Organic Process Research & Development **2000**, *4*, 427–435

Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities

Richard J. Bastin,[†] Michael J. Bowker,*[,†,§] and Bryan J. Slater[‡]

Preformulation Department, Pharmaceutical Sciences, Aventis Pharma, Dagenham Research Centre (DRC), Rainham Road South, Dagenham, Essex RM10 7XS, UK, and World-Wide Physical Chemistry Department, Discovery Chemistry, Aventis Pharma, Dagenham Research Centre (DRC), Rainham Road South, Dagenham, Essex RM10 7XS, UK

Abstract:

Selection of an appropriate salt form for a new chemical entity provides the pharmaceutical chemist and formulation scientist with the opportunity to modify the characteristics of the potential drug substance and to permit the development of dosage forms with good bioavailability, stability, manufacturability, and patient compliance. Salts are most commonly employed for modifying aqueous solubility, however the salt form selected will influence a range of other properties such as melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form, and mechanical properties. Where possible, a range of salts should be prepared for each new substance and their properties compared during a suitable preformulation program. Since it is normally possible to fully develop only one salt form, its properties should be appropriate to the primary route of administration and dosage form. An understanding of the influence of drug and salt properties on the finished product is essential to ensure selection of the best salt. The drug properties required for one dosage form may be quite different from those required for another. A well designed salt selection and optimisation study provides a sound base on which to build a rapid and economic product development programme.

Introduction

Modern drug discovery processes involve the screening of vast numbers of compounds that may have been made by the Company's research laboratories over many years. Added to these may be the many thousands of compounds that have been manufactured as libraries of structurally related series by "combinatorial chemistry" techniques. All of these compounds are generally dissolved in dimethylsulphoxide (DMSO) solution and screened in an enzyme- or receptor-based assay system. If the number of "hits" produced is large, the numbers are usually refined by further screening and selection until a manageable number of "leads" is available. Many of these leads will show only weak or moderate activity and further refinement and optimisation is invariably necessary. These optimisation procedures usually involve numerous structural modifications, aided by computational techniques, until a small number (usually 1−5) of highly active "candidates" remain.

* To whom correspondence should be sent.

[†] Preformulation Department, Pharmaceutical Sciences.

[‡] World-Wide Physical Chemistry Department, Discovery Chemistry.

[§] Current address: M. J. Bowker Consulting Ltd., 36, Burses Way, Hutton, Brentwood, Essex CM13 2PS, UK

These candidates are usually free bases, free acids, or neutral molecules, rather than their salts. Also, because of the generally higher molecular weights of modern drug substances and the increased use of DMSO solutions in the screening processes, it is becoming apparent that there is a tendency towards ever more lipophilic candidates being presented. Frequently, when first proposed as potential development candidates, they are often amorphous or partially crystalline as little effort has been made to investigate formal crystallisation procedures. The need for water-soluble candidates has been recognised[1−4] for many years before the advent of 'combinatorial chemistry.

Investigations into the Possibilities of Salt Formation

When first presented for initial preformulation investigations, normally the amount of drug substance available from Discovery Chemistry rarely exceeds 1 g. To maximize the amount of data gained from such small quantities, semi-micro techniques have been developed and are used regularly within our groups. Invariably, the first information generated for each candidate is the calculated pK_a value of each ionisable group in the molecule.[5−8] This is quickly checked against the value determined experimentally on 1−2 mg of sample by potentiometric titration (e.g., Sirius Model GLpKa apparatus, Sirius Analytical Instruments Ltd.). Knowledge of the pK_a value enables potential salt forming agents (counterions) to be selected, for each candidate, based on lists that are available in the literature.[2,9−11] For the formation of a stable salt, it is widely accepted that there should be a minimum difference of about 3 units between the pK_a value

(1) Hirsch, C. A.; Messenger, R. J.; Brannon, J. L. *J. Pharm. Sci.* **1978**, *67*, 231.
(2) Gould, P. L. *Int. J. Pharm.* **1986**, *33*, 201.
(3) Morris, K. R.; Fakes, M. G.; Thakur, A. B.; Newman, A. W.; Singh, A. K.; Venit, J. J.; Spagnuolo, C. J.; Serajuddin, A. T. M. *Int. J. Pharm.* **1994**, *105*, 201.
(4) Anderson, B. D.; Flora, K. P. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press Ltd., 1996; Chapter 34.
(5) *Remington: The Science and Practice of Pharmacy*, 19th ed.; Gennaro, A. R., Ed.; Mack Publishing Co.: Easton, Pennsylvania, 1993; Vol. II, p 1456.
(6) Hammett, L. P. *Chem. Rev.* **1935**, *17*, 125.
(7) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981.
(8) Albert, A. A.; Serjeant, E. P. *Ionization Constants of Acids and Bases*; Wiley: New York, 1984.
(9) Wells, J. I. *Pharmaceutical Preformulation*, 2nd ed.; Ellis Horwood: Chichester, 1993; p 29.
(10) Martindale, W. In *The Extra Pharmacopoeia*, 30th ed.; Reynolds, J. E. F., Ed.; The Pharmaceutical Press: London, 1993.
(11) Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. *J. Pharm. Sci.* **1977**, *66*, 1.

10.1021/op000018u CCC: $19.00
Published on Web 07/19/2000

Table 1. **Classification of common pharmaceutical salts**

salt class	examples
	Anions
inorganic acids	hydrochloride, hydrobromide, sulfate, nitrate, phosphate
sulfonic acids	mesylate,[b] esylate,[c] isethionate,[d] tosylate,[e] napsylate,[f] besylate[g]
carboxylic acids	acetate, propionate, maleate, benzoate, salicylate, fumarate
anionic amino acids	glutamate, aspartate
hydroxyacids	Citrate, lactate, succinate, tartrate, glycollate
fatty acids	hexanoate, octanoate, decanoate, oleate, stearate
insoluble salts	pamoate (embonate), polystyrene sulfonate (resinate)
	Cations
organic amines	triethylamine, ethanolamine, triethanolamine, meglumine, ethylenediamine, choline
insoluble salts	procaine, benzathine
metallic	sodium, potassium, calcium, magnesium, zinc
cationic amino acids	arginine, lysine, histidine

[a] Based on data from various sources.[9−11] [b] Methane sulfonate. [c] Ethane sulfonate. [d] 2-Hydroxyethane sulfonate. [e] Toluene sulfonate. [f] Naphthalene sulfonate. [g] Benzene sulfonate.

of the group and that of its counterion, especially when the drug substance is a particularly weak acid or base. Occasionally, exceptions may be found where a salt has an acceptable stability, despite there being a smaller difference in the pK_a values.

A microplate technique has been developed for the screening of salts; this involves dissolving approximately 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing about 0.5 mg of sample, into each microplate well. Concentrated solutions of each potential counterion in equimolar proportion, or other appropriate stoichiometric ratio, are prepared and a few microlitres of each is added sequentially to each well. Thus, all of the wells in line 1 ($x-$direction) will contain the same combination of sample and counterion 1; all of the wells in line 2 contain the same combination of sample and counterion 2, etc. Different, potential crystallising solvents can be investigated methodically in the $y-$direction. The wells are inspected using an inverted microscope (Leica, Model DMIRB) at regular intervals for the appearance of crystals. Occasionally, crystallisation can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry nitrogen gas.

Once the combinations of counterion and solvent(s) are identified, studies at a slightly larger scale (usually 10−50 mg, occasionally up to 500 mg) can be initiated to confirm the suitability and viability of the crystals. These studies can help identify problems with low melting points, determined by hot-stage microscopy, and hygroscopicity, if processed on a suitable apparatus (e.g., Dynamic Vapour Sorption Analyser, model DVS-1, Surface Measurement Systems Ltd.). Frequently these studies can also give preliminary information on the existence of solvates and hydrates, especially if differential scanning calorimetry (DSC, Mettler Toledo DSC, model 820), thermal gravimetric analysis (TGA, Mettler Toledo TGA, model 850) and hot-stage microscopy are also used in the evaluation process.

In parallel with these studies, a preliminary high performance liquid chromatographic (HPLC) method is quickly developed to give an estimate of the purity of the sample, whilst infrared and other spectroscopic techniques may be used to define the salt and the stoichiometry. Knowledge of the approximate purity is important at this stage as the presence of high levels of some impurities can often hinder crystallisation or alter the polymorphic form obtained.

Therefore, from these preliminary, small-scale studies, a range of potential salt formers and recrysallisation solvents can be quickly identified. Following further scale-up to gram quantities, more comprehensive data can be obtained to evaluate their suitability for use in formulations.

Choice of the Salt Former

Although the choice of salt is governed largely by the acidity or basicity of the ionisable group, safety of the counterion, drug indications, route of administration and the intended dosage form must also be considered. Toxicological and pharmacological implications of the selected salt former must be considered as well as the effects of the parent drug. Salt formers can be subdivided into a number of categories, depending upon their functionality and purpose. Some of the most frequently used examples are listed in Table 1.

The vast majority of salts are developed to enhance the aqueous solubility of drug substances. For weakly basic drug substances, salts of an inorganic acid (e.g., hydrochloride, sulphate, or phosphate), a sulphonic acid (mesylate or isethionate), a carboxylic acid (acetate, maleate or fumarate), a hydroxyacid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. Hydrochloride salts have often been the first choice for weakly basic drugs, since as a consequence of the low counterion pK_a, salts can nearly always be formed, and recrystallisation from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts may include unacceptably high acidity in formulations (e.g., parenteral products), the risk of corrosion, less than optimal solubility due to the risk of salting out and the potential for poor stability if the drug is acid labile and hygroscopic.[2]

Occasionally, salts may be also prepared to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent "Ostwald ripening", for taste-masking, or to prepare an extended release product. Embonate salts have been used in suspension

Table 2. [a] **Preformulation studies that are normally considered for comparison of salt forms and parent compound for oral dosage forms**

test	suitable techniques	comments
dissociation constant and basic physico−chemical properties	potentiometry, solubility, UV spectroscopy	determine pK_a for parent drug
melting point	capillary m.pt., hot stage microscopy, differential scanning calorimetry	perform on each salt and compare to parent
aqueous solubility	overnight equilibration at 25 °C; analysis by UV spectroscopy or HPLC	Perform on each salt and compare to parent
pH of solution		Examine pH of saturated solution if quantities permit.
cosolvent solubility	overnight equilibration at 25 °C, analysis by UV spectroscopy or HPLC	Determine solubilities in ethanol, poly(ethylene glycol), propylene glycol and glycerol and compare to parent.
common ion effect on solubility	overnight equilibration at 25 °C in suitable media and analysis by UV spectroscopy or HPLC	compare solubility in demineralized water with 1.2% NaCl for salts and parent
hygroscopicity	use DVS apparatus or expose to various RH values and measure weight gain after 1 week	perform at 53, 93, and 97% RH, and other values of interest; assign hygroscopicity classification to each salt[13]
intrinsic dissolution rate	use Wood's apparatus[14]	compare dissolution rates at various pHs (can provide data on wettability)
crystal shape and appearance	SEM or optical microscopy	Compare crystal habits and levels of agglomeration
particle size	SEM and laser diffraction	Examine particle size distributions.
polymorphism/pseudopolymorphism	recrystallizations, HSM, DSC, TGA	preliminary exploration
powder properties	bulk density measurement	determine Carr's compressibility index
stability	various	perform on parent drug and undertake preliminary tests on appropriate salts

formulations to increase the duration of action (e.g., chlorpromazine embonate). On some occasions, the selection of a salt with only modest aqueous solubility may be more suitable for use in tablet products prepared by wet granulation since the use of highly soluble salts can be detrimental to the granulation process. Depending on the dose required, aqueous solubilities in the range 0.1−1.0 mg/mL will normally be sufficient to satisfy the dissolution requirements for standard, solid, oral dosage forms of drugs with good to moderate potency. However, for parenteral solution products, higher solubilities, perhaps 10 mg/mL or greater, depending on the required dose and dose volume, may be required. For parenteral formulations, the pH of solution (normally within an acceptable range of 3−10 for intravenous solution) should be monitored to help ensure that the formulation will be well tolerated.

Salts are also frequently prepared for the reasons other than solubility modification; it is frequently necessary to prepare a specific salt to either achieve adequate physical stability or for taste masking (e.g., dextropropoxyphene napsylate suspension). Manipulation of drug substance solubility by selection of salts may also be employed to modify the pharmacokinetic profile of the drug (e.g., benzathine penicillin and insulin zinc complexes used in parenteral formulations). Salt formation may be also advantageous where the melting point of the active moiety is low, and it is necessary to mill or micronise the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50−100 °C higher than the free acid or free base. Also, being more ionic, the crystals are likely to be less plastic and more easily deformed by brittle fracture.

Scale-up of the Formation of Salts

The information from the preliminary crystallisation studies is communicated to the Process Chemistry group, who by this time will have started their investigations into possible manufacturing routes for each of the candidates remaining. At this stage in the development process, Process Chemistry usually aim to quickly manufacture 50−200 g of the one or two candidates that may remain to progress them towards initial clinical evaluation. The manufacturing route may be the same as used by the Discovery Chemistry group but usually is significantly different. The aims of both the Process Chemistry and Preformulation groups for the following 12−18 months is to collaborate extensively to ensure that, for the chosen candidate, there will be a viable synthetic route to the chosen form of the drug substance.

A significant portion of this batch is destined for the preparation of 3−4 g of each of the salts that were thought to be viable from the smaller-scale studies. A similar sized portion of the free base/acid is also taken for comparison purposes. The combination of individual studies undertaken on each of these 3−4 g portions varies depending on the type(s) of dosage form ultimately required for marketing. Occasionally, it may be necessary to undertake a pharmacokinetic evaluation of each salt in comparison with the free acid/base. The dosage forms most commonly used for the drug substances encountered during preliminary clinical investigations are tablets/capsules, inhalation dosage forms and injections.

Table 3. **Tests to be considered for the evaluation of candidate salts**

test to be considered	amount required, mg
Structural Analysis	
mass spectroscopy[a]	1
^{1}H NMR[a]	5
^{13}C NMR[a]	25
Ir spectrum	1
UV spectrum	1
fluorescence spectrum[a]	1
elemental analysis	10
Physicochemical Properties	
melting range	2
pK_a [a]	5
C log *P*/log *P* [a]	5
preliminary polymorphism study	200−500
X-ray diffraction	20
aqueous solubility[b]	100
pH − solubility profile	500
cosolvent solubilities[c]	300
propellant solubility[d]	500
Physical Properties	
hygroscopicity	20
microscopy (SEM/optical)	10
particle size (Malvern)	100
size reduction (sonication)	300
Impurities (hplc)	
related substances[a]	10
degradation products[a]	10
chiral purity[a]	10
Stability Studies	
stability to hydrolysis (pH 2, 7, 10)[a]	15
stability to oxidation (peroxide/peracid)[a]	15
stability to photolysis[a]	15

[a] Determined on free acid/base only. [b] Would include solubility in saline, 5% dextrose and some buffers [c] Also solubilities in complexing agents/surfactant systems where appropriate [d] Propellants and propellant/cosolvent systems for inhalation dosage forms.

Tables 2 and 3 show the types of tests normally chosen, the information that they can produce and the amount of sample normally required for these common dosage forms.

What to Develop: Salt or Free Acid/Base?

The results obtained from each of these tests are tabulated for the free acid/base, together with each of the salts, and discussed in detail between the Formulation Scientists, Preformulation Analysts, Physical Chemists, Process Chemists, and occasionally Pharmacokineticists. The Preformulation Scientists assess the relative merits of each form for use in the proposed clinical formulations and whether the properties such as solubility are adequate to give the high concentrations required in the various pre-clinical formulations. Process Chemistry need to assess the likely yield of each salt, as salt formation creates an additional step in the manufacturing process. Usually, the decision-making process results in the proposal of a single salt for further study, although occasionally it is seen that none of the salts have optimum properties, and two different salts can be proposed for in-depth study. Also, it is occasionally found that the overall properties of the free acid/base are much better than any of the salts. This occurs more frequently where the candidate has a low pK_a value and the resulting salts are less stable than required or when the salts are particularly hygroscopic or when they exhibit complex polymorphism/pseudopolymorphism (hydration or solvation).

These relatively simple investigations give much useful information very quickly; it should be noted, however, that the preliminary polymorphism study is far from the in-depth study that is always undertaken later. This preliminary study uses a range of protic and aprotic solvents of widely differing polarity and will normally show the presence of a stable hydrate or solvate.

Once a decision is agreed upon within the group, a document that gives a précis of the discussions and the basis for the proposal is normally drafted for agreement by senior management. Examples of these salt selection studies are given below:

Example No. 1 (RPR 111423)

RPR 111423 is a candidate drug substance that has been evaluated for the treatment of symptoms related to infection by AIDS. It is a crystalline, very weak base with a pK_a at 4.25. A comprehensive screening of possible salts demonstrated only a monohydrochloride (RPR 111423A) and a mesylate (RPR 111423B) could be isolated as crystalline solids.

It was decided that the free base should be taken through the simple evaluation process in comparison with these two salts. It was expected that the drug substance could be required in the form of tablets or capsules, with an injection form needed for some pre-clinical studies and for the determination of absolute bioavailability in man. Because of its high activity in screening studies, there was a possibility that very low dose oral formulations might be needed. This may require micronised drug substance to enable content uniformity requirements to be met; this micronised material would also be expected to enhance dissolution.

The results from the relatively simple studies undertaken are given in Table 4. The two salts clearly demonstrated the predictable problems associated with a relatively low pK_a value; the salts were quite weak and dissociated to liberate the free base in media with pH values below the pK_a. The very low solubility of the free base resulted in immediate precipitation following dissociation. There was clear evidence for multiple polymorphism for each of the salts, and establishing the existence of a stable polymorph, or a suitable pseudopolymorph, may have been necessary before a decision could be made on which of the two salts could be developed further.

The corresponding results for the free base indicated that it appeared to be the better candidate; it showed no evidence of polymorphism, and it was not hygroscopic. The two major areas that required further investigation were whether it had sufficient solubility in gastrointestinal media and whether it could be micronised. Studies performed on samples of drug substance and on simple capsule formulations demonstrated that the dissolution rates of micronised free base were equivalent or superior to those of the salts under the same conditions.

Table 4. **Comparison of some simple properties of RPR111423 and its two salts**

test	result for RPR 111423 (base)		result for RPR 111423A (hydrochloride)		result for RPR 111423B (mesylate)	
appearance	off-white to cream, crystalline powder		pale yellow, highly agglomerated powder		cream to pale yellow, highly agglomerated powder	
particle size by microscopy, μm	10−100 (large rhombic crystals)		2 × 1 (microcrystalline laths)		7 × 1 (microcrystalline laths)	
melting range, °C	241−244		242		210	
preliminary polymorphism study	no other form detected		at least four polymorphs detected; metastable forms revert to original on standing		at least six polymorphs detected; phase changes detected on grinding or micronisation; reverts to original form on heating	
other thermal behavior	nothing detected		loss of HCl detected at 110−120 °C		nothing detected	
aqueous solubility, mg/mL	at 25 °C	at 37 °C	at 25 °C	at 37 °C	at 25 °C	at 37 °C
- at pH 1	11.6	14.7	25.7	28.2	131.4	204.1
- at pH 2	0.71	0.89	2.51	4.58	6.11	8.91
- at pH 4	0.03	0.05	0.05	0.13	0.01	0.02
- at pH 6	0.01	0.02	0.01	0.02	0.03	0.34
- at pH 6,8	0.01	0.02	0.01	0.02	0.01	0.02
- in demineralized water	0.01	0.02	0.36	0.99	0.33	0.50
pH of saturated solution, at 20 °C, in water	6.50		2.43		2.74	
addition of water to concentrate						
- at pH 2	no changes detected		some precipitation of free base		some precipitation of free base	
- at pH 4	no changes detected		extensive precipitation of free base		extensive precipitation of free base	
hygroscopicity (hygrostat for 14 days)	non-hygroscopic <0.2%w/w water uptake at any RH		slightly hygroscopic 2.3% w/w uptake at 53% RH 22% w/w uptake at 97% RH		moderately hygroscopic 3.7% w/w uptake at 53% RH 32% w/w uptake at 97% RH	

Example No. 2 (RPR 127963)

RPR 127963 is a candidate drug substance that has been evaluated for the treatment of cardiovascular diseases; it is a crystalline, very weak base with a pK_a at 4.10. In common with most similar drug substances intended for the treatment of cardiovascular disease, it was considered that a high-dose (up to 250 mg) solid, oral dosage form and a correspondingly high-dose (up to 50 mg/mL) injection would be ultimately required. In line with our standard protocol, a comprehensive evaluation of possible salts was undertaken, and this demonstrated that five crystalline salts (a hydrochloride, a mesylate, a citrate, a tartrate, and a sulphate) could be readily produced. It was decided to quickly profile each of these salts in comparison with the free base. The results of these studies are given in Table 5.

When the anhydrous free base was evaluated, the existence of an additional mono-, di-, and trihydrate was found quite rapidly. It was shown that all four of these forms could be interconverted under conditions that might be expected to be found in granulation processing. The other potential problem with the anhydrate was the low melting point. In considering the results obtained for the various salts, the solubilities of the citrate and the tartrate were much lower than required for an injectable form and lower than ideal for high dosage formulations. An additional problem for the tartrate salt was the high hygroscopicity. Both of these salts were rejected before completion of the full evaluation. The hydrochloride salt was also shown to have several problems such as lower than ideal solubility, probable multiple polymorphism, and the formation of hydrates.

Thus, the mesylate and the sulphate were the two salts that remained; both had high melting points, excellent aqueous solubility, and were non-hygroscopic. The free base still remained a possible candidate, if a stable hydrate could be found. It was therefore decided to undertake some additional evaluations on these three forms; the results from these are presented in Table 6.

These additional results demonstrate a slight advantage in favour of the sulphate salt because of its greater solubility in cosolvents. This would give the formulator a better chance of achieving a higher dose in an injectable formulation. It was considered that the sulphate salt (RPR 127963E) could be studied further in the more detailed evaluations that would follow over the next few months. The mesylate or the free base (if a suitably stable hydrate could be found) would provide a possible back-up, should unforeseen problems arise.

Example No. 3 (RPR 200765)

RPR200765 is a candidate drug substance proposed for the treatment of rheumatoid arthritis. It is another crystalline, weak base with a pK_a of 5.3 which formed salts with a wide selection of counterions. It was expected that doses of 100−125 mg of RPR200765 in capsules would be required for clinical studies.

Early studies suggested that RPR200765 free base was unacceptable for use in solid, oral dosage forms due to a very poor aqueous solubility of approximately 10 μg/mL and poor bioavailability in animal models. However, RPR200765 would form stable salts with hydrochloride, hydrobromide,

Table 5. **Comparison of some simple properties of RPR127963 and its five salts**

test	result for free base (RPR 127963)	result for HCl salt (RPR 127963A)	result for mesylate salt (RPR 127963B)	result for citrate salt (RPR 127963C)	result for tartrate salt (RPR 127963D)	result for sulfate salt (RPR 127963E)
appearance	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder
particle size (microscopy), μm	1−3 μm (agglomerates of microcrystals)	1−3 μm (agglomerates of microcrystals)	tightly packed spherulites of agglomerated microcrystals 18 μm diameter.	microcrystals (2−3 μm) with some aggregates (70 μm)	rounded agglomerates of microcrystals in domains (70 μm)	aggregates of microcrystals (10−15 μm)
melting range, °C	119−123	166−191 (re-grows at about 166, recrystallizes at 191, then melts at about 275	280.9−282.2	130.2−134.3	198.5−201.6	305.7−308.9
preliminary polymorphism study	several hydrates detected	two monohydrates and one anhydrate	no evidence of polymorphs	stable hemihydrate detected	unstable anhydrate	no evidence of polymorphs
aqueous solubility (25 °C), mg/mL						
- in demineralized water	n.d.[a]	3.92	108	0.83	0.89	∼50
- in 0.1 M HCl	n.d.	5.2	50.4	n.d.	n.d.	5.9
- in 0.1 M NaOH	0.020	0.019	0.022	n.d.	n.d.	0.018
- in dextrose 5%w/v	n.d.	2.84	90	n.d.	n.d.	∼40
pH of saturated solution	n.d.	2.33	1.76	2.49	2.56	1.32
hygroscopicity	n.d.	non-hygroscopic	non-hygroscopic	non-hygroscopic	very hygroscopic	non-hygroscopic

[a] n.d. = Not determined.

Table 6. **Comparison of additional properties of RPR127963 (anhydrate), its mesylate (RPR 127963B) and sulfate (RPR 12963E) salts**

test	result for free base anhydrate (RPR 127963)	result for mesylate salt (RPR 127963)	result for sulfate salt (RPR 127963)
solubility in cosolvents at 25 °C, mg/mL			
ethanol	190	0.6	0.2
propylene glycol	35.4	0.7	1.7
poly(ethylene glycol) 400	188	0.2	0.2
dimethylsulphoxide	> 500	14	110
N-methylpyrrolidone	> 400	4.4	8.5
glycerol	42	n.d.[a]	2.7
peanut oil	0.18	none detected	none detected
intrinsic dissolution rate, $mg \cdot min^{-1} \cdot cm^{-2}$			
- in water	0.01	n.d.	n.d.
- in 0.01 M HCl	0.35	7.3	7.7
powder flow properties	n.d.	Good, but becomes much worse with increasing humidity	Sticks slightly

[a] n.d. = Not determined.

methanesulfonate, and camphorsulfonate counterions. Aqueous solubility, particle size and shape, powder properties, and polymorphism profile were considered to be the key properties to permit a choice of salt to be made. In addition, it was recognised that the use of some counterions with high molecular weights, would require a large excess of drug substance to achieve the required doses.

Studies demonstrated that the solubility of RPR200765 depended on the amount of drug substance used for the study. This occurred because the counterion reduced the pH of solution and enhanced solubility of the drug base. The mesylate salt consistently produced a higher solubility than any of the other salt forms. The higher solubility resulted in an enhanced dissolution rate of the mesylate salt compared to the other salt forms. The solubility and dissolution rate of the hydrobromide salt was particularly poor. Intrinsic dissolution rate studies on compressed disks could not be carried out because a good compact could not be obtained for most of the salts, and the studies were carried out using drug powder (equivalent to 50 mg free base) in capsules.

Hygroscopicity studies demonstrated that the hydrochloride and hydrobromide salts adsorbed large amounts of moisture on exposure to humidity, resulting in the formation of multiple hydrated forms. The methanesulfonate salt however, was a stable monohydrate form which lost moisture at very low humidity (<10% relative humidity (RH)) but

Table 7. **Comparison of the physicochemical properties of RPR200765 salt forms**

test	result for mesylate salt (RPR 200765A)	result for camphorsulfonate salt (RPR 200765C)	result for hydrochloride salt (RPR 200765D)	result for hydrobromide (RPR200765E)
appearance	off-white to cream, free-flowing powder	white to off-white, crystalline, free-flowing powder	white, free-flowing powder	white to off-white, crystalline, free-flowing powder
MW	566.61	684.79	524.98	569.43
melting range, °C	214	265−267	245−248	276−277
maximum aqueous solubility at 25 °C, mg/mL	39	19.95	16.68	3.29
pH of saturated solution in demineralized water at 20 °C, mg/mL	1.93	2.22	2.16	2.63
hygroscopicity (by DVS)	non-hygroscopic with a stable, monohydrate form	non-hygroscopic	hygroscopic with multiple hydrated forms	hygroscopic with multiple hydrated forms
crystal habit and appearance	individual platelike crystals with some agglomeration.	clusters of highly aggregated, platelike crystals	platelike crystals; individual crystals contain stress lines	loosely agglomerated, flaky material
particle size by microscopy	∼45−200 μm in agglomerates of 200−350 μm	crystals ∼20−50 μm, clusters ∼80−200μm -some larger clusters up to 500 μm	∼30−100 μm particles	10−40 μm particles
dissolution studies, drug substance in capsule ($T_{80\%}$, min)				
at pH 2				
at pH 4 (in citrate buffer)	2.0	6.0	7.4	3.9
	>60% release	>60% release	>60% release	14% release

rapidly re-equilibrated to form the monohydrate form when the humidity was raised. These findings suggested that this salt would be amenable to solid dose formulation and there was little risk of changes in the hydration state on processing or storage under normal conditions. The camphorsulfonate was non-hygroscopic. The results of these studies are outlined in Table 7; in this case very little comparative work was undertaken on the free base due to the poor solubility and bioavailability.

Overall, the studies suggested that the mesylate salt was the favoured form on the basis of its low hygroscopicity, clean polymorphic profile in the preliminary screen, high solubility, and rapid dissolution rates. Another favourable factor supporting the selection of the mesylate salt proved to be the good flow properties which allowed very satisfactory capsule and tablet formulations to be developed.

The Next Steps?

Having evaluated several possible alternatives in the three cases above, using a relatively simple range of tests, a form of the drug substance has been chosen that should be possible to develop further. These simple studies have required 3−4 g of the free base and a similar quantity of each of the salts; the data for all forms normally can be generated in one month, or less. The next steps involve confirmation of the choice, by employing a further range of tests, followed by the optimisation of the form of the salt. A series of tests, analogous to those in Table 2, are used in this evaluation; these tests are given in Table 8.

Optimisation of the Drug Substance Form for Development

Having chosen what should be a reasonably stable form of the salt, free acid, or free base, one of the key activities is to start investigations into which other polymorphic or pseudopolymorphic forms exist. In the short development phase where preclinical administration occurs, only a preliminary screening of these different forms is considered necessary, as it is possible that the compound can be found too toxic for further study. Our team undertakes this on about 500 mg of sample; small portions are recrystallised from anhydrous and hydrated solvents of differing polarity. Any crystalline product recovered is examined by a variety of techniques to determine how many different forms are produced and whether any are hydrates or solvates. Preliminary information on the inter-relationships between the different forms can often be found, even at this early stage.

The remainder of the tests are designed for two main purposes:

To define the various preclinical formulations that are required, to devise analytical methods for these, to determine their stability, and establish shelf lives.

To establish a database for the chosen form and to give an indication of the possibilities for clinical formulations.

To accomplish this, it is normal to request a minimum of 25 g of drug substance, although occasionally more may needed if the drug substance is intended for inhalation and there are difficulties with micronisation.

Table 8. **Tests to be considered for "preclinical phase" (column 2) and in preparation for initial clinical investigation (column 3) for compounds intended for use in oral, injection and inhalation products**

test to be considered	amount required, mg or g	amount required, mg or g
	Physicochemical Properties	
melting range	50 mg	50 mg
optical rotation		1 g
polymorphism	500 mg	25−50 g
X- ray diffraction	20 mg	20 mg
intrinsic aqueous solubility	400 mg	
cosolvent solubilities[a]	500 mg	2 g
propellant solubility[b]		2 g
	Physical Properties	
hygroscopicity	800 mg	-
microscopy (SEM/optical)	100 mg	100 mg
particle size (Laser)	200 mg	200 mg
micronisation	5 g	
specific surface area	2 g (R)	4 g (R)
true density	200 mg (R)	200 mg (R)
bulk powder density	2.5 g (R)	10 g (R)
wettability		1 g
	Impurities (HPLC)	
related substances	10 mg	10 mg
degradation products	10 mg	10 mg
chiral purity	10 mg	10 mg
electrophoresis/TLC	10 mg	
	Stability Studies	
hydrolytic profile (identify degradants)	100 mg	
bulk drug powder		2 g
	Excipient Compatibility	
HPLC, XRPD, and DSC	50 mg	250 mg
	Compression Properties	
for dry powder inhaler only		5 g
	Preclinical Formulation Development	
intra-tracheal suspensions	2 g	
oral solutions/suspensions	2 g	
solutions for nebulization	2 g	
IV solutions	2 g	
other routes (ip/sc)	1 g	-
	Clinical Formulation Development	
	predict suitable dosage forms 3 g	Phase I−IIa[c] 250−1200 g
microbiological controls		*d*
total substance requirements	20−25 g	depends on form and dose

[a] Also solubilities in complexing agents/surfactant systems where appropriate. [b] Propellants and propellant/cosolvent systems for inhalation dosage forms. [c] Develop and specify Phase I formulation − commence stability/compatibility studies. [d] Dependent on drug availability; (R) possible to recycle drug substance for certain other tests.

Once the drug substance is shown to be nontoxic, studies leading to the definition of a suitable series of clinical formulations can begin. For this, we normally expect to have a reasonably clear picture of the inter-relationships between the different forms of the drug substance and should have started to define the most stable form. As more batches are manufactured at increasing scale by Process Chemistry, they are examined using some of the key tests to add information to the database. Also, with the increased availability of drug substance, it is possible to initiate maturation studies as an additional technique to assist in the definition of polymorphism. If the structure of the drug substance has been determined by single crystal X-ray, under certain circumstances it may be possible at this stage to initiate the theoretical search for other polymorphic forms. This is achieved using the Polymorph Predictor software (Molecular Simulations Inc.). This software has been used successfully on several small molecules (molecular weight <500) and predicts theoretical crystal structures and their relative energies. The most stable form has the lowest energy; increasing energy signifies lower stabilities.

As larger quantities of drug substance and samples from different batches become available it is imperative that the variation in basic physical properties (e.g., crystal size and shape, specific surface area, powder flow properties, bulk and tapped density etc.) are studied for each batch. By close liaison with Process Chemistry, it is normally possible to modify the recrystallisation conditions such that greater batch-to-batch uniformity of these physical characteristics can be achieved. Also, these characteristics can often be modified such that they are closer to ideal.

The Negative Aspects of Salt Formation

One of the negative aspects of salt formation is that the percentage active content decreases markedly as higher-molecular weight counterions are used. If the free acid or base has only moderate or low activity, it may be necessary for the patient to have a relatively high dose for a clinical effect. If 20−50% of the weight of the drug substance is due to inactive counterion, the addition of suitable excipients for encapsulation or tableting may result in a powder volume that is too great, even after granulation, to fit successfully into even the largest acceptable capsule shell. This forces the formulator towards a tablet. Even with these formulations, a large tablet (or even multiple, smaller tablets) may be necessary; these do not aid patient compliance.

Other problems that are frequently created, or exacerbated, by salt formation are an increased tendency for the existence or formation of hydrates and polymorphs. Hydrates may be produced in formulations by interaction with water bound to excipients, water in capsule shells, etc.

Final Definition of the Form

As the candidate passes through initial clinical evaluation (Phases Ia and Ib), additional characterisation and refinement of the drug substance form continues in parallel with the finalisation of studies on the drug substance manufacturing process. Close liaison and teamwork between the Process Chemist and the Preformulation Scientist in an exploration of the various possible recrystallisation solvents can often result in further refinement of the crystal properties. An excellent scheme for the final characterisation of solid drug substances, prior to the final regulatory submission, has been described recently.[12] The aim of both the Preformulation and Process Chemistry teams is to finalise the definition of all of the characteristics of the drug substance in readiness for the initiation of Phase IIa clinical trials

Acknowledgment

We thank our colleagues for their scientific contributions made during these studies and for the stimulating discussions held.

Received for review February 24, 2000.

OP000018U

(12) Byrn, S.; Pfeiffer, R.; Ganey, M.; Hoiberg, C.; Poochikian, G. *Pharm. Res.* **1995**, *12* (7), 945.
(13) Callahan, J. C.; Cleary, G. W.; Elfant, M.; Kaplan, G.; Kensler, T.; Nash, R. A. *Drug. Dev. Ind. Pharm.* **1982**, *8*, 355.
(14) Wood, J. H.; Syarto, J. E.; Letterman, H. J. *J. Pharm. Sci.* **1965**, *54*, 1068.