APPLICATION OF HIGH THROUGHPUT TECHNOLOGIES TO DRUG SUBSTANCE AND DRUG PRODUCT DEVELOPMENT

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Abstract

This presentation describes the application of novel high throughput physical-chemical technologies to the pharmaceutical discovery and development process. The rationale for such platforms is described in light of the changes that have occurred in the biological and chemical processes leading to drug target evaluation and lead identification. Several high throughput platforms are described for identification and evaluation of solid forms and formulations of drug candidates including salt, hydrate and solvate selection and polymorph discovery and evaluation. The application to the development of oral and intravenous formulations for animal model and human clinical evaluation is also discussed. The importance of informatics to design experiments and capture and analyze data is highlighted. Examples are described showing the power of high throughput systems to discover knowledge that enables pharmaceutical scientists to make more informed and better decisions about product development choices.

Keywords

Pharmaceutical development, crystallization, formulation, high-throughput, informatics, polymorphs

Introduction

With the astounding findings and developments in biology over the last two decades, the drug discovery process has been re-invented. Whereas, in the 1980's it took years to go from a biochemical or pharmacological concept to a compound that could be tested for efficacy in a disease state, to-day that process has been greatly accelerated. The identification of novel, specific biochemical targets such as enzymes and receptors has enabled much of the discovery effort to be conducted in the test tube (*in vitro)* rather than in animals (*in vivo).* These targets have been readily converted to high throughput (HT) assays to rapidly screen thousands of molecular entities for activity. Contemporaneously, the use of novel high throughput combinatorial and parallel chemical synthetic processes has permitted medicinal chemists to prepare massive libraries of compounds that can be funneled through the HT assays to identify lead candidates for drug development. In addition, the capability to assay not just for potency at the target site, but to determine selectivity versus non-target sites has provided the drug discovery scientists with the means to identify novel potent and selective compounds in a few months. Yet we continue to read that productivity in the pharmaceutical industry is dropping and that, despite huge research budgets, there are actually fewer new drugs being approved. Why is this? Put quite simply, potency and selectivity are requisite but not sufficient criteria for a successful drug candidate. The other criteria include:

- Validation of the biochemical target as implicated in the disease state;
- An acceptable safety profile;
- Appropriate metabolic and pharmacokinetic properties; and
- Physical/chemical characteristics that ensure that the compound can be synthesized, scaled-up and formulated effectively and economically.

The drug discovery /development process is outlined schematically in Figure 1 highlighting in particular the impact points of the physical/chemical properties.

This presentation will show how novel HT methodologies are just beginning to find application and validation in this space.

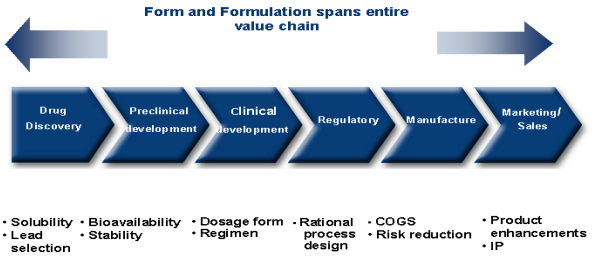

Figure 1. The drug Discovery / Development Process

Pharmaceutical properties of successful drug candidates

Almost without exception, small molecular weight drugs (called active pharmaceutical ingredients or API's) are isolated as crystalline materials principally for two reasons – purity and reproducibility. This is not true of most peptide and protein drugs which are difficult to crystallize and are almost invariably produced as amorphous solids.

Most small molecular weight drugs are relatively complex organics structures and many contain functional groups that lend themselves to the preparation of salt forms through reaction of the functional groups with pharmaceutically acceptable acids and bases. Others contain functional groups that do not lend themselves to salt formation but, like the salt formers they are susceptible to crystallization in a variety of polymorphic forms, or as hydrates or solvates. These various forms of the API can have very different physical and chemical properties that may have significant impact on the performance of the API during isolation, processing, storage and in the biological milieu in which it is administered (figure 2). In particular, the solubility and dissolution properties of the API can dramatically affect the rate and extent of absorption of the compound from the gastrointestinal tract or across other biological barriers, or may determine if the compound can be administered via the intravenous route, all potentially critical characteristics of successful drugs.

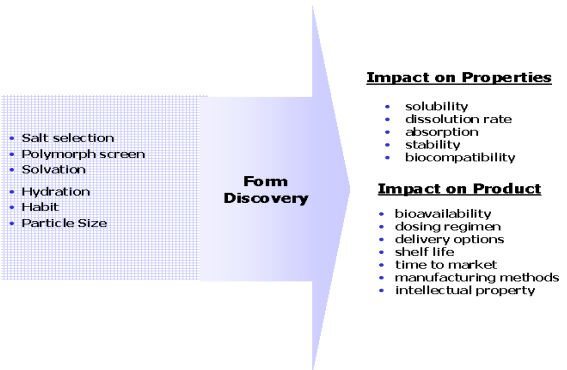

Figure 2. Effect of physical/chemical properties on pharmaceutical performance

Discovery

For the most part, the discovery scientists have paid only limited attention to the physical/chemical properties of drug candidates during most of the discovery phase, relying on solubilizing agents or pH control to "force" the compound into the animal models for efficacy or pharmacokinetic studies. The current drug targets and the use of combinatorial libraries of compounds has led to the identification of highly potent and selective compounds but with physical properties that make them difficult to formulate (Lipinski, 2002). Only when a compound, or a small series of compounds, approaches the final selection decision to initiate full scale development, does the full impact of the physical properties become apparent. In recent years, the more progressive drug companies have come to recognize the cost of ignoring these characteristics as their lead candidates fall by the way-side due to inability to administer them at appropriate doses in toxicology studies and in humans, with consequent need to recycle through the process to build in new chemical modifications to create "developability" (Mendenhall, 2001) in addition to potency and selectivity.

One of the major reasons for this disconnect between discovery and development has been the availability of HT technologies in the discovery process and the lack of corresponding capacity and throughput in the development functions such as metabolism, toxicology and formulation design. The development of novel high throughput platforms to explore physical properties and create new forms and formulations of interesting candidate compounds, permits the development scientists to provide the discovery researcher with feed-back that can be used to design "developability" into the molecules they are creating and to provide acceptable, non-toxic vehicles that can be used to deliver the compounds to animals to determine which properties are acceptable and which require modification. This is illustrated in Figure 3 where the concept of globally optimizing the candidate molecules depicts how the knowledge of physical properties can be used to determine which chemical scaffolds are suitable for development and to give real-time feed-back to the medicinal chemists to use in concert with the potency and selectivity data that can be obtained from the currently available HT platforms.

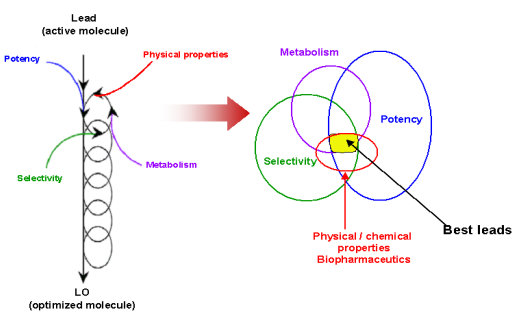

Figure 3. Global optimization of drug candidates

Preclinical and Early Clinical Development

Once the discovery scientists believe that they have optimized a small number of potential drug candidates for potency and selectivity, they develop a broader and deeper portfolio of properties of perhaps the last 3-5 compounds which are then evaluated to determine which one (or perhaps 2-3) should be accepted as candidates for initial toxicological and clinical evaluation.

Some pharmaceutical companies do not expend much effort to optimize the physical/chemical form of the API that will be tested in the first toxicological (and phase I clinical) studies, preferring to wait until the compound has shown the desired biological/pharmacological activity before investing significantly in the evaluation of the various forms (salts, hydrates, polymorphs) that might be possible. Some companies even wait till the start of phase III (i.e. pivotal) clinical studies before finalizing the forms, formulations and processes that they hope will define the marketed product. The danger of this delay is that the regulatory agencies (particularly the FDA) require a company to demonstrate that the API and its formulation(s) that were used to demonstrate safety and efficacy in pivotal clinical studies are the same as, or at least equivalent to, those that they file in the New Drug Application (NDA) for approval to market the drug. Since the manner in which the form and formulation of a drug affects its performance in the body is not always fully understood, these companies are taking great risks that they may demonstrate an effective product in clinical trials but be prevented from marketing it until they can demonstrate to the agencies the equivalence of the performance of the trial and marketed forms and formulations. This can have a very significant impact on revenues, and in some cases, has permitted competitors who did not have this problem to reach the market first.

To avoid these issues some of the more progressive companies have, over the last several years, established mechanisms for members of their development groups to actively participate in the later stages of the drug discovery process, enabling them to provide valuable input on the metabolism, toxicology and physical/chemical properties of lead compounds prior to their acceptance as clinical candidates. However, regardless of the commitment to this concept, the development groups do not have high throughput technologies that permit their scientists to comprehensively evaluate the possible options for each potential development candidate. Most activities are manual or employ low throughput automation.

Forms:

Comprehensive investigation of the options for physical forms of drug candidates has two significant benefits:

- Optimization of performance;
    - E.g. oral bioavailability, suitable intravenous formulation, maximum chemical stability:
- Avoiding the risk of developing a metastable form which could later convert to a more stable form with physical properties that adversely affect the pharmaceutical performance of the formulation of the drug.

Salt formation:

When a compound has an ionizable functional group then the possibility exists to make salts. There are several constraints on the options:

- The pK_a's of the ionizable group in the API and in the acid or base must be such that proton transfer is energetically favorable. When this is not the case, a solid "complex" may form but may rapidly disproportionate once placed in an aqueous environment.
- The number of pharmaceutically acceptable acids and bases for salt formation are limited by acceptable toxicity levels and prior product experience.

Nevertheless, the possible conditions for salt formation are quite large. When one adds the ratios of API to salt former, the "pH" of the mixture and the various possibilities for solvents and solvent mixtures from which the salt may be crystallized, the number of potential experimental conditions becomes large. For example, with 16 pharmaceutically acceptable acids and 25 approved solvents used as single solvents or binary mixtures, and three ratios of API to salt forming acid, the number of potential conditions to be evaluated is 15,600. It is inconceivable that one could run such a series of experiments using the traditional low throughput methodologies available in the industry to-day. One also has to take into account the total mass of API which would be required to conduct such studies when the amount used per trial is of the order of 5-10mg (the industry standard). In the late stages of drug discovery and early stages of pre-clinical development, such amounts are usually unavailable.

Polymorphic and pseudopolymorphic (hydrates, solvates) forms:

In the case of polymorphic forms, the situation is more challenging. Despite the availability of chemo-informatic programs there is, as yet, no failsafe method to predict the extent of polymorphism of a given compound. Hence the only manner in which one can be assured of having a complete knowledge of the polymorphic landscape on which to base a development choice (usually the most thermodynamically form) is to subject the API to a variety of crystallizing conditions that can expose the diversity of forms. This applies to the free form as well as any salt forms of the API.

The polymorphic form can also be affected by additives in the crystallizing solution. This is especially important in the scale-up and technology transfer of the API synthetic process. The presence of process impurities

or degradates can provide templates on which novel forms can nucleate. Consequently, any decision made in early development should be re-confirmed as process scale-up and transfer within the manufacturing plant are conducted. A similar analysis can be conducted for evaluation of hydrate and solvate formation.

The challenge:

The traditional methods of evaluating the possible forms of an API cannot do justice to an exploration of the possible diversity of forms. Therefore, the development scientists are forced to make decisions on incomplete data. It is not surprising, therefore, that unexpected and undesired outcomes can, and do, occur. While most companies could point to such examples in their history, none was so publicly visible as the fate that befell Abbott Laboratories shortly after the launch of their HIV protease inhibitor, Ritonavir (trade name Norvir ^{TM}). In 1998, the company became aware that some batches of the product were not meeting the dissolution specifications filed in the NDA. An investigation revealed that the API used in these lots was of a different polymorphic form than had been initially registered, and it was a thermodynamically more stable form. Since this drug had poor solubility to begin with, the lower solubility of this novel form resulted in slower dissolution of the drug from the formulation with consequent adverse implications for the oral bioavailability and efficacy of the product (Bauer et al., 2001). Abbott could not release these batches to the market and were forced to conduct a lengthy internal investigation. While this was ongoing they could not supply a very demanding market with the approved product. Only after extensive investigation were they able to find a new formulation of the more stable polymorphic form and gain approval from the regulatory agencies to put this new product on the market. This was a particularly important event because the protease inhibitors were seen as providing much needed treatment for AIDS patients and moreover, Ritonavir had properties that made it well suited to include in cocktails of anti-HIV drugs since it inhibited the rapid metabolism of other protease inhibitors, thereby prolonging their half-life in the body and providing the patient with improved anti-viral protection with less frequent dosing. Clearly, if Abbott had been able to explore the polymorphic space more thoroughly during the development phase they could have found the diversity of polymorphic forms of Ritonavir and made a more informed decision on which form to take into full development. However, the lack of high throughput methodologies to conduct these studies, coupled with the speed with which the anti-HIV drugs were being developed, hampered any desire to conduct the appropriate evaluations.

Formulations:

As high throughput techniques have been applied in discovery to improve potency and selectivity against specific biological targets, the tendency has been for lead compounds to have higher molecular weight and to be more lipophilic, properties that make them less "developable" (Lipinski, 2002., Mendenhall, 2001). In many cases compounds with these characteristics are not well absorbed from the gastrointestinal tract when delivered in the form of solid particles. Techniques such as salt formation (discussed above) and particle size reduction to the nanometer range (Liversidge and Cundy, 1995) have been applied with some success, but there still remain drug candidates which are only effectively absorbed at an acceptable rate if they are administered in the dissolved state, often in pharmaceutically acceptable organic excipients such as oils, polymeric glycols, surfactants or combinations thereof (e.g. Cyclosporin, Physicians' Desk Reference, 2001). This is also true of poorly soluble compounds which have to be administered by the intravenous route (e.g. Taxol, Singla et al., 2002). In both these scenarios, the challenge for the formulation scientist is to find an appropriate vehicle that dissolves the drug at a concentration that makes a clinically and commercially viable product, with satisfactory chemical and physical stability, and using excipients at concentrations that are acceptable from a toxicological and regulatory perspective. Since it is not possible to predict the solubility and stability properties of API's in the wide diversity of excipient mixtures, the development scientist is forced to select a few excipients and do a limited number of studies to try to find an appropriate solution. Here again, the available technology is manual or low throughput and could benefit greatly from the ability to screen hundreds or thousands of excipient mixtures from which to select, for further evaluation, those more likely to meet the target .

Process Development and Regulatory Filings:

Over the last decade, the world-wide regulatory agencies (including the FDA) have focused much more attention on the manner in which pharmaceutical products are developed and manufactured resulting in the so-called pre-approval inspections or PAI's. There is now a much stronger focus on the form of the API, the process to make and control it as a drug substance and as a drug product, with particular attention being paid to the reproducibility of the polymorphic form of the drug in the final marketed formulation. Consequently, it is important for the development groups in the industry to ensure that they can justify the selection of the drug form used in clinical trials and in the marketed product, and to show that this form is not subject to change upon scale-up or transfer between manufacturing sites. Since isolation of a particular form from a crystallization process can be influenced by many processing variables and by the presence of other components such as impurities and degradates, it is important for the scale-up engineer to be able to understand the sensitivity of the process to these factors. Likewise, in the formulation process which may involve wet or dry granulations, the impact of the process variables and the role of excipients are equally important.

With to-day's capabilities, these relationships can only be evaluated with low throughput technologies and cry out for the ability to explore the process space in a much more comprehensive manner.

Such a capability would provide two benefits:

- A fuller understanding of the process so that scale-up and process transfer can be conducted with the benefit of a sound data base:
- An ability to demonstrate to the regulatory agencies that process and manufacturing site changes can be made with greater confidence that the ultimate product characteristics will remain unchanged.

Product Life-Cycle Management

Given the very large risks, long development timelines and high costs of bringing a pharmaceutical product to market, it is not surprising that companies are looking for ways to protect their investment and extract full value from the franchise that a product has created. The speed to market is critical, but speed has its downside, namely it robs the development groups of the ability to pursue all of the options for product and process definition. Therefore, post launch, there exist numerous opportunities to pursue options that can add clinical and commercial value to the product line. These have traditionally included additional clinical claims, new routes of administration, combination products, and novel delivery systems. The capability of exploring the influence of form and formulation on the value to the patient is an area that has been under-resourced in the past, largely because the companies have not invested in a comprehensive assessment of the impact of changes in these parameters on the product performance. Here, again, the availability of high throughput technology enables such exploration in a rapid and cost-effective manner with the potential of pay-off in product enhancement of significant value.

High Throughput Form and Formulation: Discovery and Development

Over the last two and a half years we, at TransForm, have focused on developing high throughput platforms for selection of forms and formulations of pharmaceutical candidates and products. The technology has three components:

- An automated, high throughput crystallization platform;
- A suite of high throughput formulation platforms and
- An informatics platform that envelopes the processes to permit experimental design, control of the automation, data capture on line and data analysis and mining.

In the next sections, these capabilities will be described and examples of their use delineated.

I Crystallization:

Figure 4 provides an overview of the crystallization platform, CrystalMax. Its features are highlighted in figure 5.

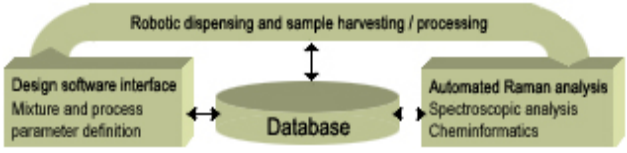

Figure 4. Overview of the CrystalMax platform

CrystalMax™

- Discovers diversity of solid forms
  - Polymorphs, salts, hydrates, solvates and methods to make them
  - Identifies best forms – solubility, dissolution rate, hygroscopicity
- Efficient / fully automated
  - Sub-mg amounts: many more experiments per mg API
  - >10,000 parallel crystallizations: faster and higher throughput
- Comprehensive
  - Ability to explore diverse experimental space using broad variation in inputs / process conditions
- On-line data capture
  - Permits knowledge mining
  - Rigorous process control

Figure 5. The features of CrystalMax

Each individual CrystalMax unit is capable of screening up to 18,000 crystallization conditions in parallel; the capacity can be used to conduct thousands of studies on one API or it can simultaneously evaluate several API's with fewer experiments per compound. Experimental design software defines combinatorial test conditions on the basis of the selection of solvent properties, additives and methods used to drive super-saturation (thermal, evaporative, anti-solvent addition). Experiments are executed in arrays of individually addressable tubes into which the API, appropriate solvents and additives are combinatorially dispensed, and which can be hermetically sealed at the start of the experiment (except for evaporative crystallization) to ensure that the composition of the solvent stays constant during the study. The amount of API per tube can be varied up to 10's of mgs but typically is between 250μg and 1mg. During the incubation process, the tubes are monitored by a vision station and samples that have crystallized are selected from the original array, the solvent is removed by aspiration, and the residue is dried with a nitrogen flow; the other tubes are returned to the incubation platform to

continue the crystallization process. Figure 6 shows a typical crystallization rate plot.

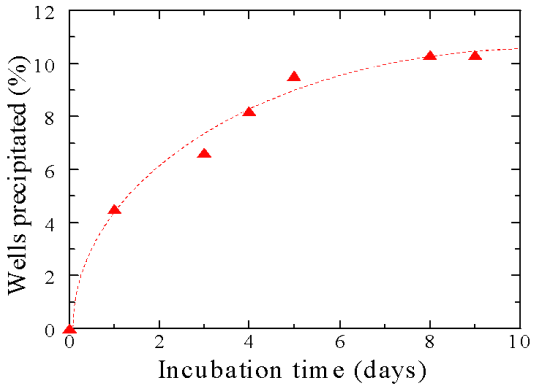

Figure 6. Typical rate of appearance of solids during a thermally driven CrystalMax run .

Optical imaging and *in situ* Raman spectroscopy are used to characterize newly formed crystals. Since the number of solid forms to be evaluated from one experiment is large, the primary screen has to be rapid. For this reason Raman spectroscopy was selected over X-ray powder diffraction. Figure 7 demonstrates a comparison of dependence of acquisition time on mass of material available for the two techniques.

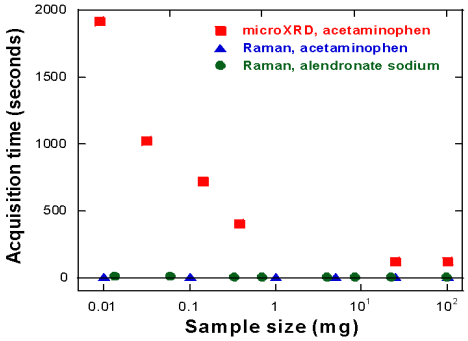

Figure 7. Comparison of acquisition times of Raman and X-ray powder diffraction data as a function of mass of API.

A classification process aids the analysis of the large number of spectra generated during the experiment. Similarity coefficients are calculated for all pairs of spectra, the coefficients are sorted, color-mapped and displayed as an n x n matrix for easy visualization. The plot in figure 8 illustrates that the Tanimoto matrix derived from the spectral data is a simple visual way of differentiating polymorphs of acetaminophen (Peterson et al., 2002) made by three processes – thermal, evaporative and melt crystallization. The first panel illustrates the results of a 7,776- tube, thermally-driven experiment which resulted in 723 crystallizations (9.3%). A particularly interesting observation is that form II was only discovered in a very few tubes (approx. 5 % of the positives), indicating that it is a rare occurrence and would not have been found if the diversity of the solvent space had not been explored. This figure also highlights another feature of the process, namely the use of informatics to guide iterative experiments to find polymorphic forms. From the results of the first evaluation, evaporative crystallization conditions were identified for use in the second round in order to expand the space around the conditions which produced form II – the results are shown for evaporation at 54C. None of these solution crystallizations produced the elusive form III of acetaminophen (Burger et al, 1982). The third panel shows how changing the process to melt crystallization was used to find that form. The structure predicted from the powder x-ray pattern of form III was evaluated against chemo-informatic predictions of polymorphic forms of acetaminophen (Beyer et al., 2001) and a novel structure of form III proposed (Peterson et al., 2002).

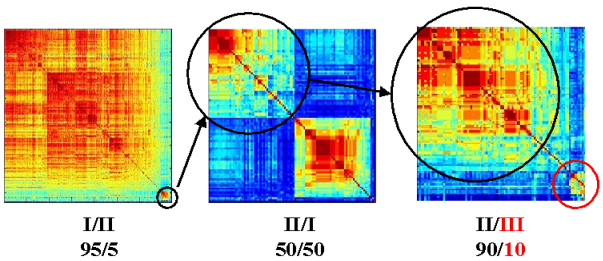

Figure 8. A representative Tanimoto matrix comparing Raman spectra of acetaminophen polymorphs. Each pixel represents the Tanimoto value for pairs of spectra, according to a color scheme where red indicates high similarity and blue low similarity.

While this study demonstrated the scientific power of high throughput crystallization and of the use of informatics to guide iterative crystallization experiments, the real value to the pharmaceutical industry comes from the ability to find forms that have not previously been identified or to be able to confirm that all of the known forms have been found. The following sections will give several examples of marketed products which have been examined by the CrystalMax platform and which have revealed novel findings.

Polymorphs: Ritonavir

As discussed earlier, Ritonavir provided a sobering example of polymorphic transition with significant adverse consequences for the product. As an early study of the power of CrystalMax, we obtained Ritonavir (Norvir™) from the marketplace and isolated the API with >99% purity by HPLC. This material was then evaluated in a CrystalMax study using 2000 tubes each containing approx 10mg of API and a range of solvent mixtures, employing a thermally driven crystallization process.

We found the two published forms of Ritonavir (form I with which Abbott launched the product in 1996 and the thermodynamically more stable form II which appeared in their product in 1998), and identified multiple solvent mixtures which yielded these forms. But we also discovered one more true and previously unreported

polymorphic form (form III) which is a higher energy polymorph, and one hydrate and one solvate (formamide). It is interesting to speculate what impact the knowledge of this polymorphic landscape would have had on Abbott's decision if it had been available in the years 1992-96 when the product was in development. It is likely that they would have selected form 2 for development, or potentially have chosen a different development candidate. Either way they would have been spared considerable anguish and embarrassment.

Hydrates: Alendronate

Many API's exist as anhydrous and hydrated forms. Some form single hydrated states, while others form multiple stoichiometric hydrates, while yet others form non-stoichiometric hydrated forms. The properties of hydrates can be very significantly different from those of the anhydrous forms, particularly with respect to their solubility and dissolution rates. This can have enormous impact on oral bioavailability or on the ability to prepare aqueous, intravenous formulations. Additionally, the patent office and the courts have deemed these as non-obvious and patentable entities. Therefore, there is now significant interest in finding all the hydrate as well as the polymorphic forms to assess differences in properties and to protect the intellectual property invested in the basic API. A recent example is the case of alendronate sodium trihydrate which is sold by Merck and Co as Fosamax™ for the treatment of osteoporosis. A generic company, Teva, has filed a patent on an additional hydrate of alendronate sodium (the monohydrate) and is claiming that it is free to market this form of the drug in countries where Merck's product is protected only by the patent on the trihydrate (Curtis, 2002). We have used CrystalMax to examine the hydrate forms of sodium alendronate with the results shown in figure 9.

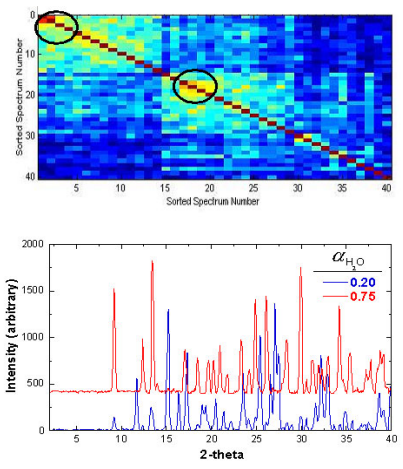

Figure 9. Identificaton of hydrate forms of sodium alendronate using in situ Raman spectroscopy in the CrystalMax platform and verification by use of X-ray powder diffraction.

In a screen involving 96 experimental conditions covering 12 water activities from 0.05 to 1, the two hydrated forms were identified by *in situ* Raman spectroscopy in the crystallization tubes, and later confirmed by x-ray powder diffraction. This again shows the power of this high throughput technology to rapidly discover novel and patentable forms of API's with only a small amount of drug being used.

Salt forms: sulfathiazole:

Sulfathiazole, a weak acid, was used to demonstrate the ability to find salt forms using pharmaceutically acceptable bases. While this was a relatively small study involving only 96 crystallization tubes a number of interesting potential salts were found (figure 10) and subjected to further secondary analysis to evaluate their physical properties.

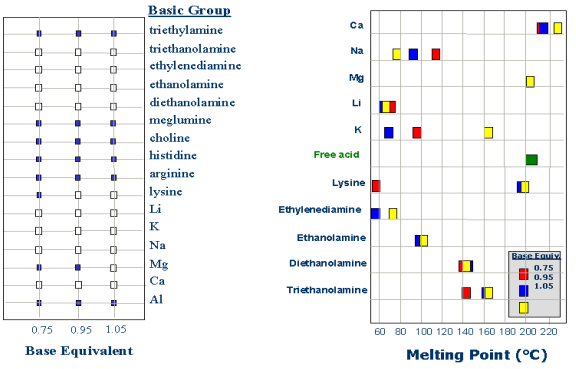

Figure 10. Panel A: salt forms of sulfathiazole found in a CrystalMax study are shown by filled symbols: Panel B: the melting points of the identified salt forms showing the diversity of results which can be used in conjunction with other data to assess the optimal form for development.

II Formulation design:

We have also developed a suite of platforms using combinatorial mixtures of solubilizers and stabilizers to identify stable, liquid formulations that can be used to deliver drugs by the intravenous and oral routes. For oral delivery (SFinX™) the challenge is to find combinations of pharmaceutically acceptable excipients that can solubilize the API at concentrations that permit the clinically effective dose to be dissolved in a reasonable volume (say 1 ml or less), while maintaining chemical and physical stability of the formulation to permit a required shelf life. Such formulations might be used in soft gelatin capsules or distributed on the surface of carrier materials which are encapsulated or tableted, or the formulation may be provided as an oral solution, though in this case taste also plays a major role. These formulations may be used in early clinical trials to validate the biological concept behind the drug program or may ultimately become a marketed product. By a modification of this technology

(AquaSFinX™) it is also possible to identify semi-aqueous vehicles that can be used in early studies during the discovery phase to demonstrate efficacy in animal models or to conduct toxicology or metabolic studies in animals. Another formulation platform (FAST™) was designed to identify combinations of pharmaceutically acceptable excipients that can solubilize API's for intravenous administration of poorly water soluble compounds. These formulations can be used to obtain critical information about the metabolism and pharmacokinetics of the drug in humans, to assess absolute oral bioavailability of an oral product or they can potentially become marketed products. We will demonstrate this capability with results from the FAST™ platform for intravenous formulations.

Paclitaxel is an anti-tumor drug that is extremely water insoluble and was formulated as Taxol™, a concentrate in ethanol and cremophor EL, a castor oil derivative that has good solubilizing properties but can also produce adverse effects in humans, including severe anaphylactic responses. This concentrate is chemically and physically stable but is diluted in saline or 5% dextrose solution before administration to the patient. The diluted vehicle is super-saturated with respect to the API, but the product has a post-reconstitution shelf live of several hours before precipitation occurs. We used the FAST™ platform to identify substitutes for cremophor EL that would retain the solubilizing properties, maintain chemical stability of the concentrate but have a potentially better safety profile. Combinatorial experiments using single, binary and ternary mixtures were conducted with 24 GRAS (generally regarded as safe) excipients to identify which combinations could maintain solubility of the paclitaxel in the diluted vehicle for at least 48 hours. In all 32,000 combinations were examined in triplicate for a total of 96,000 individual experiments.

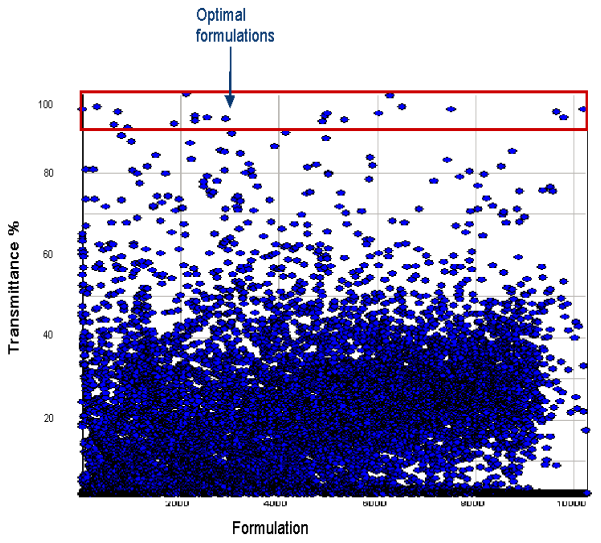

Figure 11. Evaluation of individual formulations for ability to solubilize paclitaxel. Samples with high transmittance maintained the paclitaxel in solution for ≥ 48 hours .

After analysis of the results and consideration of the nature of the excipients, their concentrations and their known safety profile, only a few met the solubility criteria and 4 were found to be potentially viable (figure 11). Two of these were administered to rats and were demonstrated to be better tolerated than the marketed formulation. This result demonstrates the scarcity of solutions to this type of formulation problem and validates the use of high throughput combinatorial approaches to identify rare positive outcomes.

III Informatics

In order to facilitate high-throughput experimentation in form and formulation, we have developed a sophisticated informatics technology suite called InForm. InForm is comprised of four components:

- a design of experiment application,
- station controllers,
- an analytical application, and
- a set of process, analytical, and chemical databases.

The design of experiment application allows scientists to set up experiments on their desktops for all of our automated platforms. Primary experiments can be designed in a combinatorial manner in order to diversely cover a property space. As discussed earlier, for maximum efficiency of the thermally driven crystallization process in CrystalMax, it is necessary to have an estimate of the solubililty of the API in the various mixed solvents as a function of temperature. This is accomplished in InForm using data on solubility in selected individual solvents and using computational methods to calculate the solubilites required. This capability has permitted a five-fold increase in the efficiency of the thermally induced crystallization process.

Once an experiment has been designed, all parameters necessary to run the experiment are stored in a database. Proprietary station controllers that are connected to all of our laboratory instruments poll this database for instructions about which experimental steps are ready to be performed, and store the process data and results back to the database. The analytical application reads information from an analytical database and allows users to explore data, cluster results, and create predictive models in concentration or property space. Models built in concentration space attempt to fit a response surface for an output variable of interest (such as solubility) to the concentration levels of mixture components. Such a model can be used to assess synergies or predict concentration levels of tested compounds for an optimal formulation from a limited set of mixtures. This hypothesized optimal formulation can be tested in a later experiment. Models built in property space, on the other hand, attempt to fit an output variable to physical properties or descriptors using techniques similar to those used for traditional QSAR. These models can be carefully extended to mixtures containing compounds not included in the original experiments if validation suggests that the models are

fairly stable. Significant models that are found in the analytical application can be stored in a "knowledge repository" in the analytical database. These models can be recalled later and used to direct iterative experiments.

The power of this approach becomes increasingly more visible when several properties are being co-optimized. This can be very important in the pharmaceutical discovery process where potency, selectivity and "developability" need to be co-optimized (figure 3). Likewise in development, oral bioavailability, stability and processability require co-optimization.

The process can be extended beyond small molecular weight drugs to biologicals such as proteins, gene transfer vectors and vaccines where in many cases the scientific knowledge relating physical properties to biological function is less obvious and the power of combinatorial, high throughput evaluations can provide leads for further evaluation and refinement.

Conclusions

We have designed and developed novel, high throughput platforms for application to form and formulations of pharmaceutical products. These platforms are applicable to the solution of challenges in discovery, pre-clinical and process development, scale-up, process transfer and improving and protecting marketed products. The use of informatics to design, control and analyze the data is a key feature. Extension of this concept to biological products and other pharmaceutical processes, e.g. lyophilization, is ongoing.

References

Lipinski, C.A. (2002). Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol. Toxicol. Methods, 44, 235.

Mendenhall, D.W. (2001). Trends in formulation science: building in developability. AAPS Newsmagazine, 13.

Bauer, J., Spanton, S., Henry, R., Quick, J., Dziki, W., Porter, W., Morris, J. (2001). Ritonavir: an extraordinary example of conformational polymorphism. Pharm. Res., 18, 859.

Liversidge, G.G. and Cundy, K.C. (1995) Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. Part 1, Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int. J. Pharmaceut. 125, 91.

Cyclosporin, Novartis Pharma AG, Basel, Switzerland. (2001). Physicians Desk Reference.

Singla, A.K., Garg, A., Aggarwal, D. (2002). Paclitaxel and its formulations. Int. J. Pharm., 235, 179.

Beyer, T., Day, G.M. and Price, S.L. (2001). The prediction, morphology and mechanical properties of the polymorphs of paracetamol. J. Am. Chem. Soc. 123, 5086.

Peterson, M. L., Morissette, S.L., McNulty, C., Goldsweig, A., Shaw, P., LeQuesne, M., Monagle, J., Encina, N., Marchionna, J., Johnson, A., Gonzalez-Zagusti, J., Lemmo, A.V., Ellis, S.J., Cima, M.J., Almarsson, O. (2002). Iterative high-throughput polymorphism studies on Acetaminophen and an experimentally derived structure for Form III. J. Am. Chem. Soc., 124, 10958.

Burger, A. (1982). Interpretation of polymorphism studies. Acta Pharm. Technol., 28, 1.

Curtis, G. (2002). Patent challenge muddles Merck picture. TheStreet.com, 8/26/2002.