An Automated Approach to Salt Selection for New Unique Trazodone Salts

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Purpose. The purpose of this study was to establish an automated approach to salt selection and to search for unique trazodone salts for new applications.

Methods. Automated procedures were developed on a Biomek 2000 automation workstation with stacker and plate reader capabilities. Trazodone was dispensed into 96-well plates, and an automated method was set up to form 104 trazodone salts. Salts were observed under a polarized light microscope to determine crystallinity. After stepwise eliminations, the remaining salts were scaled-up and subjected to differential scanning calorimetry (DSC), powder x-ray diffraction (PXRD), hygroscopic, pH-solubility, density, surface area, and particle size analyses.

Results. Oils formed in several cases resulting in preliminary elimination of mesyl and esyl salts and four crystallizing solvents. Crystallinity was observed in 34 of 44 scaled-up trazodone salts. PXRD, DSC, and hygroscopic analyses indicated a number of new salts that were comparable in physicochemical parameters to the marketed HCl salt. Among them, the tosylate salt showed uniqueness for new applications.

Conclusions. Automated procedures can be developed to increase the efficiency of pharmaceutical salt selection. The new tosylate salt gave a unique pH-solubility profile with low solubility over the entire pH range making it a potential candidate for a suspension or prolonged action formulation.

KEY WORDS: automation; high-throughput; preformulation; trazodone.

INTRODUCTION

In the past two decades, high-throughput screening (HTS) and automated method development have gained much attention with over 7000 articles published on these topics since 1980. In the pharmaceutical industry, most recent publications on these topics have only focused on the drug discovery process (1–6). However, since time restrictions and product deadlines can result in potential delays with a cost of as much as $1 million per day of revenue reduction in the first year (7), more effort should be exerted on other areas of pharmaceutical research and development. The need for automated, highly efficient steps in every part of a drug development program has become evident. High-throughput drug screening and selection assays have already proven invaluable to many pharmaceutical companies by allowing them to scan rapidly and efficiently numerous compounds from tens of thousands of plants, microorganisms, and insects in an effort to find an optimum drug candidate or "lead" compound for a specific application (8). The drug discovery area demands coordinated efforts from many different fields of study including pharmacology, chemistry, biology, and biochemistry, among others. One author suggests that the future of HTS will no longer be random searching for compounds, but computer-defined drug design for a specific biological target (9). Much of the knowledge gained from this well-researched area of drug discovery could be transferred to other areas of pharmaceutical research and development, such as preformulation development. This paper focuses on the automated approach to preformulation development with an emphasis on salt selection using the model drug trazodone.

Trazodone is currently marketed as the hydrochloride salt under several brand names. It is known as Desyrel, Trazon, and Trialodine in the United States with a former controlled-release tablet marketed as Molipaxin CR in the United Kingdom (10,11). The chemical structure of trazodone is shown in Fig. 1 (12). It is indicated for the treatment of depression and for alleviation of the symptoms of agoraphobia, insomnia, essential tremor, repetitive screaming, and some pain syndromes . The mechanism of action is not well understood, but in animals it works to increase the available serotonin levels in the brain by selectively inhibiting reuptake at the brain synaptosomes (10,13,14). Trazodone is chemically and pharmacologically unrelated to other currently marketed antidepressants. Several studies have shown it to be a superior antidepressant and anti-insomniac in the elderly with few side effects (14). To improve patient compliance in the elderly, a prolonged action product or a suspension dosage form containing a drug with low solubility may be beneficial. The advantages of lowering the solubility of the active instead of using an excipient to slow dissolution could be to gain patent rights on a new drug product, to achieve better stability, to optimize manufacturability, or achieve taste-masking in certain dosage forms (15). However, the objective of this research is to search for a new salt form of trazodone with lower aqueous solubility with a potential for this type of dosage form, not to develop and test a dosage form of this salt.

In the current study, we have implemented an automated procedure to screen efficiently a large number of pharmaceutical salts using the Biomek 2000 workstation (Beckman Instruments, Fullerton, CA, USA). The high-throughput procedure allows the screening of many different combinations of salts and crystallizing solvents with minimum time and effort on a small scale, minimizing drug usage. Therefore, we were able to screen efficiently various trazodone salts while searching for a new salt form with lower aqueous solubility which may be used to formulate a prolonged action or suspension product. Preformulation characterization of various trazodone salts was also performed.

MATERIALS AND METHODS

Materials

Trazodone hydrochloride (98%), all acids, and sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ninety-six well deep-well plates and robotic supplies were obtained from Beckman Coulter via VWR (South Plain-

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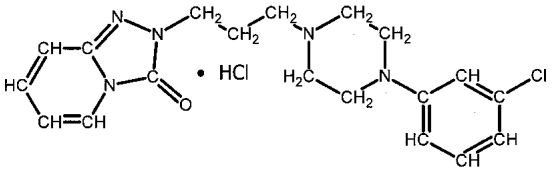

Fig. 1. Chemical structure of trazodone hydrochloride.

field, NJ, USA) and Beckman Instruments. All other chemicals were analytical grade and obtained from J. T. Baker (Phillipsburg, NJ, USA).

Extraction to Free Base

Trazodone hydrochloride was extracted to its free base form through a liquid/liquid extraction procedure. Three grams of trazodone hydrochloride were dissolved in distilled water to slightly below saturation, and the solution was placed in a 125 ml separatory funnel. The pH was adjusted to about 13 using a saturated sodium hydroxide solution. Approximately 10–15 ml of chloroform was added to the separatory funnel, and the mixture was shaken to extract the free base into the chloroform layer. Extraction was repeated 10 times. The collected chloroform layers were washed with saturated sodium chloride solution to remove excess base and then treated with anhydrous sodium sulfate to remove water. Solvent evaporation was performed using a Rotovapor until only a viscous yellow oil remained in the flask. Petroleum ether was used to salt-out the free base, giving an off-white precipitate. Around 95% yield was obtained and purity was indicated using differential scanning calorimetry (DSC) analysis against the supplied bulk trazodone hydrochloride.

Automated Salt Formation

The tools available for the Biomek 2000 include a single-channel pipettor, multichannel pipettor, gripper tool, and a multichannel wash tool, among others. There is also a four-station stacker carousel capable of storing extra tips, plates, lids, or reservoirs. The work surface of the Biomek is capable of holding up to nine plates, tip racks, reservoirs, or other labware needed to carry out a specific experiment. In the event that more than nine items are required to complete an experiment, the stacker carousel together with the gripper tool can be used to clear used labware from and place new labware onto the work surface. The automated salt formation method only required eight spaces on the work surface with a set-up as shown in Fig. 2. Each space is labeled according to row A or B and columns 1–6 to make it easy to identify where labware and tools are located.

Automation of salt formation was performed using two 96-well deep-well plates, eight different crystallizing solvents, and 13 different acids. Trazodone free base was dissolved in acetone at a concentration of 50 mg/ml and placed in a 75 ml ½-module reservoir at position B5 on the workbench of the Biomek 2000 workstation. For a 100 μl delivery per pipette tip, 5 mg of free base would be placed into each well, allowing for sufficient volume per 250 μl pipette tip, located at positions A5 and A6, to reduce error and a minimum amount of trazodone for the initial screening. Solutions of acids in acetone were prepared such that 15 μl of each acid solution gave a 1:1 molar ratio of acid to free base. The following acids

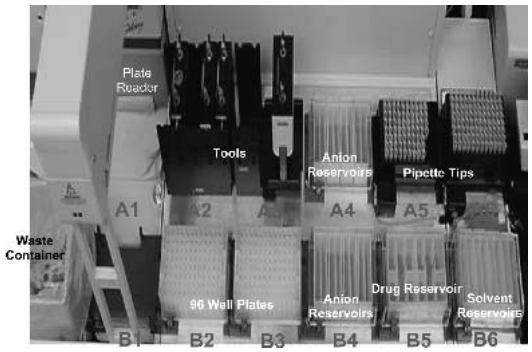

Fig. 2. Automated salt formation set-up on the Biomek 2000 workbench.

in acetone were placed in 19 ml ¼-module reservoirs at positions A4 and B4 on the workbench: hydrochloric acid (as a control), acetic acid, phosphoric acid, sulfuric acid, methane sulfonic acid, ethane sulfonic acid, *p*-toluene sulfonic acid, citric acid, propionic acid, tartaric acid, *n*-decanoic (capric) acid, pamoic acid, and elaidic (trans-oleic) acid. Initially, pamoic acid was dissolved in dimethylformamide (DMF), and citric and tartaric acids were dissolved in deionized water. Eight solvents were used, one for each row of the 96-well plate. The following solvents were placed in 19 ml ¼-module reservoirs at position B6 on the workbench: ethanol, methanol, chloroform, acetone, isopropanol, tetrahydrofuran (THF), ethyl acetate (E), and propylene glycol.

The robotic method was set up to deliver 100 μl of the free base drug solution (5 mg of free base) to all 12 wells of the first 96-well plate and the first column of the second corresponding to the 13 different anions chosen. Subsequently, it delivered 200 μl of each solvent to the plate, one solvent for each row, and then 15 μl of each acid solution, one acid for each of the 13 columns. Built into the method was a temporary halt so that the machine would stop all actions until the user clicked "Continue." This pause was implemented to allow the reactions to occur while solvents evaporated and drug salts precipitated. The 96-well plates were allowed to sit at room temperature in a hood for 12 h and then placed back onto the workbench of the Biomek at positions B2 and B3. The method was then continued. Each well was washed with petroleum ether to remove excess acids or solvents and to salt-out the final drug salt, if necessary.

Salt Screening Procedures

Microscopic observations were made immediately and 48 h after the above process to determine whether precipitation had occurred. A small amount of solid was placed on a microscope slide and observed under a polarized light microscope to view crystallinity. Any salt that either did not form a solid after 48 h or was completely amorphous was eliminated. Amorphous solids tend to be undesirable in pharmaceutical applications due to their instability relative to crystalline forms. If an amorphous drug product is used in physicochemical characterizations, for example in the NDA approval of a particular product, and later on during manufacture of the product a crystalline drug product pops up, the entire NDA approval process may require repeating because the properties of the crystalline drug may be substantially different than that of the initial amorphous drug. There are means of stabi-

lizing an amorphous drug product (16), but we decided to eliminate amorphous candidates.

A 25 mg scale-up was done on the remaining salts involving four solvents: acetone, chloroform, ethyl acetate, and THF; and 11 anions: citric, tartaric, *p*-toluene sulfonic, pamoic, *n*-decanoic, elaidic, hydrochloric, acetic, phosphoric, sulfuric, and propionic acids. Citric acid was dissolved in acetone at this time, but tartaric acid required an acetone–deionized water mixture. Pamoic acid was dissolved in THF. Powder x-ray diffraction (PXRD), DSC, and hygroscopic analyses were performed on these salts. Finally, a scale-up to 1–2 g quantities was carried out, and density, pH solubility, surface area, and particle size analyses were used to obtain physicochemical characteristics on the final salts.

Powder X-Ray Diffraction

X-ray powder diffraction patterns were collected using a SCINTAG XDS 2000 powder diffractometer (SCINTAG, Cupertino, CA, USA) with Co Kα1 radiation generated at 35 mA and 40 kV. Approximately 25-mg to 1-g samples were ground under petroleum ether and placed on a flat quartz holder. Data was collected from 5–50° 2θ at 0.02° intervals and 2°/minute using a germanium solid-state detector cooled under liquid nitrogen.

Differential Scanning Calorimetry

A differential scanning calorimeter, DSC 7, (Perkin Elmer, Shelton, CT, USA) was used. It was initially calibrated using indium and zinc reference standards. Two to five milligram samples were prepared by sealing them in small-volume sample pans, followed by piercing the top with a straight pin to allow vapor to escape. They were heated from 25°C to 120°C at 10°C/min, cooled to 25°C at 30°C/min, heated again to 300°C at 10°C/min, and cooled to 25°C at 30°C/min. Due to some difficulties with the sulfate salts, the method was modified to heat only from 25°C to 300°C at 10°C/min.

Melting Point Apparatus

A Mel-Temp melting point apparatus (Laboratory Devices, Cambridge, MA, USA) fitted with a 0–360°C mercury thermometer was used. About 1-mg samples were lightly packed into 1.5 × 90 mm capillary tubes and observed through the viewing glass as temperature was increased at a rate of about 10–20°C/min.

Hygroscopic Analysis

A humidity chamber was constructed out of Plexiglas, and a Cahn microbalance along with a humidity/temperature probe was placed within the chamber to perform the hygroscopic analysis. Inlet and outlet pipes were placed on opposite corners of the chamber to allow air or steam circulation. A BASIC program was written to collect automatically data from the balance. Data was saved in a text file at user-defined collection intervals. Five to ten milligram samples were prepared by allowing them to equilibrate within a vacuum-sealed desiccator containing Drierite for at least 48 h. They were then placed on the sample pan of the balance within the dry (~6% RH) chamber, which had previously been purged with dry air to obtain the low humidity starting condition. A collection interval of 60 s and a total duration of 170–200 min were used for each hygroscopic analysis session. Humidity and temperature data were manually collected from the humidity meter. All the weight gains were corrected after the empty pan weight gain was taken into account.

Density Analysis

Immediately after synthesis and wash, samples were dried to ensure they were free of solvent. Bulk and tapped density data was collected in accordance with the USP (17) using a 5 ml glass graduated cylinder and 0.8–2.25 g of sample. For tapped density, manual tapping was done at a height of 14 ± 2 mm until no volume change occurred. In order to obtain an estimation of powder compressibility and flow, the compressibility index and the Hausner ratio were calculated according to the formulas below (17):

$$\text{Compressibility index} = \frac{100\,(V_0 - V_f)}{V_0} \tag{1}$$

$$\text{Hausner ratio} = \frac{V_0}{V_f} \tag{2}$$

where V_0 is the unsettled apparent volume and V_f is the final tapped volume. True density was obtained using a helium pycnometer, Accupyc 1330 (Micromeritics, Norcross, GA, USA), and data was collected in triplicate to obtain an average density. The density and compressibility values for each salt were compared to each other and to the hydrochloride bulk material to aid in identifying any potential manufacturing (flow or compressibility) problems. This is most useful if the intended dosage form is a tablet or capsule.

Surface Area Analysis

Surface area was obtained using a Flowsorb II 2300 (Micromeritics, Norcross, GA, USA) fitted with a dual-channel mass flow controller (model 5841, Emerson, Hatfield, PA, USA) set up to deliver a gas mixture of 30% krypton and 70% helium. The machine was calibrated with krypton gas before each use. Desorption and adsorption values were recorded in triplicate for each salt. The average of the desorption values was taken to calculate the surface area of the material because the machine more accurately integrates as gas is desorbed rather than adsorbed.

Particle Size Analysis

An ATM sonic sifter (model L3P, ATM Corporation, Milwaukee, WI, USA) equipped with four sonic sieves and a fraction collector was used to obtain the particle size distribution in accordance with the USP Method I, a dry sieving method (17). The size of the openings in the mesh and types of sieves used were 90 μm ATM standard sonic sieve, 63 μm ATM standard sonic sieve, 40 μm ATM precision sonic sieve, and 25 μm ATM precision sonic sieve (ATM Corporation, Milwaukee, WI, USA). Each sieve along with the top assembly and bottom collector assembly were weighed on a balance before a 3 g sample was added to the top sieve. Each sample underwent a 10 min sift/pulse at amplitude 5–6 followed by a 5 min sift. The sieves and top and bottom assemblies were reweighed to obtain weight fraction retained, and the sieve fractions were recovered for further testing. Each piece

Table I. Summary Table of the Microscopic Observations of Trazodone Salts (Preliminary Eliminations Depicted with Boldface Type)

	Counter-ions												
Solvents	Hydrochloric acid	Acetic acid	Phosphoric acid	Sulfuric acid	Methane sulfonic acid	Ethane sulfonic acid	*p*-Toluene sulfonic acid	Citric acid	Propionic acid	Tartaric acid	*n*-Decanoic acid	Pamoic acid	Elaidic acid (plate 2)
Ethanol	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Little solid AW: Wet MS: Xtal Clusters**	**I: Little solid AW: Wet MS: Xtal clusters**	**I: Nothing AW: Oil, no solid**	**I: Little solid AW: Oil, no solid**	**I: Little solid AW: Sticky MS: Xtal shards**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Little solid AW: Yellow MS: Xtal clusters**	**I: Solid AW: Flaky, white MS: Needle Xtals**
Methanol	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Brown solid MS: Fine Xtal clusters**	**I: Nothing AW: Wet solid MS: Xtal sheets**	**I: Nothing AW: White solid MS: Xtal clusters**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Wet Gel MS: Needle Xtals**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Gooey, brown MS: Xtal shards**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Gooey, orange MS: Needle Xtals**	**EMPTY**	**I: Solid AW: Sticky, white MS: Needle Xtals**
Chloroform	I: Oily Film AW: Oil, no solid	I: Nothing AW: Sticky, orange MS: Xtal shards	I: solid AW: Wet, white MS: Xtal clusters	I: Oily film AW: Orange solid MS: Xtal clusters	**I: Oily film AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	I: Solid AW: Flaky, white MS: Needle Xtals	I: Oily Film AW: Oil, no solid	I: Nothing AW: Flaky, brown MS: Needle Xtals	I: Oily film AW: Oil, no solid	I: Nothing AW: Flaky, peach MS: Needle Xtals	I: Nothing AW: Oil, no solid	I: Solid AW: Sticky, white MS: Needle Xtals
Acetone	I: Solid AW: Wet MS: Plate Xtals	I: Nothing AW: Flaky, brown MS: Xtal shards	I: Solid AW: Flaky, white MS: Xtal clusters	I: Solid AW: Flaky, white MS: Xtal clusters	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	I: Solid AW: Sticky, white MS: Needle Xtals	I: Nothing AW: Oil, no solid	I: Nothing AW: Flaky, brown MS: Needle Xtals	I: Nothing AW: Oil, no solid	I: Nothing AW: Gooey, peach MS: Needle Xtals	I: Nothing AW: Yellow solid MS: Needle Xtals	I: Solid AW: Sticky, orange MS: Needle Xtals
Isopropanol	**I: Nothing AW: Sticky, brown MS: Xtal clusters**	**I: Nothing AW: Oil, no solid**	**I: Solid AW: Flaky, white MS: Xtal clusters**	**I: Nothing AW: Flaky, white MS: Xtal clusters**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Solid AW: White MS: Xtal shards**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Sticky, peach MS: Needle Xtals**	**I: Nothing AW: Yellow solid MS: Xtal clusters**	**I: Solid AW: White MS: Needle Xtals**
THF	I: Solid AW: Crumbly, white MS: Xtal clusters	I: Nothing AW: Sticky, brown MS: Xtal clusters	I: Solid AW: Flaky, white MS: Xtal clusters	I: Oily film AW: Flaky, white MS: Xtal clusters	**I: Nothing AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	I: Solid AW: Sticky, white MS: Needle Xtals	I: Nothing AW: Oil, no solid	I: Nothing AW: Flaky, peach MS: Needle Xtals	I: Nothing AW: Oil, no solid	I: Nothing AW: Sticky peach MS: Needle Xtals	I: Little solid AW: White MS: Xtal clusters	I: Solid AW: Flaky, peach MS: Xtal clusters
Ethyl acetate	I: Solid AW: Flaky, white MS: Xtal clusters	I: Nothing AW: Flaky, orange MS: Xtal clusters	I: Solid AW: Flaky, white MS: Xtal clusters	I: Oily film AW: Flaky, white MS: Xtal clusters	**I: Oily film AW: Oil, no solid**	**I: Nothing AW: Oil, no solid**	I: Solid AW: Sticky, white MS: Xtal clusters	I: Oily film AW: Oil, no solid	I: Nothing AW: Sticky, orange MS: Fine Xtals	I: Oily film AW: Oil, no solid	I: Nothing AW: Sticky, orange MS: Needle Xtals	I: Nothing AW: Yellow solid MS: Xtal clusters	I: Solid AW: Flaky, white MS: Needle Xtals
Propylene glycol	**I: Little solid AW: Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**Empty**	**I: Little solid AW: Empty**	**I: Nothing AW: Yellow solid MS: Xtal clusters**	**I: Solid AW: Wet solid MS: Oil dissolved**

I is initial observations; AW is observations after washing with petroleum ether; MS is after microscopic observations; Xtal is abbreviation for crystal. Boldface type indicates conditions eliminated during preliminary run.

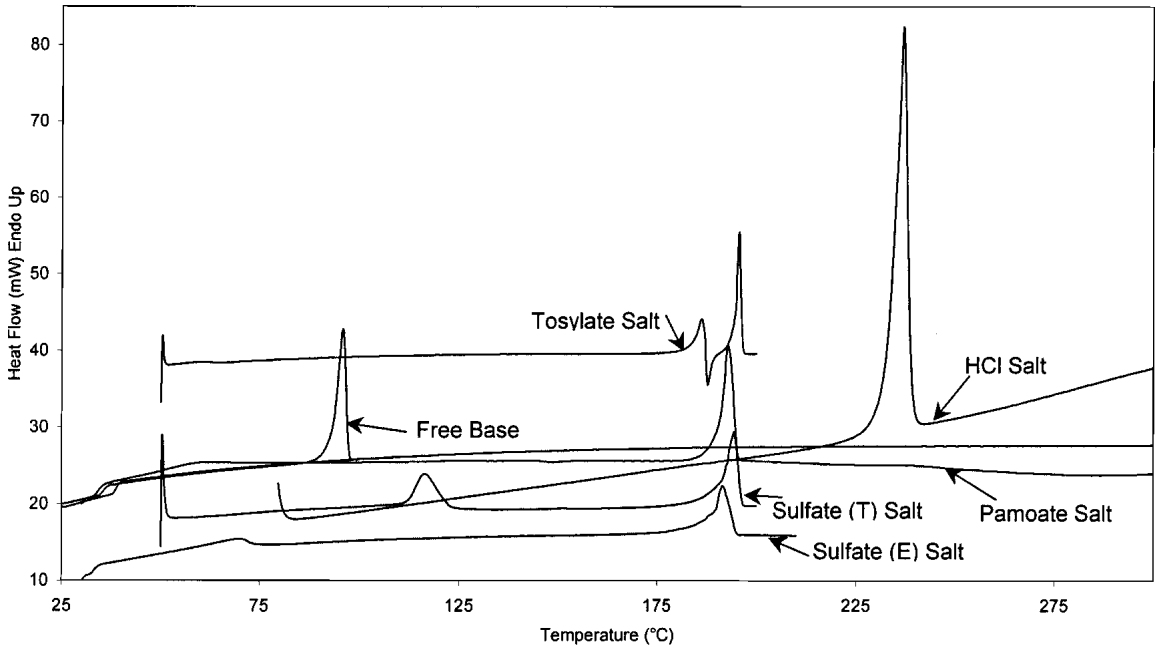

Fig. 3. DSC thermograms of scaled-up salts of trazodone compared to free base and marketed HCl salt.

of the sieve assembly was carefully washed in a sonic bath containing mild detergent, rinsed thoroughly with distilled water, and dried before the next analysis.

pH-Solubility Analysis

A modified form of the phase solubility technique was used (18,19). A saturated solution of each salt was prepared by allowing excess solid to equilibrate in distilled, deionized water for 24 h. The pH was recorded, and a 10 μl sample was taken after being filtered through a 0.2 μm filter. To ensure filter membrane saturation, the sample was taken after 1 ml of fluid had been filtered and taken up in a syringe. HCl or NaOH was added to adjust pH. After 2 h of constant agitation via stir bars, the pH was noted, and another sample was taken. This process was continued at each pH value ranging from 1–12. The samples were properly diluted and analyzed for concentration using UV spectrophotometry at 246 nm (Biomate 5, Thermospectronic, Rochester, NY, USA) or fluorescence for the very low concentrations of drug salt products

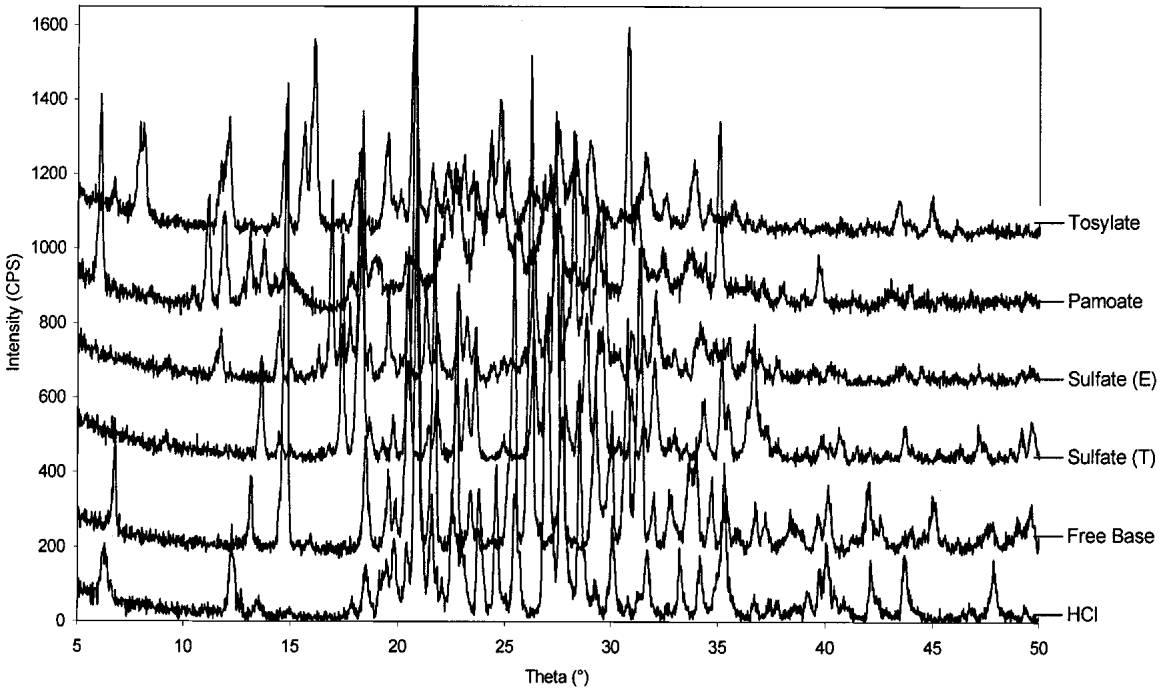

Fig. 4. X-ray powder patterns of scaled-up salts of trazodone compared to free base and marketed HCl salt.

Table II. Physical Properties of Trazodone Salts Compared to Marketed HCl Salt

Physical properties of scaled-up salts of trazodone					
	Density (g/ml)			Compressibility	Hausner
Trazodone salt	Bulk	Trapped	True	index	ratio
Pamoate	0.199	0.339	1.507 ± 0.0078	41.25	1.70
Tosylate	0.208	0.428	1.448 ± 0.0029	51.43	2.06
Sulfate (THF)	0.388	0.598	1.545 ± 0.0058	35.14	1.54
Sulfate (E)	0.344	0.538	1.537 ± 0.0050	36.11	1.57
Hydrochloride bulk	0.431	0.594	1.358 ± 0.0013	27.40	1.38

(DyNA Quant 200, Hoefer Pharmacia Biotech, San Francisco, CA, USA) (20).

RESULTS AND DISCUSSION

Salt Screening

Microscopic observations indicated no solid present in a number of cases leading to preliminary elimination of almost every well of the mesyl and esyl salts. A summary table of these observations is shown in Table I with boldface type representing the conditions eliminated before the first scale-up. I indicates an initial visual inspection into each well to see if solid material was present; AW indicates a second visual inspection after washing with petroleum ether to determine if any salt had precipitated out; and MS is observations made under a polarized light microscope to see the shape of the crystals and determine amorphous content based on the fact that birefringent crystalline materials exhibit the ability to rotate the plane of polarized light (21). The citric acid and tartaric acid salts were not initially eliminated because they had been dissolved in water, which possibly resulted in incomplete mixing with the acetone-dissolved trazodone forming oils initially. Citric acid was dissolved in acetone for the scale-up run, but tartaric acid was only slightly soluble in acetone and required a mixture of water and acetone for solubilization. The four alcohol solvents gave a larger percentage of oily products than the other solvents and were eliminated.

A scale-up was carried out on the remaining 11 counterions and four solvents to obtain about 25 mg of each salt. Among these salts, the citrate and tartrate salts did not give solid precipitates regardless of the crystallizing solvent used, and, therefore, they were not involved in the solid-state characterization. In addition, one acetate salt and one propionate salt, both using acetone as the crystallizing solvent, gave oils instead of solid precipitates. As a result, only 34 of the 44 scaled-up salts were further evaluated with the order of analysis being PXRD, DSC, hygroscopicity, and melting point apparatus to maximize data collecting efficiency and preserve usable drug salt quantities for subsequent evaluation (22–25).

DSC results of the acetate and propionate salts showed that none of the trazodone free base reacted to form a salt. All thermograms gave peaks at 95–96°C corresponding to the melting point of the free base, and all PXRD patterns were identical to the pattern of the free base. In addition, DSC thermograms and PXRD patterns of the elaidate and caprate salts showed that a mixture of the reactants was present. The phosphate salt gave a mixture of products due to its polyprotic nature. This is undesirable as it will be difficult to separate the salts to obtain a pure product. Therefore, all five of these salts were eliminated.

A final scale-up was performed to obtain gram quantities of the remaining four salts. To allow sufficient time for the reaction to reach completion, the reaction time was increased from 12 h to 48 h. For tosylate and pamoate salts, chloroform was used as the crystallizing solvent in order to produce a purer product as found by DSC. For sulfate salts, ethyl acetate and THF were used to make two different salt forms. Only these four trazodone salts were used for the final preformulation characterization.

Table III. Physicochemical Properties of Trazodone Salts Compared to Marketed HCl Salt

Physicochemical properties of scaled-up salts of trazodone			
Trazodone salt	Surface area (m^2/g)[a]	Particle size (d_{avg}) (μm)	Solubility (pH; g/ml)
Pamoate	2.54 ± 0.040	70.32	5.8; 0.077
Tosylate	1.02 ± 0.016	32.93	5.37; 2.68
Sulfate (THF)	0.79 ± 0.025	74.01	1.05; 37.73
Sulfate (E)	1.73 ± 0.024	64.61	1.14; 32.52
Hydrochloride bulk	0.24 ± 0.000	69.31	4.96; 22.12

[a] Data reported are the average of desorption values.

Preformulation Characterization of Scaled-Up Salts

Trazodone hydrochloride from Sigma was used in the preformulation characterization tests to compare to the scaled-up trazodone salts. Figures 3 and 4 show the DSC thermograms and x-ray powder patterns, respectively, for the scaled-up salts compared to trazodone hydrochloride and free base. The pamoate, tosylate, and two sulfate salts had much higher melting points than the free base, but lower than trazodone hydrochloride. The melting points of these four salts appeared around 186–195°C, which was about 40°C below that of the HCl salt.

Several interesting observations were noted. For the tosylate salt, an apparent salt melting peak was observed at 186°C. Immediately following the melting peak is an exothermic peak and another apparent salt melting peak. One possible explanation for this observation is that the tosylate salt undergoes a phase transition at 186.2°C, recrystallizes into a different polymorphic form, which then melts at 197.7°C. This type of transition has been noted in other pharmaceutical salts (26). For the pamoate salt, a small transition was found at 140°C. It appears that this is an amorphous glass transition

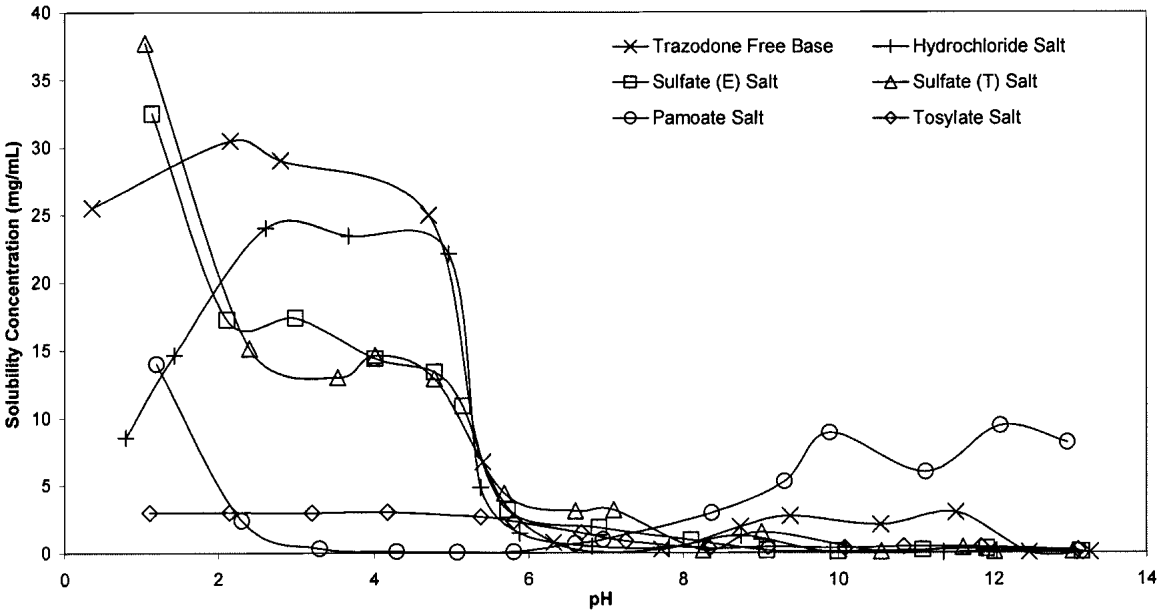

Fig. 5. pH-solubility plot of trazodone salts compared to free base and marketed HCl salt.

temperature because a small portion of the pamoate salt appeared amorphous when it was viewed under polar microscope, and amorphous character was seen in the x-ray powder pattern corresponding to an overall curved baseline throughout the theta angles measured. A similar transition was noted at 80°C and 120°C in the two sulfate salts crystallized using ethyl acetate and THF, respectively. However, little amorphous content was observed in either case, leading to the possible presence of solvates or hydrates. The temperatures at which these transitions occur were different in the two sulfate salts, potentially indicating boiling points of two different solvents. This can help explain the differences seen in the PXRD patterns and DSC thermograms. The excessive noise present in the PXRD patterns hides low intensity peaks and makes comparisons very difficult.

The hygroscopic results of the four salts compared to the HCl salt and free base were very similar and indicated that each salt had a low hygroscopicity with a maximum weight gain of 2.6%, 0.9%, and 2.6% at up to 75% relative humidity for tosylate, pamoate, and sulfate (E) salt, respectively. Lower hygroscopicity of the pamoate salt was expected because pamoic acid is considerably lipophillic. The other preformulation parameters measured for each salt are shown in Tables II and III. All salts exhibited similar true densities of about 1.5 g/ml with bulk and tapped densities ranging from 0.2 g/ml to 0.6 g/ml. The surface area and compressibility index of the marketed HCl salt were significantly lower than the other four salts. This indicates that the HCl salt has slightly better flow properties with less interparticulate interactions, but the low surface area may make dissolution rate slightly slower than the new salts. The tosylate salt had the smallest average particle size of 32 μm and the highest compressibility index indicating poorer powder flow properties due to high interparticulate interactions. The pamoate salt had the second largest average particle size of 70 μm with the largest surface area indicating possible particle agglomeration. The sulfate salts were very similar in nearly every physicochemical property except surface area in which the sulfate (T) salt was 2.2 times lower than the sulfate (E) salt. A very low initial pH value of 1 was noted upon dissolution of the sulfate salts indicating that they would probably not be good candidates for oral tablets or capsules as they may cause damage to the GI membrane upon dissolution.

The pH-solubility profiles for these trazodone salts are shown in Fig. 5. The free base gave a similar profile as the HCl salt below pH 5 because HCl acid was used to implement the pH change, thus causing formation of the HCl salt. Below pH 3, the solubility began to drop off due to common ion effects (27). The free base showed a slightly higher solubility at higher pHs than the sulfate or HCl salts, but the pamoate salt showed the highest solubility at the higher pHs approaching 10 mg/ml at pH 10. However, the pamoate salt was the least soluble with a solubility value of only 0.077 g/ml at pH 5.8, which occurred upon adding distilled, deionized water to the solid powder. The sulfates were the most soluble salt with a solubility of 33–38 mg/ml at pH 1. The most interesting profile is the tosylate salt with a low solubility throughout the entire pH range. The solubility values range from 3 mg/ml at pH 1 to 0.2 mg/ml at pH 12.

SUMMARY

Many pharmaceutical operations involve tedious and repetitive procedures, which potentially can be more efficiently performed using robotic equipment. The operations of the Biomek 2000 workstation can be programmed to perform automatically predetermined experimental steps, allowing precise and efficient operations and significantly reducing experimental error, meeting cGMP requirements. Automation of salt formation, scale-up, and analysis procedures saves time and money, and it may lead to additional application opportunities, which are not feasible if performed manually.

Pharmaceutical salt selection programs are generally designed to obtain the maximum amount of information using the smallest quantity of drug product. They involve systematically analyzing and eliminating salt candidates in a mul-

tistep process. In the current study, the Biomek 2000 workstation carried out a series of salt formation and screening procedures in an effort to search for a new trazodone salt that has a low solubility at pH <7. This salt may be beneficial in a prolonged action or suspension type dosage form of trazodone. Through the use of the Biomek 2000 workstation, we were able to screen 104 potential salts through the combination of 13 counter-ions and 8 crystallizing solvents in a three-stage process. The first stage involved deciding which combinations of counter-ions and solvents gave solid crystalline drug products and how long the reaction should be run to consume all reactants. The solid drug products from this first stage were scaled up to 25 mg quantities for the second stage and analyzed via DSC, PXRD, and hygroscopic stability to determine which conditions gave a new, pure drug salt vs. mixtures of products or reactants. In the final stage of screening, only four remaining salts were scaled up to gram quantities and tested for particle size, pH-solubility, density, and surface area. From these four salts compared to the marketed hydrochloride salt and free base, one salt was chosen as the best for a potential prolonged action or suspension type dosage form based on its lower aqueous solubility.

Out of 104 salts formed in the preliminary run, 44 were scaled up for further analysis, 34 formed solids and were tested with DSC, PXRD, and hygroscopic analyses. The results showed that out of the 34 salts, 20 reactions involving 5 different counter-ions and 4 solvents successfully formed salts. Those were the hydrochloride control, tosylate, pamoate, phosphate, and sulfate salts formed using acetone, chloroform, THF, and ethyl acetate. The most significant finding was the pH-solubility profile of the tosylate salt, which exhibited a low solubility throughout the entire pH range. This characteristic makes it the best candidate, compared to the other salts, to develop a potential prolonged action or suspension trazodone product to improve patient compliance in the elderly.

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