolysis of a Paliperidone Ester Prodrug to Active Alcohol*



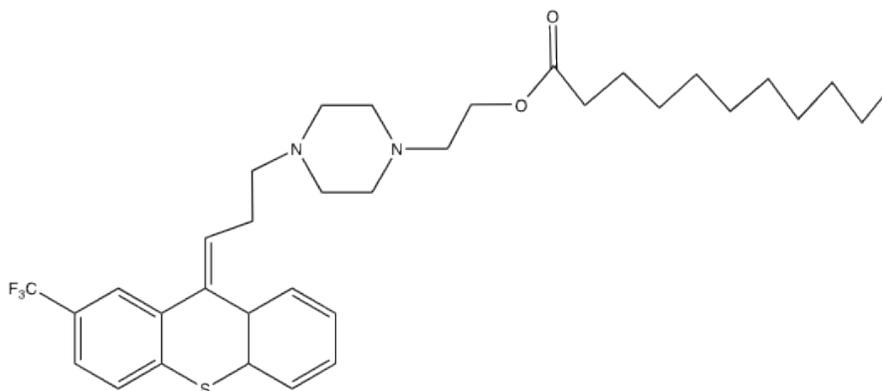
Prolixin® Decanoate, marketed by Bristol Myers Squibb is as a sesame oil solution for injection that is indicated for the treatment of schizophrenia and various psychoses. Fluphenazine decanoate possesses a molecular weight of 591.8 daltons. Fluphenazine, a phenothiazine derivative that is known to exhibit antipsychotic activity in patients, is insoluble in water<sup>19</sup> and has a partition coefficient of about 9<sup>21</sup>. This compound is structurally similar to flupentixol decanoate, differing only by the addition of a heteroatom (nitrogen) on the center ring of the molecule. Dosing with the decanoate prodrug decreased the frequency of administration from every 6-8 hours with the active drug to once every two weeks, while the half-life of the drug increased from about 33 hours to 7-10 days<sup>16</sup>.

Figure 5: Fluphenazine decanoate



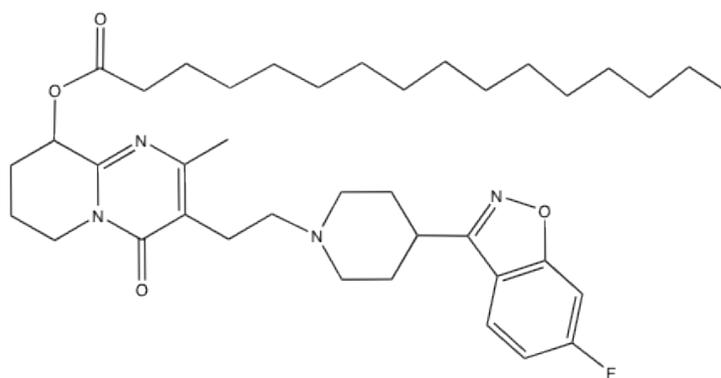
Fluanxol®, a depot containing flupentixol decanoate is another antipsychotic used to treat schizophrenia and is produced as a vegetable oil solution. Sold in the UK and in Canada by Lundbeck, the active drug flupentixol is a classical typical antipsychotic that was initially approved in December of 1995 in Canada. This compound experiences a half-life of about 35 hours *in vivo*. Flupentixol is a thioxanthene derivative that acts as an antagonist on the dopamine, serotonin, adrenaline and histamine receptors in the central nervous system<sup>22</sup>. Possessing a molecular weight of 588.8 daltons, a partition coefficient of approximately 6, and is only slightly soluble in water<sup>21</sup>. Upon the release of this formulation, dosing frequency was improved from once or more daily to once or twice monthly.

Figure 6: Flupentixol decanoate



Invega Sustanna®, marketed by Ortho-McNeil -Janssen Pharmaceuticals is prepared as a nano-suspension of paliperidone palmitate. The active drug, paliperidone, is classified as a benzisoxazole, which is in the same class as risperidone (highlighted later in this review). An atypical antipsychotic, with a molecular weight of 664.9 daltons, it is indicated for the treatment of schizophrenia and bipolar disorder<sup>23</sup>. Also known as 9-hydroxy-risperidone, paliperidone is an active metabolite of risperidone. This palmitate prodrug is insoluble in water, with a partition coefficient of approximately 10.1<sup>21</sup>, making this compound highly hydrophobic. With a half-life of 23 hours, Invega Sustenna® allowed the dosing frequency to be decreased from once daily (compared to oral Invega®) to a once monthly injection.

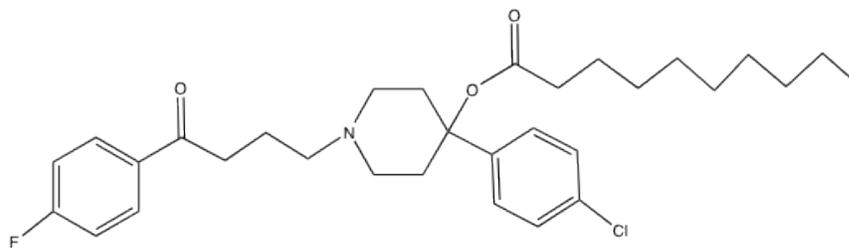
Figure 7: Paliperidone palmitate



Perhaps the most well known antipsychotic, haloperidol, or “haldol”, has been extensively used to control psychosis since its approval in 1967. First marketed by Janssen Pharmaceuticals, this traditional small molecule (a butyrophenone derivative) has a molecular mass of 530.1 daltons<sup>24</sup>. The decanoate prodrug is used primarily to treat schizophrenia and other psychoses. It has a half-life of approximately 1 day *in vivo* and has a partition coefficient value of 7.9<sup>21</sup>. Haloperidol decanoate is almost insoluble in water (solubility is

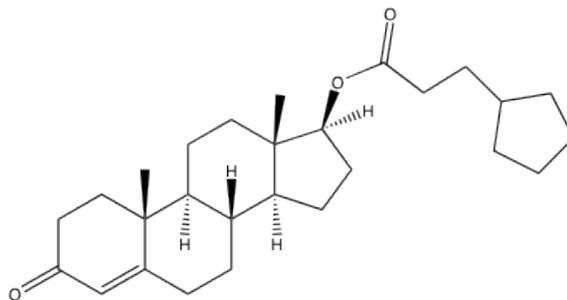
0.1 mg/mL) as is delivered as an oil solution<sup>24</sup>. With the long-acting formulation, this drug's dosing decreased from two to three times daily (for the oral dose) to once every three weeks with the extended-release formulation.

Figure 8: Haloperidol decanoate



Testosterone cypionate is the cyclopentylpropionate ester of the androgenic hormone testosterone. This compound is reported to be insoluble in water and possesses a molecular weight of 412.6 daltons. Depo-Testosterone® is most often used for the indication of primary hypogonadism and hypogonadotropin hypogonadism, where hormone replacement therapy is necessitated. The half-life of this compound is under two hours, with the depot lasting from two to four weeks, depending on the indication<sup>25</sup>. Due to the cyclopentyl ester, this drug is considerably more hydrophobic than the active testosterone, with a log P value of 6.4<sup>21</sup>, compared to testosterone's 3.3<sup>21</sup>. This drug was first developed by Pharmacia and Upjohn and is now marketed under Pfizer.

Figure 9: Testosterone Cypionate



Contained in Table 3 is a summary of the formulation improvements that were achieved through the use of prodrugs. Doses that had to be administered anywhere from one to three times daily can now be given once or twice monthly.

Table 3: Summary of Dosing Changes with Prodrug Products

<u>Parent Compound</u>	<u>Half-life</u> <u>(hrs)<sup>16</sup></u>	<u>Treatment</u> <u>frequency<sup>16</sup></u>	<u>XR Half-life</u> <u>(days)<sup>16</sup></u>	<u>XR Treatment</u> <u>Frequency<sup>16</sup></u>
Fluphenazine	33	Multiple daily	7-10	2 weeks
Paliperidone	23	Daily	25-50	1 month
Haloperidol	18	Multiple daily	24	3 weeks
Flupentixol	35	Multiple daily	21	2-4 weeks
Testosterone	<2 hours	Variable Multiple daily	<2 hrs	2-4 weeks

## MICROSPHERES

The use of microsphere technology has experienced much growth in the past several years in the area of pharmaceuticals. They are used primarily with underivatized small molecules and peptides. These drugs are typically fairly soluble in water and control release of a therapeutic compound through the entrapment of a molecular dispersion of the compound in a spherical polymer matrix.

When considering the use of microspheres for a controlled release application, it is important to note that polymer selection for the microspheres is limited (compared to other types of excipients) because many new polymers are not approved for pharmaceutical usage and numerous regulatory clearances need to be passed for new excipients for use in drug delivery systems<sup>7</sup>. However, microsphere formulations prove extremely valuable for compounds that do not contain an alcohol functional group, or other easily modifiable group that would facilitate the use of prodrug methodology. This might be an important consideration as well if for some reason the active parent molecule needs to be administered, or no suitable prodrug moiety is discovered.

The sheer number of factors that control the release rate of a compound from the polymer spheres makes the development of a microsphere drug product challenging. For example, the release rates of the microspheres can be differentiated between lower and higher molecular weight polymers<sup>7,26</sup>. Polymers that use lower molecular weights release compounds primarily through degradation of the sphere, while microspheres made from heavier polymers release mostly through diffusion of the drug through the matrix.

If the molecular weights of the polymers are not a suitable way to control the release of the drug from the microsphere, linking or blending of two different polymers can be combined in two ways: through the covalent linking of the two polymers or by blending the two polymers *in situ*. Through the covalent linking of two polymers, a larger and more porous microsphere can be prepared, while blending two polymers will produce harder and smaller microspheres<sup>26</sup>, that may be more desirable for specific applications. Evidence of the impact of this interaction can be seen in Figure 11, where polyglycolic acid (PGA) and polylactic acid (PLA) ratios are varied to show the effect of drug release in a poly-lactic-co-glycolic acid (PLGA) matrix (depicted in Figure 12).

Also important to consider for this type of delivery is the uniformity of drug dispersed in the matrix can also be a factor in the release rate of microspheres, where a uniform dispersion can increase the effect of the initial burst of the compound in the bloodstream<sup>27</sup>. A higher concentration of drug further inside the sphere allows for a slower release by having a smaller amount located on the surface of the sphere where dissolution rates are at the maximum for the particle.

Figure 10: Effect of Varying Copolymer Ratios in Risperidone Microspheres (Lactide:Glycolide)

Courtesy of Michael Palmieri, Jr., Alkermes

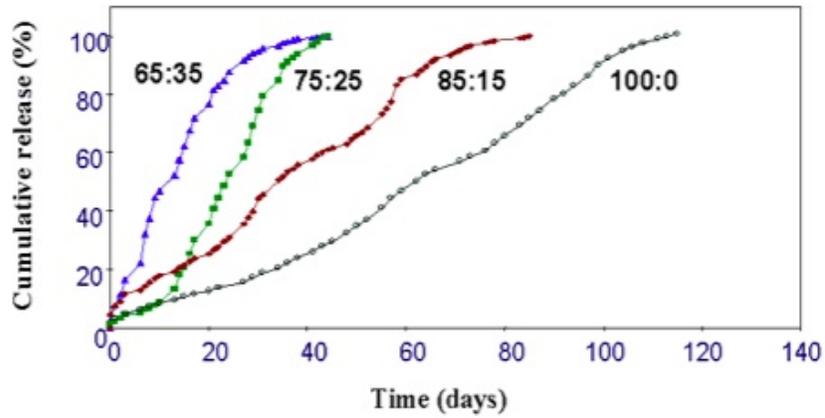
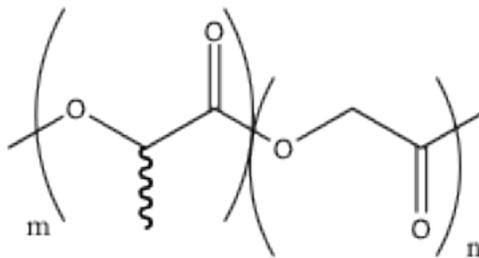


Figure 11: PLGA molecule



One important characteristic for a microsphere is its encapsulation efficiency, which is calculated using the following relationship:

$$E_e = \frac{\Delta D}{D_t}$$

Where  $\Delta D$  is the difference in masses of drug used and recovered and  $D_t$  is the total drug used

Another important calculated attribute for characterizing microspheres is the loading capacity, defined as:

$$C_l = \frac{\Delta D}{M_s}$$

Where  $C_l$  is the loading capacity of the microsphere,

$\Delta D$  is the difference in masses of drug used and recovered and

$M_s$  is the mass of the spheres

Table 4 shows the melting point and glass transition temperatures of some of the most commonly used biodegradable polymers used for pharmaceutical applications. These properties must be factored into the overall properties of the final drug product.

*Table 4: Polymers used in Microsphere Formulations*

<u>Polymer</u>	<u>T<sub>g</sub> (°C)<sup>28,29</sup></u>	<u>Melting Point (°C)<sup>28,29</sup></u>
Poly(lactic acid)	58	163
Poly(glycolic acid)	36	225
Poly-ε-lactone	-60	59

As with all technologies there are of course, are a few caveats in microsphere formulations that must be considered. One is the penchant that microspheres have to be associated with an initial burst release, which in certain cases can release as much as 30% of the total drug load<sup>10</sup>. Another stipulation is that microsphere products generally must be packaged as a dry powder, for the ability of the aqueous vehicle to hydrolyze the polymer's

covalent bonds is a concern for ensuring a product that is shelf stable for a period of months or years. Finally, it is not uncommon for microsphere products to have to be kept at refrigerated temperatures, due to the instability of the polymer matrix at warmer temperatures (observe polyglycolic acid's low glass transition temperature above).

Chemical compatibility of the polymer and the drug may also present a concern, especially when destabilization of the microsphere may occur through nucleophilic degradation, which can be observed with drug molecules that contain a strong nucleophilic functional group<sup>26</sup>. This type of interaction has the potential to increase the release rate of the compound that is being delivered and can be observed with drugs containing amine functionalities, particularly if they are not sterically hindered, as Cha and Pitt have identified<sup>30</sup>.

Currently, the most common microsphere formulations use the polymer PLGA<sup>31</sup> (Figure 13 shows an SEM image of PLGA microspheres), which is a co-polymer comprised of polylactic acid and polyglycolic acid, all of which are illustrated below. The co-polymerization of these two polymers allows developers to combine the desirable properties of each polymer, while minimizing the non-desirable effects. For example, polylactic acid is a hydrophobic polymer that is responsible for the rigid nature of the matrix that forms the microspheres, but by itself it could not adequately interact with a hydrophilic drug compound to deliver a controlled-release product. Polyglycolic acid, conversely is hydrophilic in nature making it a suitable matrix for hydrophilic materials. This compound is hydrolytically unstable, presenting concern should storage in aqueous media need to occur.

Figure 12: SEM Image of Microspheres (courtesy Rajesh Kumar, Alkermes)



Depending on the properties required for the matrix/formulation, there is room to manipulate the ratio of hydrophobic and hydrophilic polymers used in order to yield a custom drug delivery device. The two most common polymers are illustrated in Figures 14 and 15 to and demonstrate the differences in chemical compositions that are utilized for different drug compounds to obtain differing release rates.

Figure 13: Polylactic acid

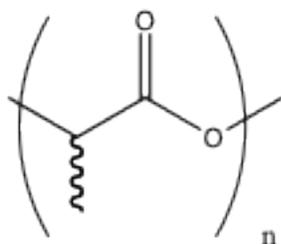
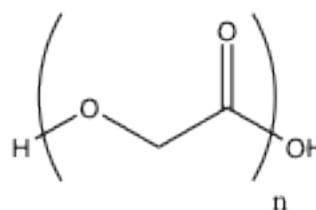


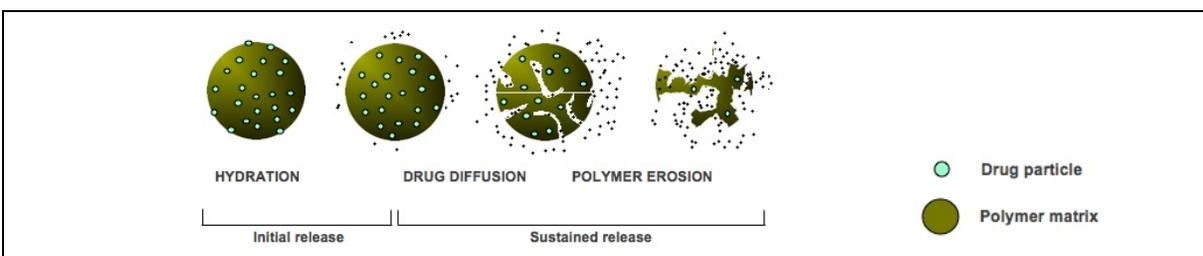
Figure 14: Polyglycolic acid



After these microspheres are administered and begin to release the active drug from their matrix, the erosion of the polymer matrix soon begins. The total release of polymer microspheres occurs over three phases: hydration, diffusion and polymer erosion, depicted in Figure 16. After the drug is solubilized from the outer surface area, the drug from the inner layers begins to diffuse out of the matrix and subsequently is also solubilized. Concurrently, the polymer matrix experiences hydrolytic degradation and begins to physically erode,

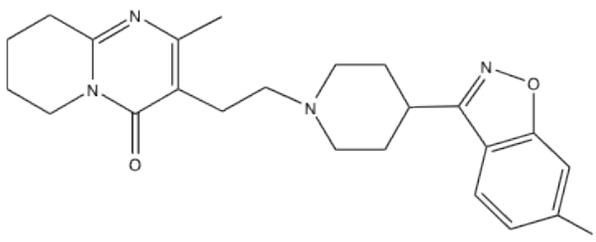
allowing the center layers of the sphere to expel their drug load, until the microsphere is completely degraded and the therapeutic effect is diminished.

Figure 15: Polymer Microsphere Degradation, courtesy Michael Palmieri, Jr., Alkermes



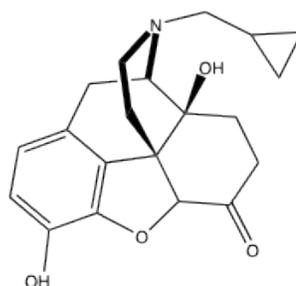
The active ingredient in Risperdal Consta®, risperidone (another atypical antipsychotic) is also used extensively in the management of schizophrenia. The molecular weight of risperidone is 410.5 daltons and the compound is delivered as a suspension of microspheres. Risperidone is encapsulated in this formulation in a 75:25 co-polymer of polylactide-co-glycolide<sup>32</sup>. This compound has limited solubility in water and with its partition coefficient of 3.04<sup>21</sup>, is relatively hydrophilic in nature<sup>33</sup>. The improved dosing with this extended-release formulation allowed patients to decrease dosing from once daily or twice weekly to a once-a-month dose. It is interesting to note that this molecule does not have any available alcohol groups from which ester moieties could be easily derivatized.

Figure 16: Risperidone



Vivitrol®, the extended-release formulation of naltrexone, is an oxymorphone compound that antagonizes the opioid receptor. This compound, marketed by Alkermes and Cephalon, is used to as a treatment to help control alcoholism. The molecular weight of this compound is 341.4 daltons and the naltrexone is delivered via a 75:25 polylactide-co-glycolide (PLG) microsphere suspension<sup>34</sup>. This hydroxy-containing molecule has a lower partition coefficient of 2.07<sup>35</sup>, making it fairly hydrophilic in nature. Naltrexone experiences a half-life of about 4 hours *in vivo*, however this new formulation allows for the traditional once or twice daily dosing to be decreased to once-monthly injections.

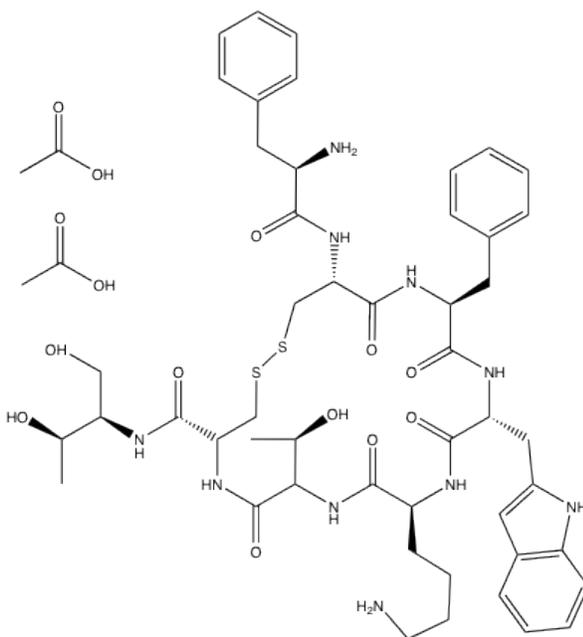
Figure 17: Naltrexone



Marketed by Abbott Laboratories, Lupron® was the first biologic to be approved for a controlled-release formulation<sup>11</sup>. Lupron® is an extended-release product containing leuprolide acetate, a synthetically prepared nonapeptide which is a derivative of gonadotropin-releasing hormone (GnRH). This peptide has a molecular weight of 1269.5 daltons and is the salt of the active oligiopeptide, which has been shown to block gonadotropin hormone secretion. For this purpose, it is used for palliative treatment of prostate cancer<sup>36</sup>. The compound is delivered in a polylactic acid polymer to deliver a 3-month sustained-release injection<sup>36</sup>.

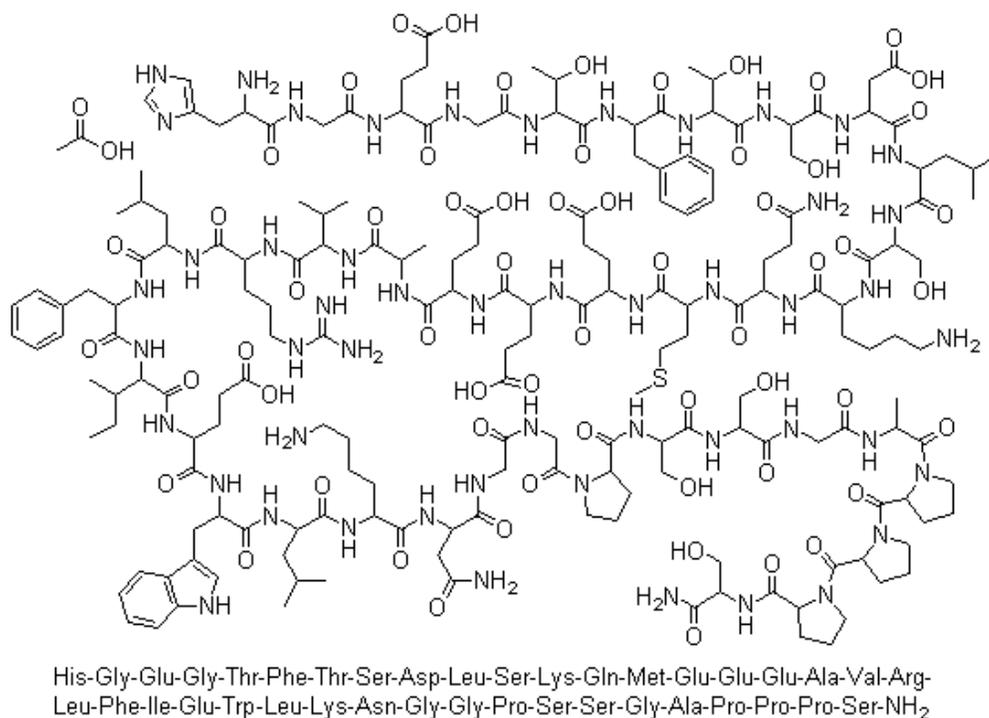


Figure 19: Octreotide Acetate



Exenatide-once weekly is an incretin mimetic that is currently under review by the US FDA. It is a 39-oligopeptide hormone that is indicated for the treatment of diabetes mellitus type 2. This formulation uses a PLGA copolymer in order to slow the release of the peptide, allowing for a once-weekly injection—a considerable improvement over the current twice-daily injection therapy available<sup>38</sup>. The partition coefficient for exenatide is given at  $-21^{21}$ , making this very hydrophilic and water soluble (about 25 mg/mL)<sup>39</sup> and without the use of a physical matrix to control the dissolution of the drug into the bloodstream, would have a very short *in vivo* half-life of 2.4 hours.

Figure 20: Exenatide<sup>40</sup>



As summarized in Table 5, there are a number of different compounds that may be applied to microsphere formulations to achieve sustained-release profiles. Biodegradable polymers can be exploited in order to deliver a dose that is given over a two week period, or for up to six months. This type of technology offers drug formulators much flexibility in designing drug products that best fit with a patient's need.

*Table 5: Summary of Dosing Changes with Microsphere Products*

<u>Parent Compound</u>	<u>Half-life (hrs)<sup>16</sup></u>	<u>Treatment frequency<sup>16</sup></u>	<u>Polymer</u>	<u>XR Half-life<sup>16</sup></u>	<u>XR Treatment frequency<sup>16</sup></u>
Risperidone	20	1-2X Daily	PLG	3-6 days	2 weeks
Naltrexone	4	Daily- 2X Weekly	PLG	5-10 days	4 weeks
Leuprolide Acetate	New drug	New drug	PLA	3 hours	1/3/4/6 month
Octreotide Acetate	6-12	3 x daily	PLGA	2 hours	4 weeks
Exenatide	2.4	2X Daily	PLGA	2.4 hours	1 week

## DIFFUSION

### *Depots*

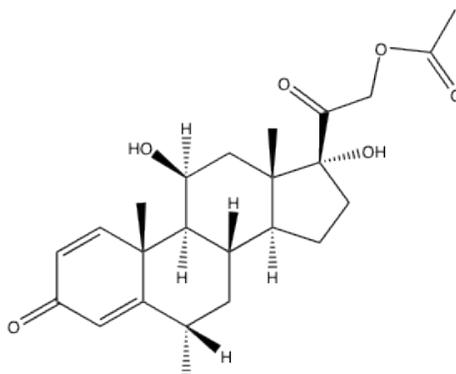
The deposit of active compound, usually administered intra-gluteally, is the simplest way to deliver an extended-release dose of drug. This delivery system, however, is not always feasible for many reasons. Compounds that can get absorbed into the bloodstream easily pose a significant threat to delivering a burst dose when lacking a system to control the release. The main mechanism of action is the diffusion of the drug from the depot in the tissue and into the aqueous bloodstream. Without the inclusion of a delivery or control system, the efficacy and practicality of this release method depends heavily on the compound's partition coefficient and the half-life of the compound.

Microcrystalline particles, commonly used with depot injections of an active drug, help to control the release of the drug to the surrounding tissue. Depending on the size of particles used, dissolution rates may increase (for smaller particles), or decrease (with larger particles). Release may also be controlled in this situation through the size of the injection volume. Smaller injection volumes have been shown to absorb more quickly, while larger volumes minimize surface area to volume, allowing the drug to slowly diffuse out of the depot<sup>5</sup>. Compounds that are mostly insoluble in aqueous media may be able to rely on this simple release mechanism, without the need for another release modifier such as prodrugs or microspheres.

Depo-Medrol® is a formulation of methylprednisolone acetate, which is a common anti-inflammatory glucocorticoid that may be used for a number of indications as an anti-

inflammatory and as an immunosuppressive<sup>41</sup>. Methylprednisolone acetate has a mass of 416.5 daltons and is practically insoluble in water, with a relatively hydrophilic partition coefficient of 2.7<sup>21</sup>. This formulation, marketed by Pfizer, possesses a half-life of 12-17 hours and only requires dosing every one to two weeks, compared to the one to multiple times a day needed for the immediate release product. This sustained-release was achieved by balancing the solubility of this compound with polyethylene glycol (molecular weight of about 3500 daltons). Polyethylene glycol, through the use of differing molecular weight polymers, can help slow the release of compounds into water for products such as these<sup>42</sup>.

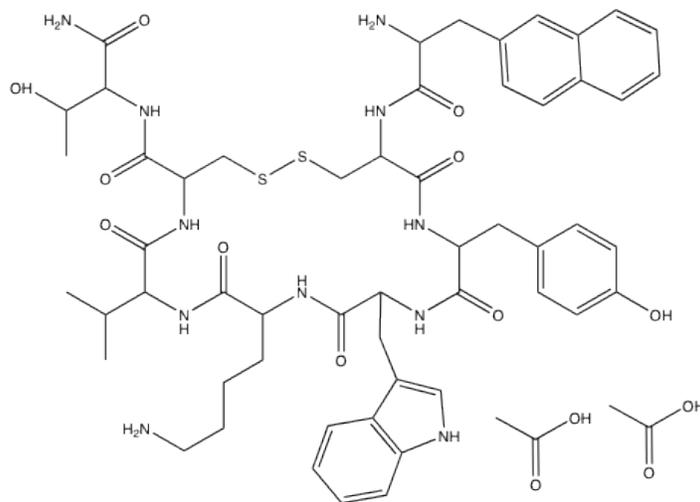
*Figure 21: Medroxyprogesterone Acetate*



Somatulin®, marketed by Ipsen Pharmaceuticals, is the acetate salt of a somastatin analogue (lanreotide). Lanreotide acetate has a molecular weight of 1096.3 daltons for the free base of the octapeptide<sup>43</sup>. Similar to octreotide, this water-soluble analogue is also cyclized at the two cysteine residues through a disulfide bond and is also used for the treatment of acromegaly<sup>43</sup>. This compound, possessing a half-life of about 3 hours, is delivered once monthly as a supersaturated solution in water that forms a gel matrix upon injection into the body. Based on the physical properties of the peptide at physiological

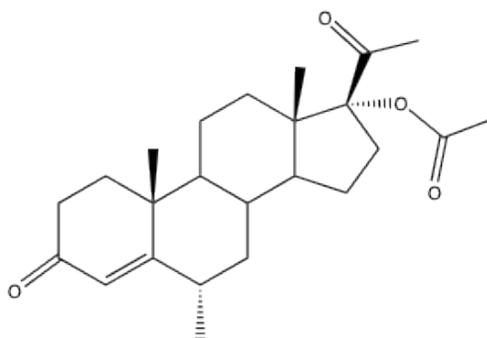
temperature, the compound creates its own semisolid matrix from which the release is retarded through entrapment in its own matrix.

Figure 22: Lanreotide Acetate



Depo-Provera®, developed by Pfizer, is an injection for contraceptive use and is formulated with medroxyprogesterone acetate. This compound, which is the acetate derivative of the common progesterone, has a molecular weight of 386.5 daltons and a log P value of about 3.8<sup>44, 45</sup>. Medroxyprogesterone acetate is insoluble in water and shows an *in vivo* half-life of about 50 days, making it amenable to an extended-release dosage<sup>45</sup>. Depo-Provera® is formulated to be administered once every three months rather than the traditional once-daily oral contraceptive. It is also formulated with polyethylene glycol of molecular weight 3350 daltons in order to help slow the release the hormone, again utilizing the controlled release properties of polyethylene glycol.

Figure 23: Medroxyprogesterone Acetate



The information shown in Table 6 summarizes the depot formulations highlighted above and shows the decrease in treatments from once or more daily to once weekly (for some indications), up to three months. Lanreotide and Medroxyprogesterone were new chemical entities developed and did not have directly comparable immediate-release products.

### *Salts*

Salt formation of a compound is the most straightforward and commonly used technique used to improve the solubility of insoluble compounds<sup>5,46</sup>. The use of preparing salt drug conjugates can be incredibly useful for compounds possessing ionizable functional groups such as amines for active cations drug or acidic groups, creating an active anion<sup>46</sup>.

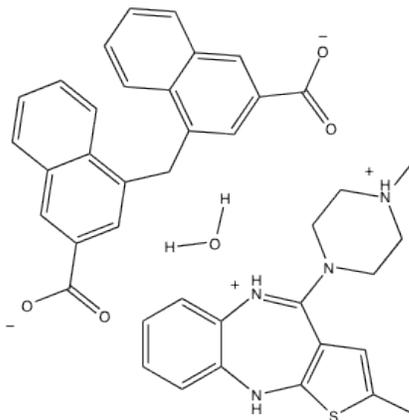
Insoluble salt forms can serve as a relatively simple way in which a drug compound can be modified to yield a sustained release profile. For parenteral products, the drug salt is typically prepared as a microcrystalline suspension. Common anion counterions (the cation usually being the active drug) include acetate and sodium phosphate<sup>5</sup>. Utilization of insoluble

salt complexes to control drug release generally alleviates some of the toxicological concerns that can be associated with derivatizing the drug and can make the assessment of the compound's pharmacokinetic data much easier<sup>5</sup>.

The aqueous solubility of an acidic or basic compound can be described by a function of the compound's pH, which can serve as an indicator of whether the formation of suitable salt derivatives can occur<sup>46</sup>. Controlling the release of a compound is dependent on the solubility profile of the desired compound. During the 1980s, a solubility of less than 20  $\mu\text{g/mL}$  was incomprehensible to formulators, and now many new drug compounds have an aqueous solubility of less than 1  $\mu\text{g/mL}$ <sup>46</sup>. This trend, should it continue, will leave formulators looking for ways to increase the solubility and improving the release of the lead compounds instead of the current trend of controlling release.

Zyprexa Relprevv®, marketed by Eli Lilly is a dry powder of olanzapine pamoate monohydrate. Olanzapine is classified as a thienobenzodiazepine, another potent antipsychotic, indicated for patients diagnosed with Bipolar I disorder or with schizophrenia<sup>46</sup>. Like many of the new atypical antipsychotics, olanzapine exhibits both setoronic and dopaminergic affinity<sup>48,49</sup>. This compound has a relatively low partition coefficient for extended-release compounds, 2.23<sup>21</sup>; however this salt, weighing 718.8 daltons, is practically insoluble in water<sup>47,49</sup>. With this formulation, the dosing frequency was decreased from once daily to once every two or four weeks.

Figure 24: Olanzapine Pamoate Monohydrate

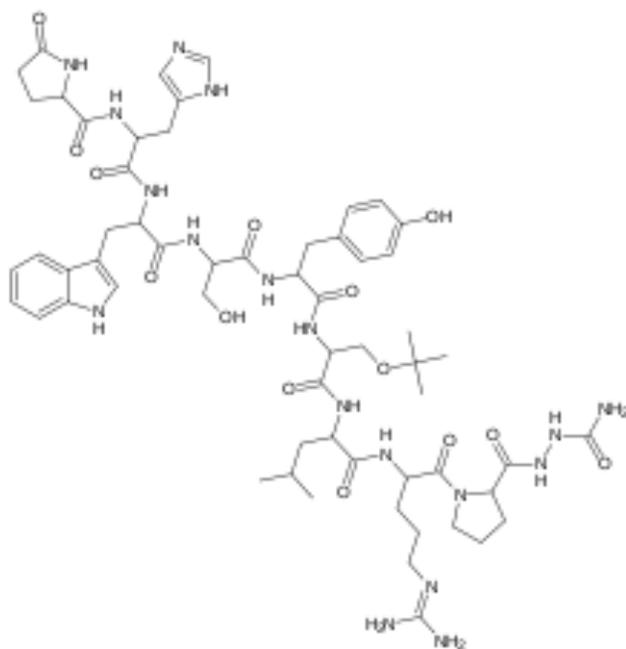


### *Injectable Implants*

With the advent of biodegradable polymers, injectable implants have received attention recently for the unique formulation advantages<sup>11</sup>. The injection of a polymer drug-delivery implant has many of the advantages of a traditional implant, without the need for surgery pre and post-administration<sup>50</sup>. With polymer injectable implants, the polymer is injected with a biocompatible, water miscible solvent that diffuses away from the immediate injection area, allowing the polymer to precipitate as a biodegradable implant<sup>50</sup>. The release of a drug compound from the device is not unlike the mechanisms observed for microsphere formulations, as it controls the release of the drug from the diffusion of the compound from the implant. Also similar to microsphere, the aqueous environment surrounding the implant degrades the polymer matrix over time, further releasing the drug in order to deliver a sustained-release of the drug that is impregnated in the device<sup>50</sup>.

Zoladex®, marketed by AstraZeneca, is goserelin acetate for subcutaneous injection formulated with a *D,L*-polylactic-co-glycolic acid polymer implant that is injected below the navel of the patient<sup>51</sup>. The active moiety, goserelin acetate, pictured below, is a synthetic decapeptide that is an analogue of luteinizing hormone releasing hormone<sup>51</sup>. This drug is primarily used to treat hormone-dependent breast and prostate cancers, but is indicated for reproductive assistance, gynecological conditions and for the treatment of precocious puberty. Available as a one or three month depot formulation, this compound has a half-life of about 4 hours and is water-soluble, demonstrating the efficacy of polymer implant in controlling the release of the drug.

Figure 25: Goserelin Acetate



*Table 6: Summary of Dosing Changes with Diffusion Products*

<u>Parent Compound</u>	<u>Half-life<sup>16</sup></u>	<u>Treatment frequency<sup>16</sup></u>	<u>XR Half-life<sup>16</sup></u>	<u>XR Treatment frequency<sup>16</sup></u>
Lanreotide Acetate	New drug	New Drug	23 days	Once monthly
Medroxyprogesterone Acetate	-	Various hormones: Once daily	50 days	3 months
Methylprednisolone Acetate	3 hours	Varied- once or more daily	3 hours	1-5 weeks
Olanzapine	21-54 hours	once daily	30 days	2 or 4 weeks
Goserelin Acetate	New drug	New drug	4 hours	3 months

## DISCUSSION

### *Preventing Burst Release*

One of the harder attributes to control with sustained-release dosages is the prevention of the “burst”, or initial release of the compound. Many current extended-release products illustrate the real challenge that is faced in this respect, for some formulations report as high as a 60% initial release *in vivo*<sup>27</sup>. Often times, it is after this initial burst that the controlled release of the drug is observed. Even if this burst concentration lies in the therapeutic window (between the therapeutic dose and a dose that approaches a toxic level), the addition of extraneous drug to the system without contributing to the therapy of the patient is not only economically ineffective, but has been associated with a negative impact the lifetime of the release system, requiring a more frequent dosing for the patient<sup>27</sup>.

There are many proposed causes and subsequent mechanisms that have been proposed and concisely summarized in 2001<sup>27</sup>. Specifically, the authors cite five main factors influencing the burst phase:

- a) Processing conditions
- b) Surface characteristics of host material
- c) Sample geometry
- d) Surface adsorption between host and drug
- e) Morphology and porous structures of dried sample

One easy and seemingly effective way to reduce the amount of excess surface drug on the polymers is to wash the samples in a suitable solvent. While this removes the drug product, it reduces the burst effect that may be seen in patients. Another viable option would be to contain the free surface drug through coating of the particles. The use of coated microspheres is beginning to appear in the pharmaceutical literature<sup>52, 53</sup>.

Uniformity of the matrix used to control the release of the compound has also been shown to have a measurable impact on the product release. Two of the main reasons outlined by Huang and Brazel<sup>27</sup> were that surface imperfections (such as cracks and pores), and heterogeneous drug dispersion concentrated the drug load in the first few layers of the microsphere as compared to the inner layers. Heterogeneous drug dispersion was caused by increased drying of surface polymers compared to the inner layers of the polymer particles.

### *Selection of Injection Vehicle*

Although selection and development of the solid drug particles are perhaps the most important facets of developing a suspension-based injectable product, the development of a compatible injection vehicle for suspension or solution needs to be carefully considered and methodically developed as well. For the suspension of solid particles, the use of viscosity-modifying agents are commonly called upon, most noticeably sodium carboxymethylcellulose. In order to facilitate the manufacturing of a drug suspension, the viscosity of any pre-mixed suspension must be high enough to ensure minimal drug loss during transfers and other processes. In addition to this consideration, the polymer sodium carboxymethylcellulose and others (such as polyvinylpyrrolidone) act as stabilizers in the

suspension, physically preventing the drug particles from coming in contact with each other and consequently agglomerating together.

Preventing agglomeration in injectable products is a key priority that is heavily investigated in the development process of suspension-based products. Despite its main use as a wetting agent, surfactants, to a smaller extent, can further increase a stabilizing effect and allow the drug particles to be readily dispersed into the drug vehicle. The final drug product must have particles that can be easily dispersed, because an excess of agglomerated or flocculated particles can increase the likelihood of injection failure (ie, needle clog). Many formulations require the administrator of the injection to either vigorously tap or shake the suspension to ensure that all the particles are dispersed, ensuring accurate dosing through the minimization of caked particles to the walls of a vial or syringe, and to break up any clog-inducing agglomerates.

Oil solutions have the ability of offering different characteristics as another option for a delivery vehicle. The release of the drug from the oil deposit is a common way in which extended release of a drug is achieved. This phenomenon is mainly described by the compounds' partition coefficient, as compounds must partition itself from the oil before it may be absorbed into the bloodstream. In Table 7, some of the most commonly used oils for injection are highlighted, along with considerations that must be realized for their storage, since many can become rancid if exposed to air, high temperatures and/or excess light.

Table 7: Oils used for parenteral injection vehicles <sup>54</sup>:

<u>Oil</u>	<u>Solidification Point</u>	<u>Viscosity</u>	<u>Storage Considerations</u>
Sesame oil	-5 °C	43 cps	<40 °C, protect from light
Vegetable oil	-	Variable	Protect from light
Cottonseed oil	0-5 °C	39 cps	<40 °C, protect from light
Ethyl oleate	-32 °C	5 cps	Protect from light

When utilizing an oily vehicle for injection, it is critical to ensure the correct placement of the injection as it forms an oily depot entering the tissue<sup>54</sup>. Both the injection site and the depth are key elements in the efficacy of the delivered dose. Injections must be delivered deep into the muscle tissue, away from fat deposits and major nerve centers. Also important to note are the different blood flows that are observed in the two most common IM areas: the gluteus and the deltoid possess distinctively different rates (9.6 ml/100 g/min) and (11.6 ml/100 g/min), respectively<sup>5</sup>. With this rate of blood flow in mind, it appears obvious to administer the injection to the muscle that receives the less blood flow, ensuring a slower uptake into the bloodstream, allowing for an extended rate of release, and is the ideology behind the administration of many extended-release depots in the gluteus tissue.

#### *Addition of Excipients*

Excipient addition to a drug product allows the developers to achieve control over how the drug compound is released, how it interacts with the biological media it encounters, and

how the drug product is manufactured and delivered, among other parameters. Additionally, excipients can enhance the bioavailability, pharmacokinetics and pharmacodynamics of the active compound through adjustment of the active's solubility<sup>55</sup>. For the use in suspensions, excipients allow the stabilization of the inherently metastable formulation<sup>56</sup>. The use of surfactants for instance, may not only increase the rate at which a dry powder is wetted by the vehicle, but can alter the solubility of the drug through physical interactions *in vivo*<sup>56</sup>.

There is a growing trend to develop more specialized excipients that are tailored to the drug product rather than the traditional roles of excipients seen for solid oral dosages. Development and acceptance of new technologies such as coated nanoparticles<sup>57</sup>, virus vectors and siRNA are new concepts that are beginning to be explored for the targeted delivery of new therapeutics<sup>58</sup>.

### *Formulating a Reasonable Injection Product*

Through the use of prodrugs, microspheres, depots and implants as release modifiers, many different types of compounds have been effectively formulated into extended-release products. The development of a drug product is usually completed through the thorough targeted design goals that are outlined early in the development process. Regardless of the final product objectives, there are a number of characteristic that can be summarized to be desirable for all sustained-release injection products.

The minimization of the total injection volume may be seen as one of the more important considerations for this type of drug product. Generally, the injection volume should be no more than 5 mL unless necessary or site reactions that may be associated with a larger

injection volume are minimized. The formation of a semi-solid to solid mass at the site of administration is not uncommon for extended release injectable therapeutics and should be minimized if at all possible to help ensure patient compliance and lower the possibilities of localized site reactions.

There are a number of considerations that must be made when developing a new drug product for injection. First and foremost, it is important to consider what the desired route of administration should be. The most common injection routes for parenteral extended release products are intramuscular and subcutaneous. Intramuscular injections are primarily delivered at the gluteal or deltoid muscle. Some formulations may be approved for administration at either area. There are a few differences that may push for one location over the other. For example a gluteal administration can accommodate a larger injection volume, yet a deltoid injection allows for a smaller needle size, but may present restrictions for the volume size and the particle size of the suspension if used.

Although intramuscular injections are a common route, and offer sites that are very rich in vasculature, they are not the only option. Subcutaneous injections offer more injection site options such as the upper arm, the waist and thigh, to name a few. Recall that Zoladex® is administered in the front of the abdomen, below the navel.

### *Biologics*

There is an increase in the developmental pipeline for biologic compounds for the treatment of a variety of disease areas. As highlighted above, the ability to control solubility with chemical modifications is much more limited with larger macromolecules. Because of

this complication, much effort is dedicated to prolonging the inherent half-life of these compounds. This can be achieved by linking them to biological moieties that have established long half-lives such as the F<sub>c</sub> portion of an antibody. Recently, the concept of protein engineering is also receiving much attention for the possibility of custom designing therapeutics that inherently display desired profile, meaning minimum formulation development. Point mutation of peptides for optimization of physicochemical properties, such as those highlighted with Somatuline®, offer ways to simplify the formulation to achieve a self-sustained controlled release.

Some of the new considerations that formulators must be aware of for future biologic drug products should include the secondary, tertiary, and possibly quaternary structures of the compound, which may present complications concerning physicochemical properties and long-term storage/stability conditions<sup>59</sup>. Equally important to note is that the dosage of many biologics must be titrated or frequently adjusted with patients (as with diabetes) and depend on multiple, sometimes highly variable factors such as daily food consumption, exercise, and/or lifestyle changes. Furthermore, disease states that are controlled with biologics have a tendency to progress over time and may see variable day-to-day or week-to-week changes, or respond to environmental factors that might require an adjustment in dosing regimen (diabetes, colitis, HIV) that is not easily achieved with an extended release dose.

### *Customizing Release Rates*

As illustrated above, controlling the release of a compound can be attained through three main ways: through the balance of physiochemical properties in order to slow absorption *in vivo*; to increase the half-life (clearance time) of the molecule by structural and/or chemical modification; or through the use of a physical matrix entrapment of a compound.

One effective way to maximize the physiochemical properties of a lead drug compound to give an extended-release profile is to select an appropriate vehicle for injection so that the diffusion of drug from vehicle to the bloodstream is delayed for a suitable time. Other solutions that may be considered are the use of microspheres, or by perhaps manipulating the colligative properties, for instance if a compound is capable of self-gelling at a specified concentration and temperature. Creative use of inherent properties of drug compounds allows the developer flexibility in developing a truly novel delivery system.

In order to increase the half-life of a compound *in vivo*, common trends observed across the compounds highlighted earlier appear to be the inclusion of a fluorine-containing substituent for drugs affecting the central nervous system, and increasing the lipophilicity (and rate of hydrolysis) with an ester prodrug group. For hydrophilic compounds with an aqueous solubility that is above the desired release rate, the use of a physically entrapping matrix is effectively used. This type of release mechanism would not be immediately suitable for use with a compound that has a low solubility because the dual release mechanism could significantly retard the diffusion release of the drug; this may prove useful if a compound needs to be released at very small dosages.

One of the major problems in formulating a new compound is to improve the solubility of the generally lipophilic molecules used for pharmaceutical active ingredients. A

unique solution to this problem is to reduce the particle size of the drug to the nanometer range, which increases the surface area to volume ratio of the particles, thus enhancing the solubility by surface interactions alone.

Perhaps one of the newer extended release prodrugs was approved in the US in July of 2009, Invega Sustanna® (paliperidone palmitate), which utilizes a nanosuspension in order to address the poor solubility of the highly hydrophobic paliperidone palmitate.

Nanosuspensions present a unique alternative because of their ability to increase the dissolution rate of a compound, due to the increased surface area of the smaller particles. Furthermore, the use of nanoparticles has been shown to decrease the inter- and intra-subject variability of patients<sup>60</sup>, allowing researchers to better understand the effects of the drug compound.

By implementing nano-sized drug particles, drugs that otherwise show poor biodistribution and weak pharmacokinetics may see improved profiles of both these characteristics<sup>6</sup>. Additionally, hydrophobic or otherwise "poorly soluble drugs" compounds, which make up approximately 1/3 of all compounds accepted by the United States Pharmacopeia<sup>61</sup> may be solubilized, thus making them amenable for parenteral (or other) administration.

Nanoparticles also have the potential to bypass biological barriers such as improved uptake across the blood-brain barrier, the highly selective barrier that many drug compounds are unable to cross<sup>6</sup>. There have been several reports of coated nanoparticles crossing the blood brain barrier in therapeutic levels<sup>62, 63</sup>. By directing the particles/drug carriers, toxicity can be localized to the desired site and systemic exposure can be minimized.

As highlighted above, it seems as though fluorine-containing compounds are a trend for this class. The addition of fluorine to a compound is known to enhance bioavailability, perhaps through the neutralization of local amine groups on the molecule<sup>64</sup>. Müller has also suggested that substituting fluorine in place of hydrogen increases the lipophilicity of a compound<sup>64</sup>, which may attenuate other biological and pharmacodynamic properties. In addition, fluorine enhances the metabolic stability of compounds by reducing the susceptibility of nearby substituents to the enzyme cytochrome P450, which is responsible for much of the metabolism of drug compounds<sup>64</sup>.

For any one drug candidate, there may be any number of polymorphisms associated with that compound. It is equally important to investigate all possible polymorphs of the compound for identification of physical properties. The difference between polymorphs may not influence their chemical or biologic properties, but it might make the difference between a drug product that is stable on the shelf at room temperature for five years versus a product that loses its crystalline quality after one hour's time in a hot car ride back from the pharmacy.

When developing drug candidates for immediate release or for extended-release, modifying the solubility of the lead compound is the most important consideration. For extended-release development, having a low solubility and dissolution profile is desirable, because this extends the release of the compound over a longer time frame. In order to achieve this, cleavable hydrophobic linkers are attached to the molecule; physical matrices are employed to trap the compound *in vivo*; physiochemical properties are exploited to take advantage of thermosensitive self-gellation; or large micron-sized particles are prepared to control the surface area-to-volume ratio. All of these affect the dissolution rate of the active compound and are used to direct the release of the compound towards a desired target.

It might be the case that a compound has a solubility that is so low it needs to be increased in order to achieve a therapeutic dose for extended-release application; or that an initial release needs to accompany the sustained-release characteristic. For situations such as these, there are a number of ways to increase the solubility of a drug. Perhaps one of the most fundamental ways to achieve this for a suspension would be utilize smaller drug particles (nanospheres or nanoparticles) to achieve a higher dissolution rate or to prepare a salt form, if possible. Yet another option could be to chemically modify the molecule with a cleavable hydrophilic group. There are numerous tools that researchers currently possess to help them in their quest to either solubilize or retard the dissolution of any number of potential therapeutics.

If the prospect of modifying the aqueous solubility is not a feasible option, improving the half-life of the drug is another feasible option. If the drug compound is able to circulate in the system without being consumed immediately, the aqueous solubility may become less important for the sustained release, although still an important consideration nonetheless. Currently, a popular option to improve half-life times is to link a therapeutic to an antibody conjugate (usually the  $F_c$  domain), which prevents the drug from being metabolized immediately and can prolong the treatment time of each dose.

Compounds that immediately lend themselves to an extended release form of dosing may be summarized to have the following properties: low solubility (on the order of 1-10  $\mu\text{g/mL}$ ) and a moderate partition coefficient, as well as a half-life that balances these two properties, ensuring that the compound is not quickly eliminated from the body. Since it is nearly impossible to design or find a drug candidate that is effective at low doses, is selective for its target, has a long half-life, possesses an acceptable side effect profile, and is easy to

formulate, modification of solubility and dissolution rates are almost always necessitated—taking into account that more emphasis must be placed on the efficacy of the molecule than its apparent formulation properties.

For biologics with molecular weights greatly exceeding 500 daltons, esterification (or other chemical modification) may not have an appreciable effect on the physiochemical properties and might not influence the solubility of the drug compound, in which case, other options must be explored. Furthermore, most of the processes highlighted that are used to control the release of smaller compounds often times present as too harsh of conditions for the delicately folded and chemically reactive protein<sup>65</sup>. Situations such as represent further and future challenges to drug developers. The necessity for novel technologies to effectively deliver these types of therapeutics for even a once-daily oral formulation therefore is very great.

## CONCLUSIONS

Based on the current trend discussed above, it would seem that many insoluble drug compounds even twenty years ago thought not “drugable” are now being brought to market. In the past, there was a written set of guidelines, famously outlined by Lipinski in 1990<sup>66</sup>, which gauged whether a drug could become a viable drug candidate. Newer drug candidates are going to be more biologically derived, in the form of proteins and peptides, thus having high aqueous solubility. In order to controllably deliver highly soluble compounds, the use of lipophilic prodrug linkers and physical entrapment in polymer matrices will become increasingly important methods, for both parenteral and oral dosage forms.

Conversely, more lipophilic and hydrophobic compounds will be developed, as an increased effort into the solubilization of these types of molecules is being witnessed. If the current trend of decreasing aqueous solubility of approved drug compounds continues, there will be the urgent need for better tools to help improve the solubility of related compounds and to increase the dissolution of those drugs into biological media.

Despite future research on the solubilization of even completely insoluble drug compounds, there are biological limits on the practicality of such highly insoluble drugs that will surely remain. Especially for extended-release applications, there are a number of crucial factors including considerations such as the half-life of drug (it must be long enough to permit realistic dosages) and the ability of the drug to be released from the delivery system at a rate that provides a therapeutic dosage of drug over desired time of action.

The formulation of new chemical entities is a field that will continue to experience growth, and perhaps to greater extent, the re-formulation of current new chemical entities will also see renewed attention. As patients begin to demand pharmaceuticals that allow more flexibility in the dosing, the market for multiple daily oral dosing and multiple daily injections will, without a doubt, begin to recede. Better formulations, armed with the preclinical and first-generation formulation knowledge will allow for re-formulations that are better suited to the patient's lifestyle and can make administering therapies for chronic conditions much easier. Since, extended-release formulations' overall goal is to optimize the pharmacokinetic properties and profile while minimizing toxicity/side effects. This will be greatly emphasized when a simple variation in one's day does not have to mean the difference between changes in a patient's pharmacokinetic profile, and is inherently more effective at managing chronic conditions.

## REFERENCES

1. Manmode, A.S.; Sakarkar, D.M.; Mahajan, N.M. Nanoparticles-Tremendous Therapeutic Potential: A Review. *Int. J. PharmTech Res.* **2009**. 1, 1020-1027.
2. Landgraf, W.; Li, N.; Benson, J.R. Polymer Microcarrier Exhibiting Zero-Order Release. *Drug Delivery Technol.* **2003**, 3, 1-12.
3. Ereshefsky, L.; Mannaert, E. Pharmacokinetic Profile and Clinical Efficacy of Long-Acting Risperidone Potential Benefits of Combining an Atypical Antipsychotic and a New Delivery System. *Drugs R D.* **2006**. 6, 129-137.
4. Lister, S.; Sorensen, S.M.; Hitchcock, J.M.; Wettstein, J.G. Effects of Drug Depot Formulations in Procedures Used to Evaluate Antipsychotic Activity in Rodents. *Drug Dev. Res.* **1999**. 47, 27-36.
5. Senior, J.H. Rationale for Sustained-Release Injectable Products. In *Sustained-Release Injectable Products*. Senior, J.H. Ed.; CRC Press: Boca Raton, FL, 2000; pp 1-4, 41-47, 66-67.
6. Mishra, B.; Patel, B.B.; BPharm; Tiwari, S.; MPharm. Colloidal Nanocarriers: A Review on Formulation Technology, Types and Applications Towards Targeted Drug Delivery. *Nanomedicine.* **2010**, 6, 9-24.

7. Park, K.M.; Mersny, R.J. Controlled Drug Delivery: Present and Future. In *Controlled Drug Delivery: Designing Technologies for the Future*. Series 752; American Chemical Society: Washington, DC, 2000; pp 2-3.
8. Degen, L.P.; Phillips, S.F. Variability of gastrointestinal transit in healthy women and men. *Gut*. **1996**. 39, 299-305.
9. Muller, R.H.; Keck, C.M. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. *J. Biotechnol.* **2004**. 113, 151-170.
10. Shah, J.C. Controlled Release-Small Molecules. In *Prodrugs: Challenges and Rewards*. Stella, V.J.; Borchardt, R.T.; Hagerman, M.J.; Oliyai, R.; Maag, H.; Tilley, J.W. Eds.; AAPS Press: New York, 2007; Part 1, pp. 357-377.
11. Langer, R. New methods of drug delivery. *Science*. **1990**. 249, 1527-1533.
12. Granero, G.E.; Ramachandran, C.; Amidon, G.L. Drug Dissolution and Solubility. In *Drug Bioavailability: Estimations of Solubility, Permeability, Absorption and Bioavailability*. van de Waterbeemd, H.; Lennernäs, H. Artusson, P., Eds. Wiley: Germany, 2003; p. 192.

13. Gibaldi, M.; Perrier, D. *Pharmacokinetics*; Drugs and the Pharmaceutical Sciences; Marcel Dekker: New York, 1982; Vol. 15, pp. 287-288.

14. Kwon, Younngil. Nonlinear Kinetics. In *Handbook of Essential Pharmacokinetics, Pharmacodynamics, and Drug Metabolism for Industrial Scientists*. Kwon, Y. Klower Academic. New York, 2001. pp. 178-184.

15. FDA Orange Book: Approved Drugs with Therapeutic Equivalent Evaluations.

[http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Search\\_Drug\\_Name](http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Search_Drug_Name) (accessed April 14, 2010).

16. Drug Information Handbook for Nursing. Turkoski, B.B.; Lance, B.R.; Tomsik, E.A., Eds. 11<sup>th</sup> ed. Lexi-Comp: Canada, 2009.

17. Hemenway, J., Stella, V.J. Prodrugs and Parenteral Delivery Systems. In *Prodrugs: Challenges and Rewards*. Stella, V.J.; Borchardt, R.T.; Hagerman, M.J.; Oliyai, R.; Maag, H.; Tilley, J.W; AAPS Press: New York, 2007; Part 1, pp. 218-281.

18. Bruice, P.Y. Carbonyl Compounds. In *Organic Chemistry*, 5<sup>th</sup> ed.; Pearson Prentice Hall: Upper Saddle River, NJ, 2007; p 757.

19. Anderson, B.D. Prodrug Approaches for Drug Delivery to the Brain. In *Prodrugs: Challenges and Rewards*. Stella, V.J.; Borchardt, R.T.; Hagerman, M.J.; Oliyai, R.; Maag, H.; Tilley, J.W, Eds.; AAPS Press: New York, 2007; Part 1, p. 579.
  
20. Fluphenazine Decanoate Injection Package Insert: Bedford Laboratories: Bedford, OH, November 1998; FLZP01.
  
21. PubChem Compound Database. <http://pubchem.ncbi.nlm.nih.gov/> (accessed March 23-April 25, 2010).
  
22. Fluanxol® Depot Product Monograph: Lundbeck Canada Inc.: Montreal, Quebec, June 2007; Control No. 11375 and 111258.
  
23. Invega Sustenna® Full Prescribing Information. Othro-McNeil-Janssen Pharmaceuticals: Titusville, NJ, March 2010; No. 976781.
  
24. Haloperidol Decanoate Injection Package Insert: Bedford Laboratories, Bedford, OH, August 2009; HAL-P07.
  
25. Depo-Testosterone® Package Insert: Pharmcia & Upjohn Company, Subsidiary of Pharmacia Corporation: Kalamazoo, MI, August 2002; 811 020 214.

26. Freiberg, S.; Xhu, X.X. Polymer microspheres for controlled drug release. *Int. J. Pharm.* **2004.** 282, 1-18.
27. Huang, X.; Brazel, C.S. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *J. Controlled Release.* **2001.** 2, 121-136.
28. Ajioka, M.; Enomoto, M.; Suzuki, K.; Yamaguchi, A. The Basic Properties of Poly(lactic acid) Produced by Direct Condensation Polymerization of Lactic Acid. *J. of Environ. Polym. Degrad.* **1995.** 3, 225-234.
29. Gunatillake, P.A.; Adhikari, R. Biodegradable Synthetic Polymers for Tissue Engineering. *Eur. Cells Mater.* **2003.** 5, 1-16.
30. Cha, Y.; Pitt, C.G.; A one-week subdermal delivery system for L-methadone based on biodegradable microcapsules. *J. Controlled Release.* **1988.** 8, 259-265.
31. Betancourt, T.; Doiron, A.; Homan, K.A.; Brannon-Peppas, L. Controlled Release and Nanotechnology. In *Nanotechnology in Drug Delivery*; Biotechnology: Pharmaceutical Aspects; AAPS: New York, 2009; p. 285.
32. Risperdal Consta® Highlights of Prescribing Information: Ortho-McNeil-Janssen Pharmaceuticals, Titusville, NJ, October 2009; No. 10130504.

33. Mannens G.; Meuldermans, W.; Snoeck, E.; Heykants, J. Plasma protein binding of risperidone and its distribution in blood. *Psychopharmacology*. **1994**. 114, 566 – 572.
34. Vivitrol® Package Insert: Cephalon, Inc., Frazer, PA, April 2006. No. 1856
35. Kuzma, P.; Quant, H.; Kuo, S. Implantable Device for the Delivery of Naltrexone and Methods of Use Thereof. U.S. Patent 12/351617. August 20, 2009.
36. Lupron Depot® 3 month 22.5 mg Package Insert: TAP Pharmaceuticals, Lake Forest, IL, December 2007; No. 3346, 03-A065-R14.
37. Sandostatin LAR® Depot Highlights of Prescribing Information: Novartis Pharmaceuticals Co., East Hanover, NJ, January 2010; T2010-23.
38. Druker, D.J.; Buse, J.B.; Taylor, K.; Kendall, D.M.; Trautman, M.; Zhuang, D.; Porter, L. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomized, open label, non-inferiority study. **2008**. *Lancet*. 372, 1240- 1250.
39. Exenatide; MSDS No. EXEND [Online]; Mallinckrodt: St. Louis, MI, November 02, 2007. [http://bulkpharm.mallinckrodt.com/\\_attachments/msds/EXEND.htm](http://bulkpharm.mallinckrodt.com/_attachments/msds/EXEND.htm) (accessed April 02, 2010).

40. Manus Aktteva, API Sourcing.  
<http://www.manusaktteva.in/Details/1/128/View/Exenatide.html>. (Accessed April 19, 2010)
41. Depo-Medrol® Package Insert: Pharmacia & Upjohn Company, Division of Pfizer Inc.:  
New York, July 2009; LAB-0160-5.0.
42. Rowe, R.C.; Sheskey, P.J.; Owen, S.C., Eds. Handbook of Pharmaceutical Excipients. 5<sup>th</sup>  
ed. Pharmaceutical Press: Chicago, 2006; p. 545.
43. Somatuline® Depot Highlights of Prescribing Information: Tercica, Inc., Brisbane, CA,  
August 2008.
44. Kozutsumi, D.; Kawashima, A. Pharmacokinetics of 9 $\alpha$ -fluoromedroxyprogesterone  
acetate in rats : Comparison with Medroxyprogesterone acetate. *Biopharm. Drug Dispos.*  
**1999**. 20: 277-284.
45. Depo-Provera® Physician Information: Pharmacia & Upjohn Company, Division of  
Pfizer Inc., New York, May 2006; LAB-0149-5.0.
46. Serajuddin, A. T. M. Salt Formation to improve drug solubility. *Adv. Drug Delivery Rev.*  
**2007**. 59, 603-616.
47. Zyprexa Relprevv® Full Prescribing Information: Eli Lilly and Company, Indianapolis,  
IL, December 2009; PV 5920 AMP.

48. Bhana, N.; Perry, C.M. Olanzapine: A Review of its Use in the Treatment of Bipolar I Disorder. *CNS Drugs*. **2001**. 15: 871-904.
49. Aravagiri, M.; Teper, Y.; Marder, S.R. Pharmacokinetics and Tissue Distribution of Olanzapine in Rats. *Biopharm. Drug Dispos.* **1999**. 20: 369–377.
50. Madhu, M.; Lewis, S.; Baig, J.A. Biodegradable injectable implant systems for sustained delivery using poly(lactide-co-glycolide) copolymers. *Intl. J. Pharm.* **2009**. 1, 103-107.
51. Zoladex® 3.6 mg Package Insert: AstraZeneca Pharmaceuticals LP, Wilmington, DE, December 2003; SIC 64216-00.
52. Callewaert, M.; Millot, J.; Lesage, J.; Laurent-Maquin, D.; Edwards-Levy, F. Serum albumin-alginate coated microspheres: Role of the inner gel in binding and release of the KRFK peptide. *Intl. J. Pharm.* **2009**. 366, 103-110.
53. Graff, S.; Hussain, S.; Chaumeil, J.I.; Charreau, C. Increased Intestinal Delivery of Viable *Saccharomyces boulardii* by Encapsulation in Microspheres. *Pharm. Res.* **2008**. 25, 1290-1296.

54. Murdan, S.; Florence, A.T. Non-aqueous Solutions and Suspensions as Sustained-Release Injectable Formulations. In *Sustained-Release Injectable Products*. Senior, J.H. CRC Press: Boca Raton, FL, 2000; pp 71-101.
55. Wilson, A. G. E.; Nouraldeen, A.; Gopinathan, S. A new paradigm for improving oral absorption of drugs in discovery: role of physiochemical properties, different excipients and the pharmaceutical scientist. *Future Med. Chem.* **2010**. 2, 1-5.
56. Chaubal, M.V.; Kipp, J.; Rainbow, B. Excipient Selection and Criteria for Injectable Dosage Forms. In *Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems*. Katdare, A. and Chaubal, M.V., Eds. Informa Healthcare: New York, 2006; pp. 271-290.
57. Kreuter, J. Nanoparticles and microparticles for drug and vaccine delivery. *J. Anat.* **1996**. 189, 503-505.
58. Chaubal, M.V. Polymeric Excipients for Controlled –Release Applications. In *Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems*. Katdare, A. and Chaubal, M.V., Eds. Informa Healthcare: New York, 2006; pp. 341-372.
59. Gokarn, Y.R.; Kosky, A.; Kras, E.; McAuley, A.; Remmele, R.L. Excipients for Protein Drugs. In *Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery*

*Systems*. Katdare, A. and Chaubal, M.V., Eds. Informa Healthcare: New York, 2006: pp. 291-306.

61. Xiaohui, P.; Sun, J.; Li, M.; He, Z. Formulation of Nanosuspensions as a New Approach for the Delivery of Poorly Soluble Drugs. *Curr. Nanosci.* **2009**. 5: 417-427.

62. Scholer, N.; Krause, K.; Kayser, O.; Muller, R.H.; Hartwig, H.; Hahn, H.; Liesenfeld, O. Atovaquone Nanosuspensions Show Excellent Therapeutic Effect in a New Murine Model of Reactivated Toxoplasmosis. *Antimicrob. Agents Chemother.* **2001**. 45, 1771–1779.

63. Kroll R.A., Pagel M.A., Muldoon L.L., Roman- Goldstein S., Fiamengo S.A. Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: a comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. *Neurosurgery*. **1998**. 43: 879-886.

64. Müller, K.; Faeh, C.; Diederich, F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Science*. **2007**. 317, 1881-1886.

65. Burke, P. A.; Putney, S. D. Microspheres for Protein and Peptide Delivery: Applications and Opportunities. In. *Sustained-Release Injectable Products*. Senior, J. H., Ed. CRC Press: Boca Raton, FL, 2000; pp 371-396.

66. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeny, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997.** *23,* 3-25.