

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 33
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogado(a)/Señor(a)
HELENA CAMARGO WILLIAMSON

Asunto: Radicación: 13-109274- -4
Trámite: 11
Evento: 378
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Oficio: 6656
Folios: 33

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2011/070099				
Fecha de Presentación Internacional	15/11/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No 13-109274-00000-0000	30/Abril/2013
Reivindicaciones:	1 – 11 del radicado No 13-109274-00000-0000	30/Abril/2013
Prioridad:	DE 10 2010 044 131.7	18/Noviembre/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

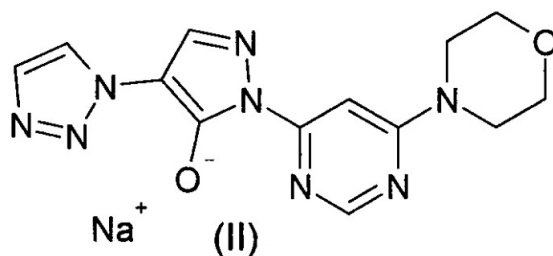
De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Dado que en la presente solicitud se evidencia pago por la reivindicación adicional, se procede a estudiar todo el capítulo reivindicatorio.

Título de la solicitud: “1H-PIRAZOL-5-OLATO SÓDICO SUSTITUIDO”.

El problema planteado en esta solicitud consiste en proporcionar 1-[6-(morfolin-4-il)pirimidin-4-il]-4-(1H-1,2,3-triazol-1-il)-1H-pirazol-5-olato de sodio, adecuado contra enfermedades cardiovasculares y hematológicas, renales y en curación de heridas.

Para resolver este problema técnico la solicitud en estudio proporciona 1-[6-(morfolin-4-il)pirimidin-4-il]-4-(1H-1,2,3-triazol-1-il)-1H-pirazol-5-olato de sodio y procedimiento para su preparación.



El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D 403/14 AC; A61P 9/00 AC; A61K 31/506.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 6 – 9 y 11 no es patentable, de acuerdo con el Art 14.

5. De la solicitud de patente

Título

El título no corresponde con el objeto de la solicitud porque es demasiado amplio, por lo cual se sugiere al solicitante hacer la corrección necesaria, por ejemplo colocando el nombre del compuesto en particular reclamado.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 18 de noviembre de 2010.

Consultadas las fuentes de información con que se cuenta, se encontró (encontraron) el (los) siguiente (s) documento (s) que anticipa (n) el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de presentación
D1	WO2008067871	"Substituted dihydropyrazolones for treating cardiovascular and haematological diseases"	12/Junio/2008
D2	Libro	Handbook of pharmaceutical salts	2002

El documento D1 ¹ (resumen y reivindicaciones) divulga derivados de dihidropirazolona sustituidos , procesos para su preparación y su uso contra patologías cardiovasculares y hematológicas, entre otros.

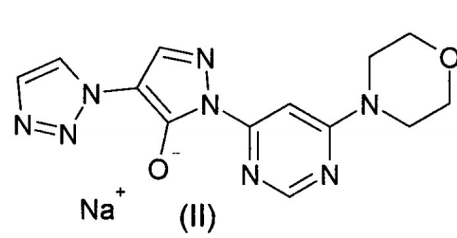
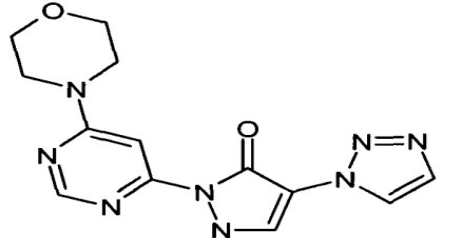
El documento D2 ² (págs. 162-177 y 183-188; págs. 19 y 28; tablas 6 y 7) divulga ácidos y bases empleados en la formación de sales farmacéuticamente aceptables, así como el mejoramiento de las propiedades fisicoquímicas y mecánicas de los fármacos mediante la formación de sales.

7. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en "1-[6-(morfolin-4-il)pirimidin-4-il]-4-(1H-1,2,3-triazol-1-il)-1H-pirazol-5-olato de sodio de acuerdo con el compuesto de fórmula II..." (ver pág. 33 (reivindicación 1) del radicado No. 13-109274-00000-0000)

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Sal sódica	No señala en particular la sal sódica pero menciona: o una de sus sales (ej. sal sódica o potásica) (ver pág. 12, líneas 19 y 20 y ejemplo 71)	Sales de compuestos activos tales como sal de sodio (ver tablas 6 y 7)
		de principios activos farmacéuticos

El documento D1 es considerado el estado de la técnica más cercano a la invención definida en la reivindicación 1, ya que revela derivados de dihidropirazolona sustituidos, procesos para su preparación y su uso contra patologías cardiovasculares y hematológicas, entre otros (D1: pág. 12, líneas 19 y 20 y ejemplo 71).

1URL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=1&at=55&locale=en_EP&FT=D&CC=WO&NR=2008067871A1&KC=A1)

DB=EPODOC&II=0&ND=1&at=55&locale=en_EP&FT=D&CC=WO&NR=2008067871A1&KC=A1

2 Handbook of pharmaceutical salts. Properties, selection and use; Chapter 12 "Monographs on acids and bases"; Wiley-VCH; 2002; pgs. 264-267, 283, 284, 290-293 y 300; Chapter 7 "A procedure for salt selection and optimization"; pgs. 162-177 y 183-188; pgs 19 y 28.

La diferencia entre el compuesto de la invención, y lo revelado en D1, consiste en que se está reclamando en particular 1-[6-(morfolin-4-il)pirimidin-4-il]-4-(1H-1,2,3-triazol-1-il)-1H-pirazol-5-olato de sodio, es decir la sal sódica del compuesto revelado en la anterioridad.

Sin embargo, el que la sal del compuesto conocido sea una sal sódica no concede ningún efecto técnico inesperado o una ventaja técnica y esto se puede demostrar a partir del arte previo, en donde se señala que para el compuesto del ejemplo 72 la actividad inhibidora de HIF prolil hidroxilasa representada en valores IC_{50} (μM) es de 0.49 (D1: ver tabla 1). Es decir, que la sal clorhidrato se comporta igual, en cuanto a su actividad, que la sal sódica porque en el capítulo descriptivo de la presente invención (pág. 27, última línea) se menciona que el compuesto de fórmula (II) de acuerdo con la invención inhibe la actividad de la HIF-prolilhidroxilasa en este ensayo con un valor de 0,47 μM . Lo anterior demuestra que la sal reclamada en la presente invención no tiene ningún efecto técnico sorprendente o inesperado frente a otras sales.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: ¿cómo modificar el compuesto 2-(6-morfolin-4-ilpirimidin-4-il)-4-(1H-1,2,3-triazol-1-il)-1,2-dihidro-3H-pirazol-3-ona, para obtener la sal sódica del mismo?

El documento D2 divulga la utilización de múltiples bases, tales como las bases fuertes de sodio, para la formación de una sal sódica farmacéuticamente aceptable (D2: págs. 264-267, 283, 284, 290-293, 300 y tablas 6 y 7). El documento D2 también enseña que el mejoramiento de la velocidad de disolución de fármacos que son ácido o bases débiles poco solubles es uno de los principales motivos para la formación de sales (D2: pág. 19 y 28), que la selección y la preparación de una sal adecuada da la oportunidad de modificar características fisicoquímicas y mecánicas de los fármacos que permiten el desarrollo de formas farmacéuticas con mejor biodisponibilidad, estabilidad, entre otros parámetros variables (D2: pág. 163) y asimismo, da a conocer que la elección de una sal en particular está determinada por la acidez o basicidad del grupo ionizable, la seguridad del grupo potencial para la formación de la sal (contra-ión), indicaciones del medicamento, ruta de administración y la dosis, ya que la mayoría de las sales se desarrollan con el fin de aumentar su solubilidad (D2: pág. 167). Por otra parte la anterioridad D1 ya revelaba que el compuesto 2-(6-morfolin-4-ilpirimidin-4-il)-4-(1H-1,2,3-triazol-1-il)-1,2-dihidro-3H-pirazol-3-ona, se podría encontrar en cualquiera de sus sales, solvatos y solvatos de las sales, y particularmente señala (D1: pág. 12, líneas 18 y 30), que los compuestos se encuentran en sales de bases convencionales, tales como a manera de ejemplo y preferiblemente sales de metales alcalinos (ej. sales sódica y potásica), entre otras.

Por lo anterior, una persona normalmente versada en la materia, al observar el compuesto conocido de la anterioridad D1, junto a sus conocimientos básicos en química orgánica y en las sales farmacéuticamente aceptables reveladas en D2, habría podido “proponer” alguna de sus sales, como ocurre con la sal sódica de la presente invención, como una alternativa para llegar a la reivindicación 1 y por tanto se considera obvia. En consecuencia la reivindicación 1 no cumple el requisito de nivel inventivo.

En cuanto a las reivindicaciones dependientes es necesario mencionar que tampoco contienen características técnicas adicionales que hagan inventiva la invención, porque se presentan reivindicaciones que reclaman procesos de preparación, pero que simplemente se refieren a una reacción entre el compuesto de fórmula (I), que presenta equilibrio ceto-enol, y que por sus características cuando se encuentra en su forma de alcohol se comporta como un ácido débil, que puede donar fácilmente su protón de hidrógeno y reemplazarlo por uno que sea mejor atraído por la fracción nucleofílica O- que presenta la molécula cuando se encuentra reaccionando con una base como la propuesta, es decir una base sódica. Estas reacciones son tan comúnmente conocidas por cualquier versado en la materia, que ni siquiera se describe en la anterioridad, porque se trata de revelaciones implícitas cuando menciona el documento del estado del arte (en este caso aplicable a D1), que las moléculas pueden estar en forma de su sal sódica. Es más, en cualquier texto relacionado con el campo químico orgánico ³ pueden encontrarse un amplio número de ejemplos de reacciones de este tipo que no constituyen ningún avance científico ni desarrollo sorprendente sino que por el contrario son consideradas de rutina y por tanto las reivindicaciones dependientes 2 – 5 no cumplen el requisito de nivel inventivo.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2008067871	1 – 5	Nivel Inventivo
D2	Handbook of pharmaceutical salts	1 – 5	Nivel Inventivo

³ **Morrison y Boyd**. “Química Orgánica”. Ed Pearson. Quinta Edición. New York. 1998. Cap. 17.1, pág. 621 – 685.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

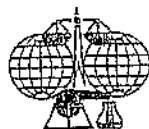
Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-06-03

Elaboró: Nelson Ricardo Velasco Gonzalez
Revisó: Gloria Jacqueline Alfonso Roza



International Union
of Pure and Applied Chemistry (IUPAC)

Handbook of
Pharmaceutical Salts
Properties, Selection, and Use

P. Heinrich Stahl, Camille G. Wermuth (Eds.)



Verlag Helvetica Chimica Acta · Zürich



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Chapter 2

Solubility and Dissolution of Weak Acids, Bases, and Salts

by Madhu Pudipeddi*, Abu T. M. Serajuddin, David J. W. Grant,
and P. Heinrich Stahl

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1. Introduction

Improvement of dissolution rate of weakly acidic or weakly basic drugs that are poorly soluble is one of the primary reasons for preparation of pharmaceutical salt forms [1–7]. Salt forms have also been used to control drug dissolution [8] [9]. This chapter examines the pH-dependencies of the solubility and dissolution rate of pharmaceutical acids, bases, and salts, and the relevance of these dependencies to the selection of the final form.

2. Solubility Behavior of Acids, Bases, and Their Salts

2.1. Measurement of Aqueous Solubility

The solubility of an ionizable compound as a function of pH, known as the pH-solubility profile, can be determined by phase-solubility techniques [10] [11]. Saturated solutions of a free acid, base, or salt are prepared by shaking an excess of the solid with an appropriate volume of deionized water at controlled temperature. Solubilities at various pH values are determined by stepwise titration of these suspensions with a relatively strong acid or base. After each addition, equilibrium is re-established by agitation. The pH of the suspension is measured, and the supernatant solution phase is analyzed for total solute concentration. The process is continued until the entire pH-solubility profile is obtained. The ionic strength of the system is not controlled in this method.

Alternatively, buffer solutions of suitable ionic strength may be used to maintain the desired pH conditions [12–14]. However, inadequate buffer capacity and suppression of solubility due to ionic-strength effects have been noticed when buffers are used for pH-solubility studies [12]. Salt formation with buffer species may also occur when buffers are used to control pH. Recently, an automated potentiometric method has been described for determination of pH-solubility profiles [15].

Establishment of equilibrium must be confirmed in all cases by verifying the constancy of solubility values at various times during equilibration. The solid phase in equilibrium with the saturated solution (referred to as 'excess solid') must be verified at selected pH values to identify potential solid-state transformations, such as polymorph/hydrate formation, or conversion of salt to free acid or base, or *vice versa*. In a routine preformulation operation, thermal analysis of the excess solid may provide a simple means to identify solid-state transformations, but powder X-ray diffractometry is usually more reliable. Elemental analysis may also be performed for further identification of the solid phase.

2.2. General Features of pH-Solubility Profiles

Fig. 1 shows a classical pH-solubility profile for a weakly acidic compound, *Flurbiprofen*, with the two key regions marked as *Region I* and *II* [2] [16]. In *Region I* (pH < 7.3 in Fig. 1), the excess solid phase in equilibrium with the saturated solution is the free acid. In *Region II* (pH > 7.3), the excess solid phase is the sodium salt. In *Region I*, the total solubility is described by the following equations:

$$S = [\text{HA}] + [\text{A}^-] \quad (1)$$

mation. Further confirmation of the nature of the excess solid phase may be obtained by powder X-ray diffractometry or elemental analysis. If the excess solid phase is entirely in the free form, the experiment must be repeated with a higher solid to solvent (H_2O) ratio until the excess solid contains the salt. In some cases, complete conversion of the salt to the free form may occur at all solid-to-solution ratios, often leading to formation of a 'gel'.

As a result of the above phenomenon, in some cases, an attempt to make a solution of a 1:1 salt at a concentration well below the solubility of the salt (as estimated from the K_{sp} value) will be unsuccessful due to precipitation of the free unionized form. To further develop this point *Anderson and Flora* [2] defined the term 'stoichiometric solubility'. The stoichiometric solubility of a 1:1 salt is the concentration of salt which can be dissolved in pure H_2O with no formation of a precipitate of the unionized free form. The authors pointed out that the difficulties of low 'apparent solubility' of a salt due to free form precipitation may be encountered in the preclinical stages of development when concentrated solutions in H_2O are required for toxicology testing. However, appropriate pH adjustment should overcome this problem.

3. Dissolution Behavior of Acids, Bases, and Their Salts

The term *dissolution* refers to the overall process by which a solid compound dissolves in a liquid medium, while *dissolution rate* is the kinetic descriptor giving the rate at which the dissolution takes place. The concept of solubility, on the other hand, implies that the process of dissolution has reached equilibrium, and the solution is saturated. Dissolution rate of solids is of paramount importance in the development of pharmaceutical products and quality control. Salt formation is one of the most commonly employed techniques to improve dissolution of weakly acidic or basic drugs.

A number of theories of the dissolution of solids have been proposed [17] [29] [30]. However, the simple diffusion model may be adequate to describe the dissolution behavior of most pharmaceutical solids in aqueous and non-aqueous media. The diffusion layer model (*Fig. 7*) assumes that a thin film of saturated solution of concentration c_s exists at the interface of the dissolving solid and the dissolution medium. The dissolution rate is controlled by the diffusion rate of solute molecules from this thin saturated film into the bulk solution. The concentration of the solute in the bulk is denoted by c_b . As the distance x increases from $x=0$ (at the surface of the solid) to $x=h$ (at the beginning of the bulk solution), the concentration decreases from $c=c_s$ to $c=c_b$. Beyond $x=h$, the bulk concentration remains uniform at c_b . In the simplest form of the theory, following *Fick's First Law*, the rate of dissolu-

Chapter 7

A Procedure for Salt Selection and Optimization

by Michael J. Bowker

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REFERENCES

1. Introduction

Until *ca.* 15 years ago the drug discovery processes adopted by pharmaceutical companies involved the screening of large numbers of compounds that had been synthesized by the company's research laboratories over many years. Alternative sources of compounds were also available to some companies from university laboratories, or as extracts from a multitude of natural sources. In the last 15 years, there has been a rapid change towards the use of combinatorial-chemistry techniques which are now being used routinely to prepare many thousands of compounds per year. As described in *Chapt. 6*, these compounds are generally dissolved in dimethyl sulfoxide (DMSO) to enable screening for activity in an enzyme- or receptor-based assay system. Initial screening usually involves a highly automated 'high throughput screen' (HTS). If the number of 'hits' found is large, the numbers are usually refined by a more involved and specific 'low throughput screen' (LTS) and other selection processes, until a manageable number of 'leads' is available. Often, several of these 'leads' show only moderate activity, and further structural refinement and optimization is invariably necessary, using a mixture of computational techniques and intuition by the chemistry team, until a smaller number (usually 1–5) of highly active potential 'candidates' remain.

These 'candidates' are usually relatively impure free bases, free acids, or neutral molecules, and are often amorphous. Because they are dissolved and stored in DMSO prior to being used in screening procedures, there is no need to ensure that they are crystalline. Also, the preparation of any salts of these compounds is rarely necessary as the neutral/covalent molecule usually exhibits better solubility in both DMSO and the assay system to be used by the pharmacology group. As can be seen readily, these new procedures favor lipophilic molecules, whereas the majority of marketed drug substances are hydrophilic. The need for water-soluble candidates for use in drug development and the vast majority of dosage forms has been recognized [1–4] for many years before the advent of 'combinatorial chemistry'.

Because drug substances are designed to bind with a receptor, enzyme, protein *etc.*, they normally possess several functional groups capable of hydrogen bonding. Some of these functional groups may give potential for salt

formation and thus increased hydrophilicity. This salt formation also gives the pharmaceutical chemist and formulation scientist the opportunity to modify the physicochemical (*e.g.*, melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form *etc.*) and mechanical (*e.g.*, hardness, elasticity *etc.*) characteristics of the potential drug substance and to develop dosage forms with good bioavailability, stability, manufacturability, and patient compliance.

For weakly basic drug substances, salts of an inorganic acid (*e.g.*, hydrochloride, sulfate or phosphate), a sulfonic acid (mesylate, esylate, or isethionate), a carboxylic acid (acetate, maleate, or fumarate), a hydroxy acid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. In the past, hydrochloride salts have often been the first choice for weakly basic drugs, since, as a consequence of the low counter-ion pK_a , salts can nearly always be formed, and recrystallization from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts include unacceptably high acidity in formulations (*e.g.*, parenteral products), the risk of corrosion of manufacturing plant and equipment, 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].

Historically, prior to the severe regulatory constraints that abound today, it had been possible to develop the neutral species (free acid or free base) together with one or more different salts. These different chemical forms were often used for specific purposes. An excellent example of this concerns the phenothiazine drug substance, prochlorperazine. The free base was used in suppositories, as it was the most lipophilic form, the mesylate salt was used in injections and in sachets for the preparation of an oral solution, whilst the less soluble maleate was used for the manufacture of tablets.

Occasionally, salts were also prepared in former times to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent 'Ostwald ripening', for taste masking (*e.g.*, dextropropoxyphene napsylate suspension), or to prepare an extended release product. Embonate salts have been used in suspension 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 of 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 solutions) should be monitored to help ensure that the formulation will be well tolerated.

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 micronize the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50–100 °C higher than that of 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.

Chapt. 6 describes some of the theoretical aspects behind salt formation. This chapter describes some of the procedures adopted by the *Preformulation* team at the *Aventis Pharma* (previously *Rhône-Poulenc Rorer*) *Dagenham Research Centre* (DRC). These procedures are similar to a systematic approach proposed by *Morris et al.* [3] but were devised empirically with one aim of how to obtain the maximum amount of information from the minimum amount of precious candidate batch. The outline of the process is shown in the *Figure*.

Where possible, we prepare a range of salts for each new candidate and compare their properties in our preformulation program, which is designed to give us the relative suitabilities of each one. Only one salt form is developed, and its properties should be appropriate to the primary route of administration and the intended dosage form proposed for initial marketing. The properties of the drug substance required for one dosage form may be quite different from those required for another. An understanding of the influence of drug substance, or its salt, properties on the finished product is essential to ensure selection of the optimum salt form. Some of these aspects are dealt with in more detail in *Chapt. 4*. The procedures demonstrated here are also described in outline form in [5].

2. Salt Formation – Is It Necessary, Is It Feasible?

Each new molecule synthesized within the *Discovery Chemistry* group has an entry prepared in the compound database. Among the first information added as an entry into this database for each compound is the calculated pK_a value of each ionizable group in the molecule [6–9]. Another early entry is the calculated $\log P$ value. Immediately, a small amount of material is made available, the values are determined experimentally on 1–2 mg of sample by potentiometric titration (*e.g.*, *Sirius Model GLpKa* apparatus, *Sirius Analytical Instruments Ltd.*), and the values determined experimental-

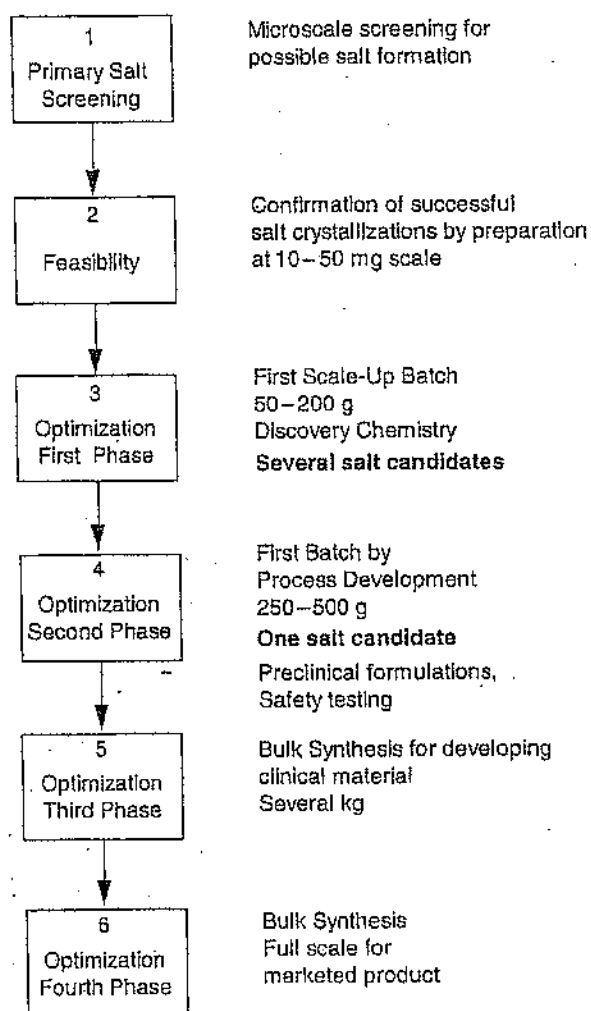


Figure. Process of salt selection and optimization

ly are again entered into the database. The $\log P$ value is usually determined concurrently by chromatography using an octanol-loaded HPLC column [10].

Synthesis of a working quantity of each new candidate is rarely undertaken at a scale that exceeds 5 g; the primary synthetic aim is to provide sufficient compound for activity screening in both cell and animal models. Once activity is confirmed in these screens, the material remaining from this first batch is usually further sub-divided across a small number of Departments. The amount of drug substance made available to *Physical Chemistry* and the *Preformulation* teams together rarely exceeds 1 g and is often less than 0.5 g.

With the pK_a value of each ionizable group in the molecule known, either by experimental determination or by calculation, a list of potential salt forming agents (counter-ions) can be selected, for each candidate, based on

lists that are available in the literature [2] [11–13] (see also the tables in the *Appendix*). Based upon studies on numerous candidates over more than 10 years, it is our considered opinion that, for the formation of a stable salt, there should be a minimum difference of ca. three units between the pK_a value of the ionizable group and a possible counter-ion. This is especially so when the drug substance is a particularly weak acid or base where disproportionation back to the component parts often occurs in solution. This then results in precipitation of the free acid or free base. Occasionally, an exception may be found where a salt has an acceptable stability, despite there being a smaller difference in the pK_a values.

To maximize the amount of data obtained from small quantities of candidate drug substances, micro and semi-micro techniques have been developed and are used regularly within our groups. A microplate technique has been devised by the *Aventis Physical Chemistry* group for rapidly and qualitatively screening whether solid salts can be prepared. This involves dissolving ca. 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing ca. 0.5 mg of sample, into each glass microplate well. Concentrated solutions of each potential counter-ion in equimolar proportion, or (if the molecule could form double or triple salts) other appropriate stoichiometric ratios, are prepared, and a few microlitres of each is added sequentially to each well. Thus, all the wells in line 1 ('x'-direction) will contain the same combination of sample and counter-ion 1; all the wells in line 2 contain the same combination of sample and counter-ion 2, etc. Different, potential crystallizing solvents can be investigated systematically in the 'y'-direction. The wells are inspected using an inverted microscope (*Leica*, model *DMIRB*) at regular intervals for the appearance of crystals or solid. Occasionally, crystallization can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry N_2 gas.

Once the possible combinations of counter-ion and solvent(s) are identified, studies at a slightly larger scale (usually 10–50 mg) can be initiated to confirm the formation of a crystalline salt or an amorphous solid and initiate a study of any polymorphism. These studies give a lead to the chemist and can help in the identification of future problems. Melting points are determined by hot-stage microscopy, and samples showing evidence of hygroscopicity can be evaluated using a dynamic vapor sorption (DVS) analyzer (Model *DVS-1*, *Surface Measurement Systems Ltd.*). There is usually sufficient material for a preliminary powder X-ray study using an environmental chamber (model *D8 Advance*, *Bruker Instruments*) to qualitatively determine the presence of crystalline or amorphous material. Frequently, these studies can also give 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. Most of these studies can be accommodated, if care is taken, on 20–25 mg of sample.

In parallel with these studies, a preliminary HPLC method is developed within *ca.* one week, and, after completion of minimal validation, it is used to give an estimate of the purity of the original sample, whilst IR 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 crystallization by 'poisoning' crystal growth or promote the formation of a specific polymorphic form. Immediately after the development of this HPLC method for use in the assessment of the main manufacturing impurities, the analytical chemists turn their attention to the development of the corresponding stability-indicating method. The development and partial validation of this method involves the use of degraded solutions of the drug substance produced in accelerated stress studies. This method takes *ca.* three weeks to complete and can then be used in the early stability evaluation of salts; their solutions and, ultimately, simple formulations.

Thus, by carefully planned studies using a small quantity of drug substance, it is possible to identify quickly the possibilities for the chemist to investigate, once larger quantities of drug substance are made. These studies may point to a small group of potential salt formers for initial evaluation; they also give the chemist ideas on possible recrystallization solvents that can be tried.

3. The Salt-Optimization Process – First Phase

3.1. Choice of the Salt Former (Counter-Ion)

The choice of salt former (counter-ion) depends on several factors. Obviously the primary factor is that it should yield a crystalline salt. Crystallinity in a salt affords a means of purification and removal of unwanted impurities. Lack of crystallinity (*i.e.*, the salt is amorphous) normally would be expected to lead to severe problems and uncertainties, if the product intended to be developed is a solid, oral dosage form. The prospect of unexpected and uncontrolled crystallization at some stage with just one batch, creating a product recall, is something that the *Quality, Regulatory, and Marketing* groups could not live with. Absence of crystallinity is often less of a problem if the dosage form intended is a liquid.

The choice of salt is also governed largely by the acidity or basicity of the ionizable group, the safety of the counter-ion and the drug indications.

Table 1. *Classification of Common Pharmaceutical Salt Formers*

Salt Formers	Examples
<i>Anions</i>	
Inorganic acids	Hydrochloride, Hydrobromide, Sulfate, Nitrate, Phosphate
Sulfonic acids	Mesylate, Esylate, Isethionate, Tosylate, Napsylate, Besylate
Carboxylic acids	Acetate, Propionate, Maleate, Benzoate, Salicylate, Fumarate
Anionic amino acids	Glutamate, Aspartate
Hydroxy acids	Citrate, Lactate, Succinate, Tartrate, Glycolate
Fatty acids	Hexanoate, Octanoate, Decanoate, Oleate, Stearate
Acids for insoluble salts	Pamoate (= embonate), Resinate (e.g., Polystyrene sulfonate)
<i>Cations</i>	
Metallic	Sodium, Potassium, Calcium, Magnesium, Zinc
Organic Amines	Triethylamine, Ethanolamine, Triethanolamine, Meglumine, Ethylene diamine, Choline
Cationic amino acids	Arginine, Lysine, Histidine
Bases for insoluble salts	Procaine, Benzathine

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*.

3.2. Scaling Up the Preparation of Salts

In parallel with the preliminary evaluation of opportunities for the formation of crystalline salts, the *Process Chemistry* group start to investigate the possibilities for economic manufacturing routes for each of the candidates remaining (usually one or perhaps two at most). The manufacturing route may

be the same as used by the *Discovery Chemistry* group, but usually is quite different as less expensive reagents with greater commercial availability start to be used. The aim is to manufacture rapidly 50–200 g of each candidate to permit their evaluation by teams from various departments, including *Toxicology*. A significant portion of this batch is destined for the preparation of 3–4-g of each of the salts that were considered viable from the early studies on the smaller batches. An equivalent portion of the free base/acid is also taken for comparison purposes. The combination of individual studies that are undertaken on each of these 3–4-g portions varies depending on the type(s) of dosage form required for initial clinical evaluation and also that ultimately required for marketing.

The most common route of administration for drug substances is orally. For the oral route, the dosage forms most commonly encountered during initial clinical investigations for the drug substances are highly dependent on company's philosophy. Some companies use simple oral solutions whenever the drug substance properties permit; they revert to capsules for less soluble candidates. Others prefer to develop tablets that would closely resemble a potential marketed formulation. The majority seem to adopt an intermediate strategy of using capsules for oral dosing, to be followed by tablets for marketing. In addition, an injection formulation is also invariably required for certain toxicology studies and at least a determination of the absolute bioavailability of the oral solid dosage form in humans. Obviously, if the candidate is required for the treatment of asthma, inhalation dosage forms would be developed, together with an injection formulation for limited studies.

To generate data that is an invaluable preliminary to the development process for the drug product(s), a basic 'checklist' has been devised. This list contains a reasonably comprehensive range of tests that give an initial database of properties to establish a firm basis for later decisions. The 4-g batches of each salt, plus the free acid or free base, are examined using each of these tests. Similar 'checklists' can be established for drug substances that are intended to be developed into a solution (injectable or oral) formulation or one intended to be delivered by the pulmonary route (solution metered dose inhaler (MDI), suspension metered dose inhaler, or dry powder inhaler (DPI)). Obviously, many of the tests give basic data that should be obtained irrespective of the type of formulation that is intended. In several cases, the test remains the same but the target value expected for the result may differ. An example of this would be aqueous solubility; the target value for a drug substance intended for use in a solid, oral dosage form would be around 1 mg/ml in a medium with a pH value within that found in the human gastro-intestinal system (a lower target solubility would be acceptable, if the candidate was shown to be highly potent). A similar candidate intended for use in an injectable formulation would have a target solubility value around

10 mg/ml, unless it was also exceptionally potent. Similarly, for a candidate intended to be developed into a solution MDI, it would be important to determine that the solubility in the new hydrofluoroalkanes (HFA 134a or HFA 227) was high enough to yield an acceptable dose from an actuator. The solubility would also be determined in these HFAs containing up to 5% ethanol. It is less important to determine it in aqueous-based solutions. Lack of adequate solubility in the pure HFAs or an ethanolic HFA solution implies that a suspension MDI should be considered. For a drug substance destined to be formulated as a suspension MDI, it should ideally be readily micronizable and also have a density similar to those of the HFAs to enable maintenance of a stable suspension.

A typical compilation of the main tests used for drug substances intended for solid, oral dosage forms is presented in *Table 2*; it shows the types of tests normally chosen and the information that these tests can yield. A more detailed tabulation of these tests and the amount of sample normally required is given in *Table 3*.

3.3. The Decision on What To Develop – Salt or Free Acid/Base?

The results obtained from each of the small batches of salt and the free acid or base form the initial 'Preformulation Database'. The results are tabulated for comparison and the collated results discussed by all the scientists concerned in the project. 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* needs to assess the likely yield of each salt, as salt formation normally creates an additional step in the manufacturing process. Usually, the decision-making process results in the proposal of a single salt for further study, though, occasionally, it is seen that none of the salts has optimum properties, and two different salts can be proposed for further, in-depth study. 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 base has a low pK_a value (or a correspondingly high value, if the candidate is an acid), and the resulting salts are less stable than required, when the salts are particularly hygroscopic, or when they exhibit complex polymorphism/pseudopolymorphism (hydration or solvation).

These simple investigations give much useful information very quickly and with low usage of drug substance. It is important to realize, however, that the polymorphism study undertaken at this stage is very limited, and a much more detailed study is always undertaken later. This very early study uses a

Table 2. *Preformulation Studies That Are Normally Considered for Comparison of Salt Forms and Parent Compound for Oral Dosage Forms*

Test	Suitable Techniques	Comments
Dissociation constant	Potentiometry, solubility, UV spectrophotometry.	Determine pK_a for parent drug
Melting point	Capillary m.p., hot-stage microscopy, differential scanning calorimetry.	Perform on each salt and compare to parent.
Aqueous solubility	Overnight equilibration at 25 °C; analysis by UV spectrophotometry 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 spectrophotometry or HPLC.	Determine solubilities in: ethanol, polyethylene glycol, propylene glycol, and glycerol (as a minimum) and compare to parent.
'Common-ion' effect on solubility	Overnight equilibration at 25 °C in suitable media and analysis by UV spectrophotometry or HPLC.	Compare solubility in demineralized water with 1.2% NaCl for salts and parent.
Hygroscopicity	Use DVS apparatus or expose to various humidity values and measure weight gain after one week.	Perform at 53%, 93% and 97% r.h., and other values of interest. Assign hygroscopicity classification to each salt [14].
Intrinsic dissolution rate (IDR)	Use <i>Wood's</i> apparatus [15].	Compare dissolution rates at various pH values (can provide information on wettability). If appropriate, consider use of ' <i>Dressman</i> buffers' [16].
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	Recrystallization from various solvents of differing polarities, HSM, DSC, TGA.	Preliminary exploration.
Powder properties	Bulk density measurement	Determine <i>Carr's</i> Compressibility Index [17 – 19].
Stability	Various	Perform on parent drug and undertake preliminary tests on appropriate salts.

Table 3. Tests To Be Considered for the Evaluation of Candidate Salts

Test	Amount required [mg]
Elemental Analysis	10
Structural Analysis	
Mass spectrometry ^{a)}	1
¹ H-NMR ^{a)}	5
¹³ C-NMR	25
IR	1
UV	1
Fluorescence spectrum ^{a)}	1
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 (<i>Malvern</i>)	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)} Includes solubility in saline, 5% dextrose solution, and some buffers. ^{c)} Also solubilities in complexing agents/surfactant systems where appropriate. ^{d)} Propellants and propellant/cosolvent systems for inhalation dosage forms.

range of individual protic and aprotic solvents of widely differing polarity, mixtures of these solvents, and mixtures containing water. More often than not, it will enable detection of a 'stable' hydrate or solvate. If a relatively stable hydrate is detected in this early study, it is usually beneficial to prepare a small sample (1–2-g) and to repeat some of the tests such as solubility, in-

trinsic dissolution, thermal analysis *etc.*, as hydrates are invariably less soluble than anhydrides. Also, it is useful to know both the dehydration temperature and the polymorphic form produced upon dehydration.

Once we have completed these initial studies, a short document is drafted that gives a summary of what has been found, the likely effect on an initial clinical formulation (*i.e.*, do any of our findings impact heavily upon the choice of formulation or manufacturing process?). The recommendations of the collective group involved in these studies on whether to develop a specific salt, or the free acid or base are placed before senior management for ratification.

3.4. The Negative Aspects of Salt Formation

One of the negative aspects of salt formation is that the percentage of active content of the drug substance decreases markedly as counter-ions with higher molecular weights are used. If the free acid or base has only moderate or low activity, it may be necessary for the patient to be prescribed a relatively high dose for a clinical effect. It is quite common to have a situation where 20–50% of the weight of the drug substance is due to inactive counter-ion. Because most drug substances have flow properties that are non-ideal, it is necessary to formulate them with suitable excipients for encapsulation or tableting. The final powder mix, or granule, may require 40–70% of excipients to enable it to be filled successfully. Addition of the contribution made by the inactive counter-ion to that of the excipients may result in a very low percentage of active ingredient in the final mixture. This often creates a powder volume that is too great, even after granulation, to fill successfully into even the largest acceptable size of capsule shell. It may mean that the unit dose is more than one tablet or capsule, a situation that does 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 slowly on storage in formulations by interaction with atmospheric water, water bound to excipients such as lactose monohydrate, or the water in capsule shells (gelatine capsule shells contain ca. 12% (*w/w*) loosely bound water) *etc.*

4. Recent Case Histories

To demonstrate how the decision-making process works in practice, three recent examples have been chosen from the many that have been studied over the last five years. These exemplify some of the principles involved.

4.1. RPR111423

RPR111423 contains a pyridine ring that is the only group capable of being ionized and a primary amide group; it has a single pK_a at 4.25 that is consistent with the presence of the pyridyl group. The $\log P$ value was determined at 2.1. Thus, it is a very weak base and would be expected to form stable salts with only the strongest acids (mineral acids and sulfonic acids); it would not be expected to form a stable salt with any of the organic acids.

The free base was found to be a crystalline solid with a high melting range (241–244 °C). A screening of possible salts was undertaken and demonstrated that there were only two salts that could be isolated as crystalline solids; these were a hydrochloride (RPR111423A), with a melting point at 242 °C determined by DSC (model DSC 820, Mettler Toledo Ltd., UK), and a mesylate (RPR111423B), with a melting point at 210 °C determined by DSC. We noted the similarity in melting points of the base and hydrochloride for later evaluation. It was decided that the free base should be taken through the initial evaluation process in comparison with these two salts and 4–5 g of each were prepared.

It was agreed that oral dosing was preferred, and it was hoped that capsules could be formulated from the drug substance form that remained to be selected; an injectable formulation would also be needed for some pre-clinical studies and for the determination of absolute bioavailability in man at a later stage in development. Because of its apparent high activity in screening studies, it became obvious that both of the above formulations could easily be very-low-dose products. For a low-dose capsule formulation, uniformity of active content may be an issue to be faced in the future. A determination of the particle-size distribution, perhaps linked with an assessment for size reduction by milling or micronization, thus became quite important.

4.1.1. Basic Physicochemical Properties of RPR111423

The free base crystallized in large laths, in the range of 10–100 μm which could be readily micronized, yielding material with a particle size range of 2–5 μm , without a change in polymorphic form. In addition to this information, numerous small-scale crystallization experiments all gave the same polymorphic form, with no evidence for the presence of amorphous material, hydrates, or solvates. Several experiments using both crystalline and micronized drug substance and either hygrometers, or a DVS apparatus (model DVS-1, Surface Measurement Systems Ltd., UK) confirmed the material was non-hygroscopic.

As expected from the pK_a value and the chemical structure, the solubility profile was shown to be markedly dependant on pH (see *Table 4* for data). The intrinsic dissolution rate was determined using *Wood's* apparatus [20] and was found to be 0.55, 0.085, and 0.017 mg/min/cm² into solution at pH 1.0, 2.0, and 4.0, respectively.

Preparation of a very simple capsule formulation containing 25 mg of the base, together with other common excipients and sodium dodecylsulfate as wetting agent, gave an acceptable dissolution profile when tested over the pH range 1.0–4.0 with $t_{70\%}$ values of ca. 12 min.

4.1.2. *Basic Physicochemical Properties of the Hydrochloride RPR111423A*

A similar investigation was undertaken on the hydrochloride. As noted earlier, the melting point was determined to be within the range found for the free base, and an investigation was undertaken using thermogravimetry (model TGA 850, *Mettler Toledo Ltd.*, UK). This technique clearly showed a weight loss, consistent with loss of HCl, between 110–120 °C and explained the similarity of melting range to that of the free base. The initial sample consisted of aggregates of micro-crystals, typically 1–2 μ m, and further recrystallizations from a variety of solvents produced at least four different polymorphic forms. None was hydrated or solvated, and no evidence was found for the presence of an amorphous form. All these forms were shown to be metastable with respect to the form originally provided and reverted to that form on standing over a few days. Recrystallization from aqueous ethanol returned the free base. The original form was shown to be slightly hygroscopic when stored at 53% r.h. (2.0% weight gain) but gave a 22% increase in weight on storage at 97% r.h.

As with the free base, the solubility was markedly pH dependant at 37 °C (28 mg/ml at pH 1.0 and 4.6 mg/ml at pH 2.0). At higher pH values, any undissolved solid was shown to convert quantitatively to the free base, and, at pH values higher than 4, the free base precipitated from solution.

4.1.3. *Basic Physicochemical Properties of the Mesylate RPR111423B*

The sample provided consisted of aggregates of fine laths, each ca. 1 μ m $\times$ 7 μ m, with a melting point at 210 °C. Hot-stage microscopy, DSC, and thermogravimetry demonstrated that the material is chemically stable upon melting. Small-scale recrystallization experiments from a range of solvents similar to those used for the hydrochloride resulted in the production of at least four new forms. Grinding and micronization each produced an additional form; all of these six new forms reverted back to the original one

Table 4. Comparison of Some Basic Properties of RPR111423 and Two Salts

Property	Base RPR 111423	Hydrochloride RPR 111423A	Mesylate RPR 111423B
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 $\times$ 1 (microcrystalline laths)	7 $\times$ 1 (microcrystalline laths)
Melting range [$^{\circ}\text{C}$]	241 – 244	242	210
Polymorphism, preliminary results	No other form detected	At least 4 polymorphs detected; metastable forms revert to original on standing.	At least 6 polymorphs detected; phase changes detected on grinding or micronization. Reverts to original form on heating.
Other thermal behavior	Nothing detected	Loss of HCl detected at 110 – 120 $^{\circ}\text{C}$	Nothing detected
Aqueous solubility [mg/ml]	At 25 $^{\circ}\text{C}$	At 25 $^{\circ}\text{C}$	At 25 $^{\circ}\text{C}$
	At 37 $^{\circ}\text{C}$	At 37 $^{\circ}\text{C}$	At 37 $^{\circ}\text{C}$
	11.6	25.7	131.4
	0.71	2.51	6.11
	0.03	0.05	0.01
	0.01	0.01	0.03
– in 0.1M HCl (pH 1)	14.7	28.2	204.1
– in 0.01M HCl (pH 2)	0.89	4.58	8.91
– in acetate buffer (pH 4)	0.05	0.13	0.02
– in acetate buffer (pH 6)	0.02	0.02	0.34
– in acetate buffer (pH 6.8)	0.02	0.02	0.02
– in demineralized water	0.01	0.36	0.33
pH (saturated solution, water at 20 $^{\circ}\text{C}$)	6.50	2.43	2.74
Addition of water to concentrate	No changes detected	Some precipitation of free base	Some precipitation of free base
	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 r.h.	Slightly hygroscopic 2.3% (w/w) uptake at 53% r.h. 22% (w/w) uptake at 97% r.h.	Moderately hygroscopic 3.7% (w/w) uptake at 53% r.h. 32% (w/w) uptake at 97% r.h.

upon standing within one week. As with the hydrochloride, recrystallization from aqueous ethanol returned the free base.

The material was slightly more hygroscopic than the hydrochloride with a 3.9% weight increase on storage at 53% r.h. and a 28% weight increase at 97% r.h. It was also more soluble than the hydrochloride but with an even more marked pH dependency (204 mg/ml at pH 1.0 and 8 mg/ml at pH 2.0). It suffered from exactly the same problems in aqueous solution with solid material converting to the free base at pH 2.0, and the free base precipitating from solution above pH 4.0.

4.1.4. *Discussion of Results*

The results from these preliminary studies 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 around the pK_a value. 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 main area that required some additional investigation was whether it had sufficient solubility in gastro-intestinal media. Studies performed on samples of drug substance and on simple capsule formulations demonstrated that the dissolution rates of micronized free base were equivalent or superior to those of the salts under the same conditions. This was confirmed in a simple pharmacokinetic evaluation in individual dogs, where the stomach pH was controlled upon dosing capsules by the administration of citrate buffer at pH 4 and 7.4. Therefore, the free base was taken forward for further study in preference to either of the salts.

4.2. RPR127963

RPR127963 has been evaluated for the treatment of cardiovascular diseases; it is a crystalline, very weak base with a pK_a of 4.10 that is commensurate with the presence of an aromatic secondary amine. The $\log P$ value was determined as 2.2. In common with most similar drug substances intended for the treatment of cardiovascular disease, it was considered that a high

hand-filled capsules was quite fast, but not as rapid as either of the sulfonates.

4.3.5. *Basic Physicochemical Properties of the Hydrobromide RPR200765E*

The hydrobromide had the lowest solubility and the slowest dissolution rate of all the salts and was particularly hygroscopic, resulting in the formation of multiple hydrated forms. It had the highest melting range of all the salts of 276–277 °C. With these problems and the disadvantageous pharmacological activity of the bromide ion, no further studies were undertaken.

4.3.6. *Discussion of Results*

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 form to be favored on the basis of its low hygroscopicity, clean polymorphic profile in the preliminary screen, high solubility, and rapid dissolution rates. Another favorable factor supporting the selection of the mesylate salt proved to be the good flow properties, which later allowed very satisfactory prototype capsule and tablet formulations to be developed.

5. Optimization – Second Phase: Drug-Substance Form for Preclinical Development

The initial studies described above require *ca.* 3–4 g of the free base and a similar quantity of each of the salts. The data for the free acid or base, and each of the different salt forms normally can be generated in *ca.* 4–6 weeks if the samples are available for simultaneous analysis. Having evaluated several possible alternatives in the three cases above, using a range of relatively simple tests, the choice of the form has been narrowed down to one candidate that should be suitable for further development. The next step is to build upon the initial database by employing a more comprehensive range of tests, which require more material. Within three months of receipt of the next batch of drug substance, it should be possible to define with some certainty whether this chosen form is suitable. A series of tests, analogous to those in *Table 2*, are used in this evaluation; these tests are listed in *Table 8*. Upon completion of this second battery of tests, the *Preformulation* team will have generated much additional, solid-state information to add to the database and

Table 7. Comparison of Physicochemical Properties of RPR200765 Salts

Property	Mesylate salt RPR200765A	Camphorsulfonate salt RPR200765C	Hydrochloride RPR200765D	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
M_r	566.61	684.79	524.98	569.43
Melting Range [°C]	214	265 – 267	245 – 248	276 – 277
Crystal habit and appearance	Individual plate-like crystals with some agglomeration.	Clusters of highly aggregated, plate-like crystals	Plate-like crystals; individual crystals contain stress lines	Loosely agglomerated, flaky material
Particle size (by microscopy)	ca. 45 – 200 μm in agglomerates of 200 – 350 μm	Crystals ca. 20 – 50 μm , clusters ca. 80 – 200 μm , some larger clusters up to 500 μm	ca. 30 – 100- μm particles	10 – 40- μm particles
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 (using DVS apparatus)	Non-hygroscopic with a stable, monohydrate form	Non-hygroscopic	Hygroscopic with multiply hydrated forms	Hygroscopic with multiply hydrated forms
Dissolution rate at pH 2 [80%, min]	2.0	6.0	7.4	3.9
% Released into citrate buffer, pH 4	> 60	> 60	> 60	14

developed all the different dosage forms required for initial preclinical evaluation. Each of these formulations will have a defined manufacturing process, analytical methods, stability data, a set of defined storage conditions, and a shelf-life.

Even at this early stage, the *Process Chemistry* group will have started a thorough evaluation of the possible manufacturing routes to the chosen molecule and a batch of 250–500 g is normally made available within 1–3 months, depending on the complexity of synthetic route, for study across a wide range of departments. The recrystallization solvent normally employed would be that used for the preparation of the 4–5-g batch in an attempt to keep the physical form the same. The particle size may differ, however, because of different cooling rates. One of the key activities for the *Preformulation Scientist* and the *Process Chemist* is to start investigations into which other polymorphic or pseudopolymorphic forms exist. In the short development phase where initial preclinical administration occurs, it is rarely necessary to undertake these 'sighting studies' to *Good Laboratory Practice* (GLP) standards. Only a preliminary screening of these different forms is considered necessary, as it is possible that the compound will be found too toxic for further development. Our team undertakes this screening on ca. 0.5–1-g of sample; small portions are recrystallized from anhydrous and hydrated solvents of differing polarity. Any crystalline product recovered is examined by a variety of techniques in order to determine how many different forms are produced, and whether any of them 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:

1. To define the various preclinical formulations that are required, to devise analytical methods for these, to determine their stability, establish storage conditions, and to propose shelf-lives.
2. 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 20 g of drug substance. More will be needed, if the drug substance is intended for inhalation, and there are difficulties with micronization.

6. Optimization – Third Phase: Drug-Substance Form for Clinical Development

Once the drug substance has successfully passed through the initial battery of safety studies, the main requirement is for the preparation of batches of drug

substance manufactured using *Good Manufacturing Practice* (GMP) for use in the various safety studies undertaken to GLP standards. Also, at this stage, work can begin on the definition of a suitable series of clinical formulations. For this, we normally expect to have a reasonably clear picture of the inter-relationships between the different solid-state forms of the drug substance and should have started to define the most stable form. As *Process Chemistry* produces more batches at increasing scale, they are also examined using some of the key tests (see *Table 8*) to add information to the database. Also, with the

Table 8. *Tests To Be Considered for the Second and Third Phases for Compounds Intended for Use in Oral, Injection, and Inhalation Products*

Test	Amount required	
	Second Phase	Third Phase
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
Micronization	5 g	—
Specific surface area	2 g (R) ^{c)}	4 g (R) ^{c)}
True density	200 mg (R) ^{c)}	200 mg (R) ^{c)}
Bulk-powder density	2.5 g (R) ^{c)}	10 g (R) ^{c)}
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 (analyzed with HPLC, XRPD, and DSC)	50 mg	250 mg
Compression properties (for dry powder inhaler only)	—	5 g

Table 8 (cont.)

PRECLINICAL FORMULATION DEVELOPMENT		
Intra-tracheal suspensions	2 g	—
Oral solutions/suspensions	2 g	—
Solutions for nebulization	2 g	—
Intravenous solutions	2 g	—
Other routes (<i>i.p.</i> ; <i>s.c.</i>)	1 g	—
CLINICAL FORMULATION DEVELOPMENT	Predict suitable dosage forms 3 g	Phase I – IIa ^{d)} 250 – 1200 g
Microbiological Controls		(?) ^{c)}
TOTAL SUBSTANCE REQUIREMENT	20 – 25 g	Depends on dosage and form

^{a)} Also solubilities in complexing agents/surfactant systems where appropriate. ^{b)} Propellants and propellant/cosolvent systems for inhalation dosage forms. ^{c)} (R): possible to recover drug substance for certain other tests; (?) : Depends on supply of sample material. ^{d)} Develop and specify Phase-I formulation – commence stability/compatibility studies.

increased availability of drug substance, it is possible to finalize the definition of the solid-state form using *ca.* 50 g of drug substance for the initiation of 'maturation studies' [14] as an additional technique to assist in the definition of polymorphism. This experimental technique involves the preparation of suspensions of each of the available polymorphic forms in a variety of different solvents. These suspensions are then stored under a suitable range of temperatures for *ca.* one month. At the end of this storage, the solid material is isolated and the solid-state form evaluated. Much useful information on the relative stabilities of each form can be obtained using this technique.

If the structure of the drug substance has been determined by single-crystal X-ray study, under certain circumstances it may be possible at this stage to initiate the theoretical search for other polymorphic forms. This is achieved, with care, using the *Polymorph Predictor* software (*Molecular Simulations Inc.*, Cambridge). This software has been used successfully [20] 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. The purpose of these *ab initio* predictions is to gain knowledge on potential polymorphic forms that could theoretically exist to attempt to minimize setbacks in development due to the unexpected appearance of a previously unencountered form part way into development or marketing. If one, or more, forms are found with comparative energies and structural characteristics to the forms already produced, an urgent and extensive search is initiated to discover conditions for their formation.

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

7. Optimization – Fourth Phase: Drug-Substance Form for Marketing

If the candidate passes through initial clinical evaluation (Phases Ia and Ib), additional characterization and refinement of the drug substance form should continue, and each batch of drug substance produced should be characterized physically. The *Process Chemistry* group normally continues to make minor adjustments to the manufacturing process and recrystallization conditions to improve the yield, purity, or particle size. It is imperative that samples of each batch produced are examined to ensure that the polymorphic form remains constant, until such time as it becomes necessary to 'lock' the *Process*. Close liaison and teamwork between the *Process Chemist* and the *Development Scientist* in an exploration of the various possible recrystallization solvents can often result in further refinement of the crystal properties.

An excellent scheme for the final characterization of solid drug substances, prior to the final regulatory submission, has been described recently by *Byrn et al.* [22]. The aim of both the *Development* and *Process Chemistry* teams is to finalize definition of all the characteristics of the drug substance in readiness for the initiation of Phase IIb.

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