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OPTIMIZATION OF IBUPROFEN FORMULATION: MOLECULAR PHARMACEUTICS OF THE BIOLOGICALLY ACTIVE STEREOISOMER

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BY

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ABSTRACT

A program of experiments comparing the formulation of rac-ibuprofen to that of (+)-S-ibuprofen was performed. Early investigations revealed that although complying with the United States Pharmacopeia compendial standards five different sources of rac-ibuprofen had different processing characteristics and as a result variable biopharmaceutical properties. Crystal habits critically influenced processing parameters. It was possible to identify low and high liquid requirement powders for the wet granulation end-point. Further analysis of rac-ibuprofen crystals was performed during the different stages of tablet manufacture. Phase diagrams confirmed that rac-ibuprofen crystallizes preferentially in the monoclinic space $P2_1c$ group as a true racemate. It was found that crystal distortion translated into an hydrophobic network of ibuprofen causing a drop of 8.5 KJ $mole^{-1}$ in the enthalpy of fusion. This is thought to be responsible for the poor performance of ibuprofen tablets. The extent of this network seemed to be dose dependent as suggested by the dissolution profiles.

Using single crystal x-ray diffraction, the crystal lattice of (+)-S-Ibuprofen was elucidated and the molecular pharmaceutics of the S and racemate investigated. The (+) isomer, although crystallizing with the same number of molecules in the unit cell, exhibited a totally independent

crystal with a melting point of 54°C and an enthalpy of fusion ΔH of 17.9 KJ mole^{-1} less. The stereoisomer of ibuprofen was more soluble than rac-ibuprofen in aqueous media. However, a study of the solution thermodynamics revealed that standard free energy of solution (ΔG^{o}_{rac} = 30.3 and ΔG^{o}_{S} = 29.5 in KJ mole) were comparable, whereas heats and entropy of solution were very different at pH 1.3 (ΔH_{rac} = 32.2 and ΔH_{S} = 51.5 in KJ mole). The small specific surface area of the S isomer (2.8.10^{-3} m^2 g) compared to the racemate (0.34 m^2 g) is probably responsible for the slower intrinsic dissolution (IDR_{rac} = 11.6 $\mu g.sec^{-1}.cm^{-2}$ and $IDR_{S(+)}$ = 8.1 $\mu g.sec^{-1}.cm^{-2}$). The study of biopharmaceutical properties of (+)-S-Ibuprofen formulations, however, indicated an excellent flow and better dissolution than the racemate. Extensive eutectic behavior of the S(+) stereoisomer might be of some concern to the formulators.

In order to formulate the pure enantiomer, the pharmacokinetic of rac-ibuprofen was investigated. Using the Stella^{tm} simulation software it was determined that 1 3 of the (+)-S-Ibuprofen was derived from the inversion of (-)-R-ibuprofen systemically rather than pre-systemically. Thus 150 mg of (+)-S-ibuprofen might be therapeutically equivalent to 200 mg of rac-ibuprofen.

ACKNOWLEDGEMENTS

I want to express my profound appreciation for not only the guidance and assistance of C.T. Rhodes, but also his support of all my endeavors. The project led me down avenues that nearly exhausted the breadth of exploration I ever thought possible. Without the help of my major professor my growth over these last three years would have been undoubtedly stunted.

I want especially to thank Ciba-Geigy Corporation for the award of a fellowship over the last three years. Under the supervision of Dr. George Lukas, Executive Director of FPT, I had guidance and advice to continue on the right direction and also the freedom to alleviate my scientific curiosities. A handful of individuals at Ciba-Geigy went beyond the call of duty to assist me, among them Lou Savastano, Russ Rackley and Franck Clarke; they made my stay in Summit most productive, fruitful and enjoyable.

I would like to extend my thanks to Dr. Bauer and Ethyl Corp. for providing me with the (-)-S-ibuprofen, thus giving me the opportunity to work on a very challenging topic.

Mrs Reba Whitford deserves particular acknowledgement. Time and again she has been the cohesion of the department, taking under her wing all the graduate students including myself. Over my tenure at URI I have enjoyed and benefited from her professionalism, care and devotion.

Finally, to all of those who have shared in the trial and anticipation of graduate work, my fellow students, thank you. The completion of this dissertation was achieved within the heart of this group. To Alex and Mary Kay with whom I have and will enjoy the greatest of friendship.

PREFACE

I elected to write this dissertation following the format of the manuscript plan described in section 11-3 of the Graduate manual at the University of Rhode Island. This option was most appropriate to present my results in several sections.

Section I consists of a general presentation of the problem with introduction and objectives of my investigations. The five manuscripts, chronologically numbered in Section II, are the core of this study. Most of the papers have been either accepted or submitted for publication. Section III, a published manuscript on the topic of clinical research, was not directly related to the core of this work, but some of the analytical method was later employed in the pharmacokinetic methodology used for the (+)-S-ibuprofen.

Section IV is a set of appendices A to D giving experimental details on this work.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Manuscript I has been published in the journal Pharm Acta Helv 66(2):34-43. (1991) and was presented, in part, at the 1989 A.A.P.S. meeting in Atlanta.

Manuscript II was presented in part at the 1990 A.A.P.S. national meeting in Las Vegas and will be submitted to Int. J. Pharm.

Manuscript III has been accepted for publication in the journal Chirality 1991.

Manuscript IV has been accepted for publication in the journal Drug Dev. and Ind. Pharm. 17(5), 1991.

Manuscript V will be submitted for publication in the Journal of Pharmacy and Pharmacology

Manuscript VI will be submitted for publication as a technological note in the Journal de Pharmacie de Belgique.

Manuscript VII has been published in the journal Clin. Res. Practices and Reg. Affairs 8(2): 123-151. (1990) and was presented, in part, at the GRASP '90 meeting in Chapell Hill, NC.

"The hard Things We Do Immediately.
The Impossible Takes A Little Longer"

Pete DeMaria
Ciba-Geigy Corporation

To my Parents and to Teri.

To Colette.

SECTION I

INTRODUCTION

The production of pharmaceutical compressed tablets is very common despite the fact that our understanding of the process is by no means complete. In many instances, the choice of formulation variables is based on an intuitive rather than a rational function. Thus the processing technology may or may not be optimal. As a result there are problems in fully validating the process as required by the Food and Drug Administration(FDA).

Ibuprofen is currently administered as a racemate and oral dosage forms are manufactured using wet granulation. This technology improves the flow and compactibility of powders by increasing the particle size and cohesion. The effect of processing on crystal and granule characteristics have been carefully discussed in the literature. The distribution of particle size depends substantially on the binder solution (1), its volume (2), the mixing time (3) and many other factors (4). The drying stage may have critical effects on the hardness (5) and other physical properties of the granules (6-9). Several authors have correlated compaction parameters to the granule characteristics (10-12) Similarly , the properties of pharmaceutical tablets such as dissolution (13-16), disintegration time (16) or hardness (14), were related to the primary processing technology. To date it is generally recognized that some characteristics of

the raw materials are responsible for certain aspects of the processing behavior (16 21). Although granule growth mechanisms have been studied rather successfully (17,21-25), the theoretical models proposed fail to explain some ambiguities of the ibuprofen formulation. There are a number of articles describing the relationship between the molecular behavior of powdered drugs and tablet processing (26-29). These studies addressed the crystal modifications of carbamazepine(28), sulfanilamide(29), phenobarbital(30), aspirin(31) or many other drugs (32) but at this time, there are no such publications for ibuprofen.

The development of ibuprofen, a non steroidal antiinflammatory agent (NSAI), with several doses strengths presents many challenges (33) to the formulators. Yet, new challenges emerge from the recent possibility of manufacturing the biologically active stereoisomer [(+)-S-ibuprofen] using an economically viable chemical synthesis.

During the course of this study (Spring 1990), we were able to obtain a substantial amount of (+)-S-ibuprofen. At the time several prestigious pharmaceutical companies (Johnson & Johnson, Merck Sharp & Dohme and McNeil) were actively investigating possible synthetic routes to obtain the (+) isomer in a large scale fashion and presently the benefits as well as possibilities of formulating this compound are under heavy scrutiny. This general interest in stereospecific drug development meets the new trends in

regulatory bodies, especially the FDA under the leadership of Carl Peck, in promoting the pharmaceutical development of pure pharmacologically active enantiomers.

With the exception of Naproxen (Syntex), all profens currently used as antiinflammatory products in the United States are marketed as racemates (34). In most of these cases, the dextrorotary or S optical isomer seems to be responsible for the therapeutic activity that is the stereospecific inhibition of the cyclooxygenase and further the prostaglandin synthetase. Various pharmacokinetic reports have been published, suggesting that for some of these aryl propionic acids bioinversion of the inactive stereoisomer could take place in vivo by enzymatic mechanisms (35).

While it was my intention to investigate the relations between processing and ibuprofen crystals at a molecular level, (in order to improve and optimize its formulation), it would have been unreasonable to consider solely the racemate at this stage. Therefore during the spring 1990 I decided to redirect my research work with an emphasis on comparing the rac-ibuprofen to (+)-S-ibuprofen crystals. As a result, a combination of several "expertise", some exclusively reserved to basic research (i.e. single crystal X-Ray diffraction) were applied to the study of the active isomer. The hypothesis were that ibuprofen crystal was modified during formulation and in turn influenced the

properties of resulting formulations. We also hypothesized that, using similar techniques the formulation of the biologically active stereoisomer was indeed possible.

My review of the published literature indicated that predicting or understanding the molecular behavior of ibuprofen under processing had never been reported. On the chirality issue, most reviews on the topic approached the problem from a pharmacodynamic or drug metabolism point of view (33,36). There were no published reports investigating the possibility of developing the pure ibuprofen enantiomers nor addressing the issue of stereospecific drug development in terms of molecular pharmaceutics. It is believed that this approach is a unique concept in the development of pure enantiomers that can be used in many other comparable cases.

The specific objectives of this research work were :

1) to demonstrate the effect of ibuprofen crystal on the processing and biopharmaceutical properties of resulting formulations

2) to assess crystal distortion qualitatively and quantitatively during formulation

3) to use these crystal properties in the development of the pharmacologically active (-)-S-ibuprofen

4) and to compare the molecular pharmaceutics of racemate and the S enantiomer

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SECTION II

MANUSCRIPT I

INFLUENCE OF DIFFERENT SOURCES ON THE PROCESSING AND BIOPHARMACEUTICAL PROPERTIES OF HIGH DOSE IBUPROFEN FORMULATIONS

ABSTRACT

It is known that depending on the manufacturing and synthetic processes, drugs may exist as different forms. As a result, physico-chemical properties, compression characteristics, intrinsic dissolution and bioavailability may vary substantially. The purpose of this study was to investigate the effect of different sources of ibuprofen on the processing of tablets and on their properties. Another emphasis of this work was to rationalize one or several key characteristics of the raw material as directly related to wet granulation parameters and to the behavior of final tablets. Commercially available ibuprofen was obtained from different manufacturers and a preformulation program, including X-ray crystallography, differential scanning calorimetry, scanning electron microscopy, determination of particle size distribution and flowability, was performed to characterize the raw material. Granules were prepared with a planetary mixer and liquid requirements for the end-point were obtained by monitoring power consumption. Tablets were manufactured on Stokes rotary and single punch instrumented presses. Data acquisition interfaces produced compression data for each formulation. Granules and final tablets were analyzed for hardness, dissolution profiles and content uniformity. Statistical evaluations using analysis of variance and multiple comparison procedures were performed

on the results to determine the significance of the variability between independent parameters. The ibuprofen tested was found to be a unique polymorphic form with some differences in the external crystallinity. The particle size characteristics of the material also allowed a differentiation between sources and although there was no differences in dissolution patterns or content uniformity, particle size was found to account for 50% of the variability in tablet hardness. Two sources of ibuprofen with lower mean particle size showed significant variations in end-point liquid requirements resulting in variable tablet crushing strength.

INTRODUCTION

It is now widely recognized that grade variability of the starting material can be responsible for major differences when processing and formulating (1) oral solid dosage forms. Inadequate control of the synthetic process can lead to the production of different polymorphs or crystal forms having variable intrinsic dissolution and exhibiting differences in bioavailability (2), compaction behavior (3) or wet granulation parameters(4). Those phenomena have been frequently addressed in the pharmaceutical literature. For example, the changes in molecular pharmaceutics resulting from grinding, compression and in general processing, have been discussed extensively (5). The

knowledge of physico-chemical characteristics of the starting materials is critical for the formulator (6), especially when high dose drugs are formulated, where the nature of the active itself can also influence substantially the processing of the final products. To date, although, industrial pharmaceutical specifications recommend two suppliers for materials used in a formulation, it has been shown that small changes may occur between products from different manufacturers and within products provided by a same supplier (7). As a result, formulation problems arise when processing the corresponding formulations (4). It is the role of the formulating pharmacist to understand and monitor the transfer of technology involved in switching sources or suppliers, in order to avoid nonideal or unexpected behavior during large scale manufacturing, therefore insuring the good quality of a drug product to guarantee the patient's safety. Ibuprofen, our model compound, is a widely used OTC Non Steroidal Antiinflammatory Agent. Different polymorphs of recrystallized ibuprofen have been shown to exhibit variable extent and rate of biological absorption (2) and this molecule could exist under different crystal forms depending on the synthetic process. As many therapeutic applications of ibuprofen may become available for children at low dose levels (8), minor changes in the crystal structure could result in dramatic changes in the pharmacologic disposition of this compound. The objectives of this investigation were

to determine if several sources of ibuprofen could exist as different forms and exhibit variations in their physical pharmacy profile. A correlation between some characteristics of the raw material, processing parameters and properties of the final products was studied. In further studies, the effect of processing on ibuprofen is investigated in more detail and at a molecular level. For example, it has been shown that particle size (9), particle morphology (4) and surface area (10) of the starting material can influence significantly granule formation and binding properties during compaction.

Fractional factorial designs were utilized to investigate the extent of variability between the different sources and multiple comparison procedures were performed on the results for processing parameters and biopharmaceutical characteristics. This study is the first of a three paper serial investigation leading to the optimization of some aspects of ibuprofen formulation. The information obtained in this work served to correlate key material characteristics to product properties, isolate them from processing parameters and support recommendations regarding the use of different validation procedures for various raw materials if they are provided by different suppliers as it is the case in most industrial pharmaceutical settings.

EXPERIMENTAL

MATERIALS

Ibuprofen was obtained through intermediate distributors and manufacturers. The identity and origin of the five different sources analyzed in this study are presented in table I. Monobasic phosphate and sodium hydroxide were of analytical grade and obtained through the Fisher Scientific Company. Ibuprofen standards for calibration purposes were obtained from the Drug Standard Division of the United States Pharmacopeial Convention in Rockville, MD. Wet granulations containing Fast flow Lactose (Schieffield), Povidone (GAF Co.), Explotab (Edward Mendell) were prepared using purified water. Lubrication was performed with Magnesium Stearate (Fisher Scientific Co.).

METHODS

GRANULATION PROCESS

Ibuprofen was formulated at three different strengths, in order to study the effect of increasing amounts of active on the processing and biopharmaceutical properties of the final products. The formulation investigated in this work may not be the most appropriate for

large scale manipulations. Nevertheless, it was the most convenient combination available, given the complexity of variables involved in the wet granulation process, thus unabling the study of pure source effect.

Blends composed of the active, the diluent, the disintegrant and the binder were dry mixed for ten minutes in a Turbular mixer. The mixture was transferred on an instrumented planetary mixer (Kitchen Aid Model K5-A Hobart) interfaced with an IBM personal computer. A data acquisition software from Extech Co. allowed the recording of Power consumption. The pre-mixed powder was then dry mixed in the planetary mixer allowing the Watt-reading to stabilize to a baseline (11). The granulating fluid was added to the mixture using a peristaltic pump at a rate adjusted to 10 ml min. with five seconds interruption every minute. Wet granulation was proceeded until the end point. In order to have a uniform distribution of liquid bridges the mixer was stopped three minutes after the end-point. The power data was plotted against the granulation time which corresponds to the real wet mixing time and the volume of water added was recorded . Although we fully understand the importance of time and rate at which the liquid is added, it was not the scope of this investigation to study this aspect of the granulating process . Granules were then gently hand screened through a number 8 mesh screen and dried at 40°C during 12 hours in a convection oven to reach a one to two

percent final moisture content. The dry granules were screened through a # 16 mesh screen, mixed for ten minutes with the lubricant and the appropriate amount of disintegrant, and compressed into tablets on an instrumented B-2 Stokes rotary press. An instrumented single punch press (F3-Stokes) was used to validate 350 mg tablets with acceptable tensile strength ranging from 8 to 15 Sc. The compaction force was recorded and the different formulations compared using this parameter. Another experiment was conducted and the hardness of tablets, made at the same level of compaction, was recorded. Three different levels of compression-force were investigated as some biopharmaceutical properties are known to be proportional to the compaction behavior (12). Compression data were recorded as fingerprints of each formulation. Table II shows the starting formulation.

PREFORMULATION AND PHYSICAL TESTING

Analytical testing was performed at different stages of the study and during the formulation process(8). The five different sources of raw material were screened through a solid state preformulation program to characterize the active. This preliminary testing included the following analysis:

-particle size analysis

-surface area determination

-differential scanning calorimetry

x-rays diffraction patterns

-scanning electron microscope photographs

This reduced physical pharmacy profile was undertaken to detect any remarkable differences between sources. The particle size was characterized on ibuprofen water suspensions (10 mg ml) with a Brinckman Particle Size Analyzer model 2334A using a laser light scattering technique. Surface area was determined using a gas adsorption monolayer method and calculated using the B.E.T. equation. This technique was available on a Quantasorb Sorption Analyzer from Quantachrome, NJ. The melting processes were measured by differential scanning calorimetry on a Perkin-Elmer Thermal Analysis Series 7 interfaced with a Perkin-Elmer P7500 E computer. X-Ray crystallography and scanning electron microscopy photographs of the raw materials were performed by the Analytical R&D services of Ciba-Geigy in Ardsley, NY. Flowability measurements were obtained with a custom designed recording powder flow meter on ibuprofen powders and granules; a powder flow linearity index was derived from the flow charts when applicable (13). Apparent tapped density was recorded with an Erweka tap density tester with 2000 taps. Statistical evaluation with analysis of variance was used to differentiate between independent

variables and support interpretations. Final granule size was measured using a conventional sieve method. the size distributions were compared by plotting the percentage oversize vs the amount of active on the screen.The median point was used as the mid-point to compare the different formulations. Moisture contents and loss on drying profiles were determined on a Computrac Moisture Analyzer from Computrac Co. NJ. Tablet crushing strength was measured on an Erweka Automated Hardness tester. Dissolution testing was performed with an Easylift model 63-734-100 from Hanson Co. The method consisted of a rotating paddle at 50 RPM in a pH 7.2 USP-phosphate buffer at 37^{o}C. In order to avoid time consuming dilutions. the working wavelength was adjusted to 264 nanometers. This technique was applied successfully in a previous work (14). Granulations were tested for moisture content. loss on drying curves. liquid requirements for granulation end point, flowability and size distribution. Ibuprofen cores were analyzed for hardness and dissolution profiles in correlation to compaction forces.

RESULTS AND DISCUSSION

PREFORMULATION

The preformulation profiles of ibuprofen showed several differences in the solid state characteristics of

the various sources. Nevertheless, it is understood that analytical testing can present some variations and one has to be extremly cauticus with interpretation. For example, the ibuprofen-water suspensions used with the particle size analyzer may be a fraction not representative of the overall sample populations and each analysis was performed in triplicate. The mean particle sizes and log-normal frequency size distributions are given in table III and figure 1 respectively. Both measurements were performed on ibuprofen particles suspended in an inert solvent and micronized for ten minutes to obtain uniform suspensions. We believe that the micronization divided the aggregates into primary particles but did not generate sufficient energy to dislocate the primary crystals related to processing characteristics. Further experiments including a study of the effect of increasing micronization time on particle size will be performed. The average particle size was obtained from the surface weighed equation (15). It is appreciated that sampling may also be subject to certain variation in this case. The Francis High density had the largest mean particle size with a right skewed tendency. The Cheminor source seemed to exhibit a narrow distribution with an intermediate average particle size. The Boots and Francis Low density sources exhibited the lowest mean particle size, with the narrowest distribution of almost 100% of the sizes below 25 micrometers. The surface area results of unmicronized

ibuprofen samples are presented in table IV. The volume-surface measurements of average particle size are mostly used for pharmaceuticals because they are inversely proportional to the specific surface. Combined with surface area measurements, it allows an accurate evaluation of physical properties of medicinal powders. Some BET results were not expected as Francis low D. and Boots, which exhibited the lowest mean particle size, had intermediate surface area values. On the other hand, Francis High D. which showed the largest mean particle exhibited the highest surface area indicating a very broad size distribution in accordance with the frequency curve. The apparent tapped densities reported in figure 2 summarize the micromeritics behavior of ibuprofen powder based on the previous experiments. According to the packing theory, as a result of size characteristics and surface area, Francis High D. exhibited the largest density. Boots and Francis Low D. showed the smallest apparent density, probably indicating the uniformity of the shape distributions. Figures 3-4 are scanning electron microscopy photographs of ibuprofen raw material. The macroscopic observation of X500 magnified ibuprofen crystals allowed rational conclusions on the differences between sources as sorted by particle size, BET values and density results. There was no significant visible difference between Ethyl and Boots, which exhibited lamellar needle type crystals. On the other hand, Francis low D. has a very uniform

distribution of the smallest needle type particles. Francis high D. has the largest rounded macrocrystals with small microcrystals and Cheminor exists as laminated square plates of intermediate size. The surface of Francis High density macrocrystals seems to be very irregular. probably an explaination of the high surface area value. Clearly. this visual evaluation indicated that the final crystallisation step of the synthetic process could be very different for the various sources leading to differences in crystal forms. This observation could not be predicted from the BET results which did not show any significant differences between Cheminor.Ethyl and Boots but appeared to vary from the surface area of the two Francis sources as indicated by an F-test.The thermal analysis gave more information on the crystal structure. All the DSC curves exhibited a unique endotherm in the range 75-76^{o}C with enthalpies of fusion ranging from 113 J g to 118 J g. An example of a typical DSC profile of ibuprofen is shown in figure 5 and all melting points are reported in table V. An analysis of variance did not show any significant differences indicating that the ibuprofen tested do not exist as different polymorphic forms and the internal crystal structure is equivalent for all sources. Nevertheless. the enthalpies of fusion are statistically different. The results of an F-test. shown in table VI. suggest that ibuprofen has variable crystal surface

structure, as anticipated from the scanning electron microscopy photographs and the X-ray diffraction patterns in figure 6. The general shapes are similar for all sources however, for low angles of the spectra, the intensity of the first deflection peak varies between materials indicating a difference in external crystallinity. Except from Francis high D, which exhibited a flow index of 18.5 (a flow index of 19 is representative of good flowability properties) all ibuprofen powder did not flow through the orifice of the flowmeter.

The wet granulation process divided the sources into two groups: raw materials with low liquid requirements for the end-point (figure 7) and crystals with high liquid requirements (figure 8). It is appreciated that the power scale does not represent means of differentiating between sources, rather the general shape of the power consumption curves was analyzed in detail with emphasis given to the inflection points where the torque required to rotate the paddle at the same speed within the wet granules increased suddenly. The increase in wattage was attributed to a change in the physical state of the wet mass, which we associated with the end-point of the granulation also represented by the arrows on the power consumption curves. The addition of water was interrupted upon observation of this increase. The arithmetic average of liquid requirements are reported in table VII.

This categorization between sources was not performed arbitrarily but using an analysis of variance and Duncan's multiple comparison procedures which divided the raw materials into two groups with a 95% confidence level (table VIII). Ibuprofen powders with high liquid requirements and ibuprofen sources with low liquid requirements. As a consequence of end point requirements, the moisture content of the wet mass, measured at 65°C before the drying step exhibited differences recorded in table IX. Loss on drying profiles (figure 9) were also different probably indicating variations in the channels and pore tortuosity of the granules in which the moisture migrates to the surface to evaporate. Granules from different sources were mixed with lubricant for ten minutes and tabletted. An acceptable compaction force was applied (10-15 KN), the hardness of the corresponding tablets measured and reported in table X. Several comparison procedures were applied to evaluate the effect of independent variables such as particle size or amount of active. Although, not using an interactive model, some inferences could be made on the size effect with the ANOVA in table XI. The sum of square due to the particle size of the raw material demonstrated that about 50% of the hardness variability among granulations was due to differences in particle size fraction and distribution of the starting material. The differentiation and classification of

the sources by liquid requirements was confirmed on the compaction versus hardness investigation. The crushing strength of tablets made with Boots and Francis Low D. sources was higher for the same level of compression forces as compared to the other powders. Dissolution profiles of ibuprofen cores did not indicate any significant differences in the release from the various formulations and based on the previous preformulation experiments. we do not forecast any problems of biological availability with the use of different sources. Further studies will include the analysis for the enantiomeres of ibuprofen. Figure 10 shows the various dissolution profiles and calibration curve for the ibuprofen cores. In order to preserve the clarity of the figure. the percentage dissolved after 70 minutes are not represented as they did not bring further information on possible differences between sources. Figure 11 shows a complete dissolution profile of ibuprofen cores made with one source (Cheminor). The low disintegrant level (1%) and its position in the tablet formulation (100% intragranular) are responsible for the slow ibuprofen release rate. Further studies include the optimisation of the concentration and position of the disintegrant when the active/diluent ratio is increased.

CONCLUSIONS

The process of chemical synthesis or isolation of drug substances and excipients used in tablet formulation although designed to produce materials of reproducible high chemical purity, may not necessarily result in batches of product with equivalent physico-technical properties. The nature of the solvents or the concentration of intermediates present in the liquors used for crystallisation can affect particle morphology including crystal dislocations, surface rugosity and surface area. Those properties, although not reflected in significant differences in melting points, solubility or crystal forms, can influence compression characteristics and possibly the amount of granulating fluid required to produce a coherent mass. This conclusion underlines the importance of the preformulation and in-process testing when using different suppliers and possibly different batches of the same material.

As the different ibuprofens tested did not exhibit major variations in physical-chemical properties and do not exist as different polymorphs, various sources of this active could be used in oral solid dosage forms without risks of altering the biological availability. Nevertheless, during the course of this study several important differences were detected. Possibilities of variations in liquid requirements for the end-point, which could be

predicted from particle size analysis and apparent density measurements, have to be kept in mind as they affected the final hardness of ibuprofen cores. To date all commercial ibuprofen tablets are coated and the ease of coatability, mostly related to friability and hardness, is a critical parameter in formulation technology. Certainly, since different sources led to substantial differences in hardness (as a result of higher end-point liquid requirements), coatability function may be affected by slight differences between sources. As a consequence, when consistent variations between two sources can be detected through a solid state preformulation program, key parameters such as density, crystal size, surface area and crystal surface morphology may be used to predict problems in the formulation behavior. It is appreciated that the conclusions of this work do not advantage one source over another, since at any moment of the formulation stage the processing parameters can be modified to obtain final products in acceptable ranges, rather those observations underline the importance of a strict and detailed physical pharmacy profile for materials from different suppliers and suggest the usefulness of two validation procedures or two standard operating procedures specific to each one of the sources, thus avoiding costly unexpected pharmaceutical behavior during large scale operations.

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Table I
SOURCE AND ORIGIN OF IBUPROFEN

Name	Origin	Abbreviation
Francis Low Density	Italy	FranL.D
Francis High Density	Italy	FranH.D.
Boots	U.S.A.	Boots
Ethyl	U.S.A.	Eth
Cheminor	India	Chem.

Table II :
STARTING FORMULATION

IBUPROFEN	57 %
FAST FLOW LACTOSE	36 %
PLASDONE	6 %
EXPLOTAB	1 %
LUBRICANT	1 %
GRANULATING FLUID (WATER)	q.s.

Table III

MEAN PARTICLE SIZE (MICRONS)

SOURCE	Surface-Number Mean	Surface-Weighted Mean
FRANCIS LOW D.	3.17	5.03
BOOTS	3.58	6.22
ETHYL	5.23	18.18
CHEMINOR	7.94	31.07
FRANCIS HIGH D.	10.54	38.25

BRINCKMAN PARTICLE SIZE ANALYZER
Micronised Water Suspensions

Table IV
SURFACE AREA
B.E.T. VALUES (SD) in m^2 /gram

FRANCIS LOW D.	0.76 (0.03)
FRANCIS HIGH D.	0.86 (0.09)
ETHYL	0.34 (0.01)
BOOTS	0.41 (0.03)
CHEMINOR	0.36 (0.04)

(*) UNMICRONIZED SAMPLES

Table V
MELTING RANGES
DIFFERENTIAL SCANNING CALORIMETRY

SOURCE	ONSET	(SD)	MELTING POINT	(SD)
CHEMINOR	73.0	(0.2)	75.4	(0.05)
ETHYL	73.2	(1.4)	75.6	(0.10)
BOOTS	73.8	(0.2)	76.1	(0.10)
F.LOW D.	72.4	(0.1)	75.1	(0.10)
F.HIGH D.	73.4	(0.1)	75.3	(0.10)

All values are reported in degrees Celsius

Table VI
ENTHALPY OF FUSION OF VARIOUS SOURCES (J/g)
ANALYSIS OF VARIANCE TABLE

	df	SS	MS	F	p
SOURCES	4	67.63	16.91	4.67	0.01
ERROR	16	57.96	3.62		
TOTAL	20	125.60			

At the 99% confidence level there is a significant difference in ΔH's of 3 to 5 J/g

Table VII

LIQUID REQUIREMENTS FOR IBUPROFEN GRANULATIONS END-POINT

SOURCE	MEAN VOLUME (SD) IN ML	
ETHYL	27.8	(4.2)
F.HIGH D.	31.2	(3.4)
CHEMINOR	31.5	(5.1)
BOOTS	44.0	(6.7)
F.LOW D.	58.2	(2.2)

Liquid requirements for 175 gm batches

The average reported was obtained from all granulations

Table VIII

EFFECT OF DIFFERENT SOURCES :

LIQUID REQUIREMENTS FOR GRANULATION END-POINT

DUNCAN'S MULTIPLE RANGE TEST

SIGNIFICANCE LEVEL α = 0.05

27.8	31.2	31.5	44.0	58.2	(ML)
ETHYL	FHIGHD.	CHEM.	BOOTS	FLOWD.	

ANY TWO AVERAGE NOT UNDERLINED BY THE SAME SEGMENT ARE SIGNIFICANTLY DIFFERENT

Table IX
WET GRANULES: MOISTURE CONTENT

ETHYL	**11 %**
FRANCIS HIGH D.	**13 %**
CHEMINOR	**18 %**
BOOTS	**23 %**
FRANCIS LOW D.	**32 %**

Loss on drying at 65 degrees C

Table X
HARDNESS LEVELS
COMPACTION RANGE (KN) 10-15:

SOURCE	FORCE(KN)	HARDNESS(Sc)
ETHYL	10-15	19-22
BOOTS	12-14	22-29
CHEMINOR	12-14	12-24
FRAN.LOW	11-13	19-28
FRAN.HIGH	10-12	18-25

Table XI
Analysis of Variance for tablet hardness
One degree of freedoom comparisons

Source	df	SS	MS	F	p-value
Among granulations	**11**	**3651.1**	**331.9**	**25.7**	**0.001**
Ethyl Vs. Boots	**1**	**127.1**	**-**	**9.8**	**0.025**
Size1 Vs. Size3	**1**	**390.1**	**-**	**71.0**	**0.000**
Size2 Vs. Size3	**1**	**891.6**	**-**	**69.1**	**0.000**
Size1 Vs. Size2	**1**	**427.5**	**-**	**33.1**	**0.000**
Within Granulations	**66**	**852.1**	**-**	**12.9**	

Size1: Sieve fraction 20/40 ; Size3: Sieve fraction 80/Pan
Size2: ibuprofen "as is"

Figure 1: Size-Frequency Distribution

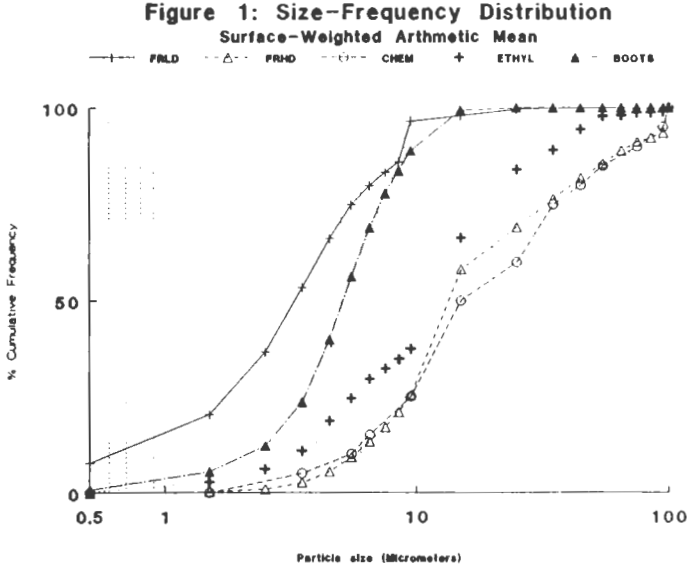


Figure 2
Apparent Bulk and Tapped Densities

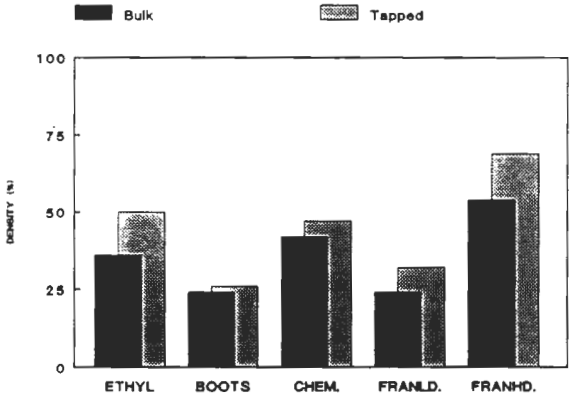


Figure 3:

Scanning Electron Microscopy Photographs of Ibuprofen Crystals

I) FLD: Francis Low Density

II) FHD: Francis High Density

III) Boots

IV) Ethyl

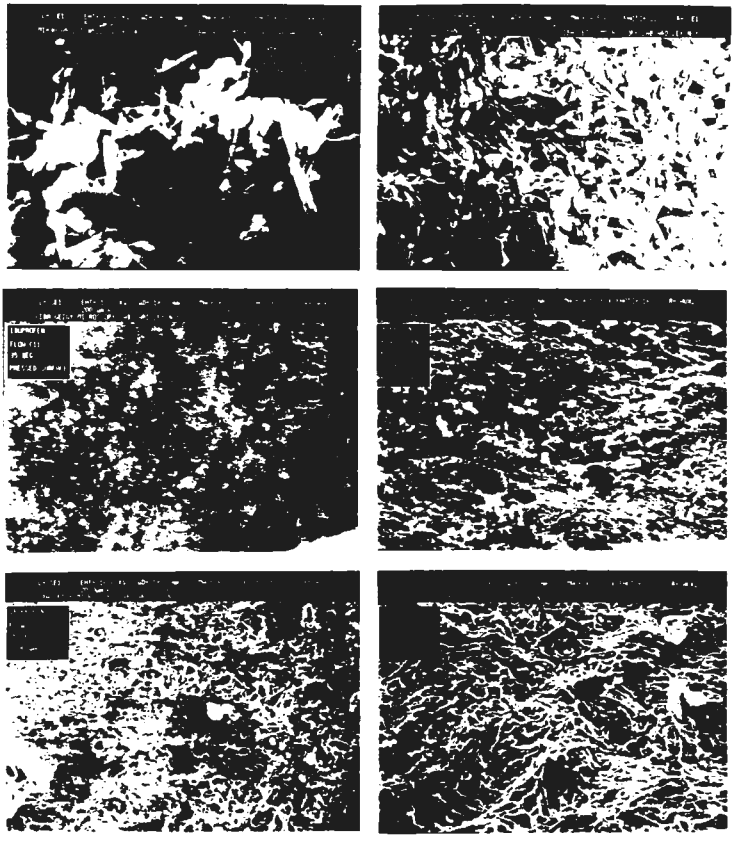


Figure 4:

Scanning Electron Microscopy Photographs of
Ibuprofen Crystals
I) and II) Cheminor
II) and IV) Ethyl: different batches

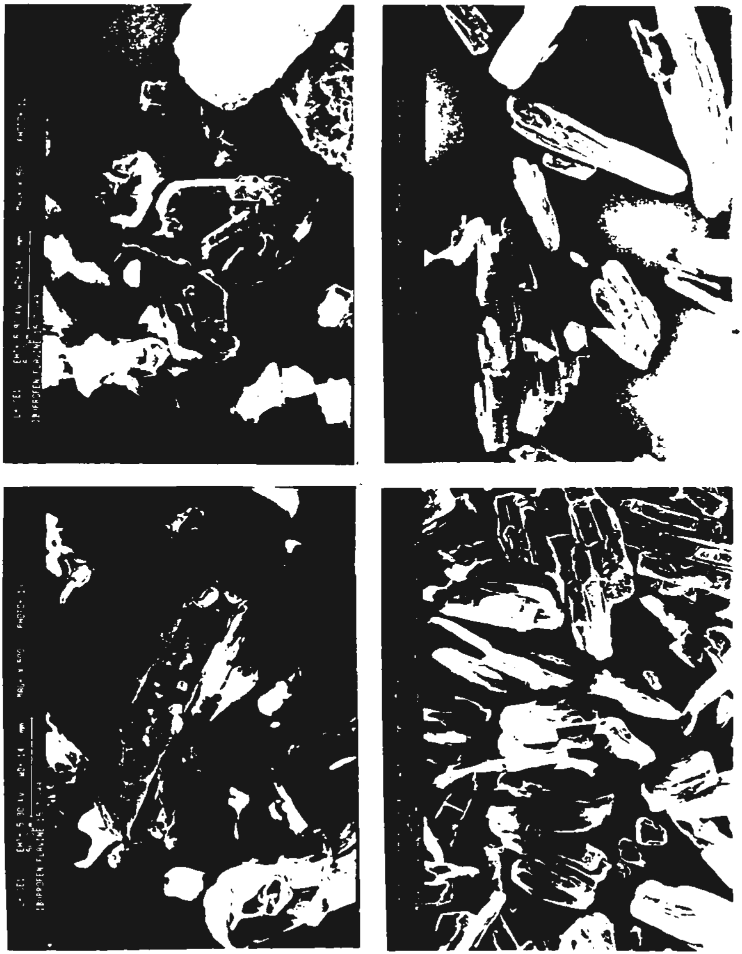


Figure 5: Typical DSC Endotherm of Ibuprofen

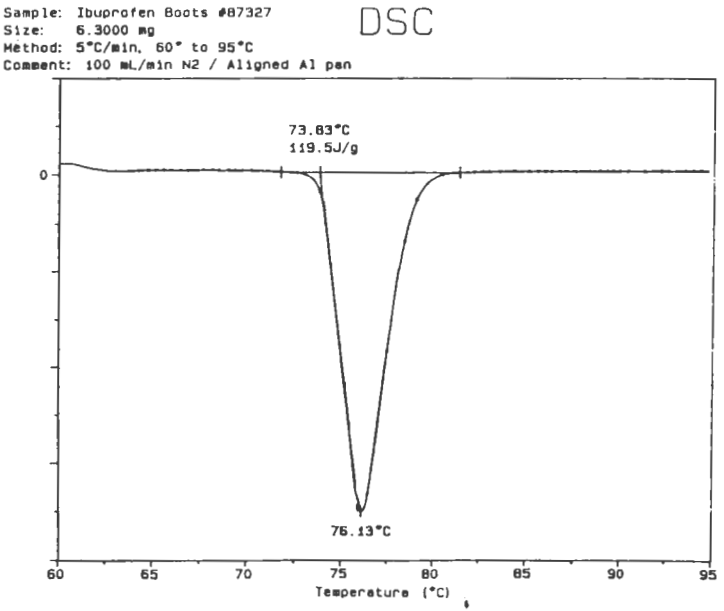


Figure 6: X-Ray Diffraction Patterns of Ibuprofen Sources
A. Francis High D. B. Francis Low D.
C. Cheminor D. Boots E. Ethyl

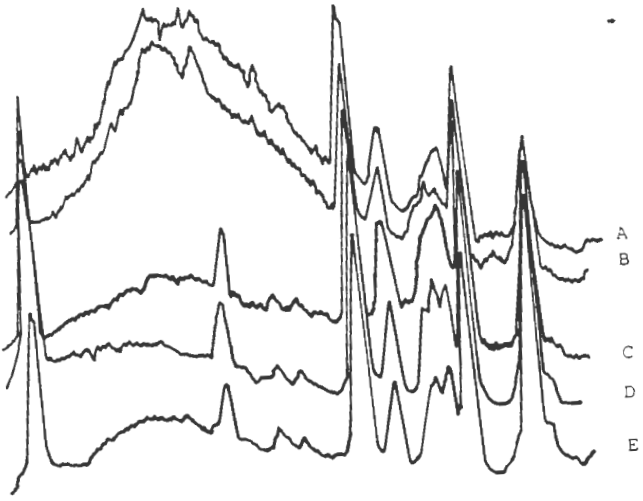


Figure 7
POWER CONSUMPTION FOR WET GRANULATION

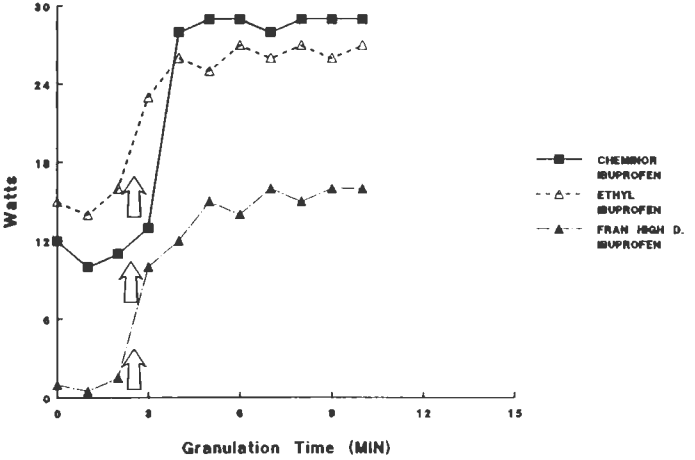


Figure 8
POWER CONSUMPTION FOR WET GRANULATION

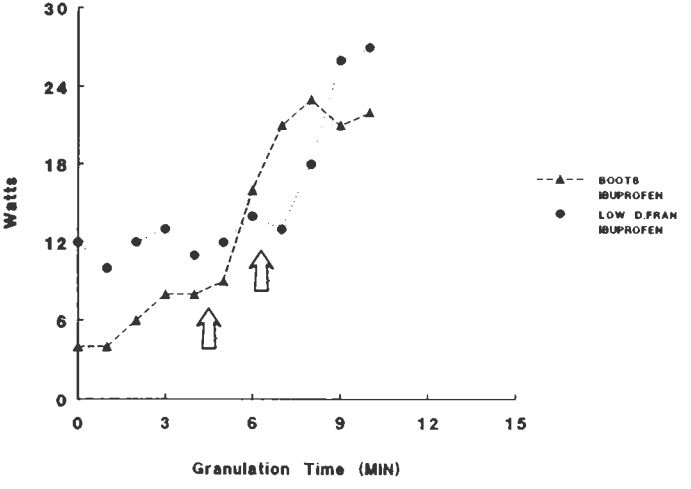


Figure 9
LOSS ON DRYING PROFILES AT 40°C

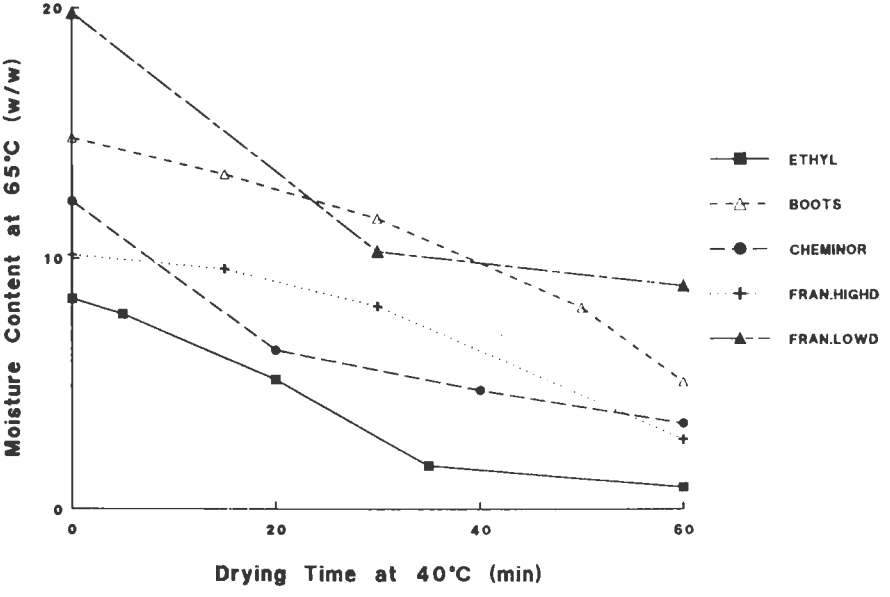


Figure 10

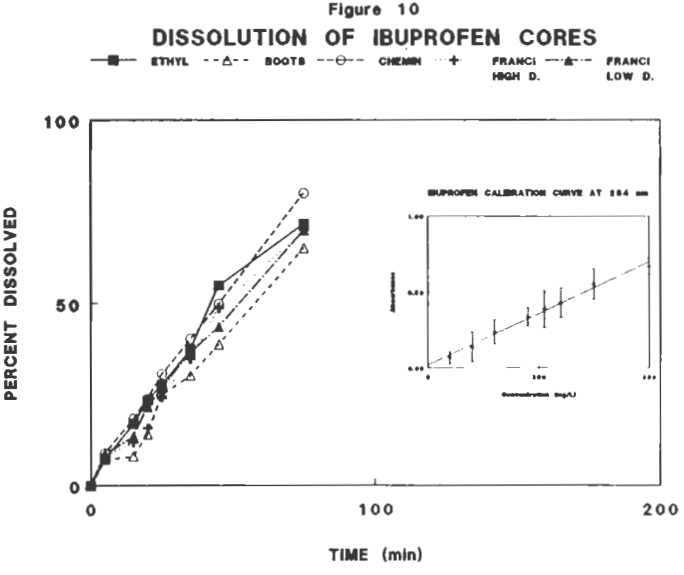
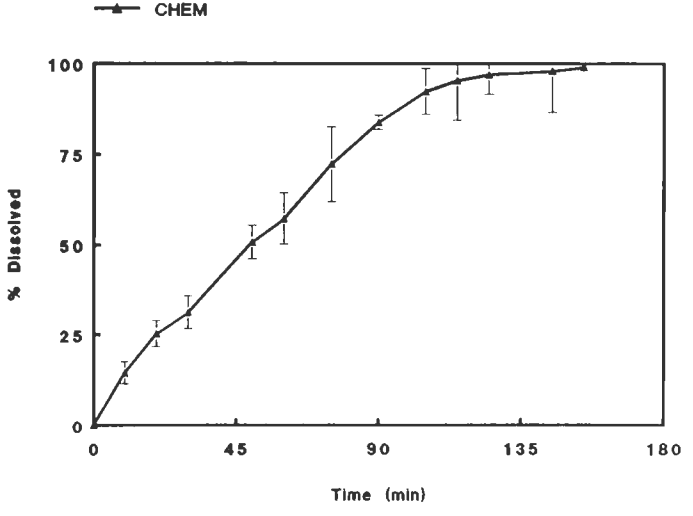

DISSOLUTION OF IBUPROFEN CORES
ETHYL
BOOTS
CHEMIN
FRANCI HIGH D.
FRANCI LOW D.
PERCENT DISSOLVED
100
50
0
0
100
200
TIME (min)
IBUPROFEN CALIBRATION CURVE AT 264 nm

Figure 11

DISSOLUTION OF IBUPROFEN CORES (CHEM)



MANUSCRIPT II

MONITORING CRYSTAL MODIFICATIONS IN SYSTEMS CONTAINING IBUPROFEN

Key words: Ibuprofen: Crystal analysis: Hydrophobic network: Intermolecular interactions: Formulation effects.

SUMMARY

Qualitative and quantitative crystal analysis, including differential scanning calorimetry, X-ray powder diffraction and scanning electron microscopy were performed at different stages of ibuprofen tablet manufacture obtained at three levels of compaction. Melting points and enthalpy of fusion were carefully monitored and compared using statistical techniques (ANOVA and one degree of freedom procedures). Drug-disintegrant interactions were investigated using a fractional factorial design. Wetting and compaction affected the crystal surface as measured by a 0.2 to 8.6 KJ mole decrease in the heat of fusion, and a shift of 2-3 ^{o}C in the melting point. The differences were too small to suggest the existence of enantiotropically or monotropically related polymorphs. The results, however, indicated a lattice modification of ibuprofen during processing. The initial dissolution rates appeared to be inversely related to the amounts of ibuprofen in the formulation and the fastest drug release was obtained for a 1:3 intragranular ratio.

INTRODUCTION

Fundamental investigations, especially in the field of compaction and wet granulation, have long established that pharmaceutical processing can modify some characteristics of raw materials in such a way that can be detrimental to the overall performance of the final drug product (Lefevbre et al 1986, Chan et al, 1985). Monitoring crystal changes has become essential in order to optimize many formulations (Haleblian et al, 1975). For example, sulfanilamide crystal habit was altered as a function of increased compression forces or exposure to liquids (Cruaud et al, 1981) and physical interactions between ibuprofen and excipients can induce eutectic behavior (Gordon et al, 1984; Mura et al, 1987). The latter does not necessarily mean adverse incompatibility but may explain handling difficulties. Many pharmaceutical manipulations will affect the crystal habit of drug substances and these modifications may have adverse consequences on the formulation (Cruaud et al, 1981) or the drug bioavailability (Aguiar et al, 1967).

Crystal properties of ibuprofen are known to influence the processing behavior (Romero et al, 1991; Hiestand et al, 1981). This aspect of ibuprofen formulation is well documented and it is generally recognized that the drug undergoes changes due to processing (Franz et al, 1986). For

example, eutectic behavior has been proposed with some pharmaceutical excipients (Gordon et al. 1984) and although never experimentally proven, surface sintering has been suggested as a theory for rearrangement of crystal lattice during compression (Alhec et al. 1990).

Nevertheless, very little has been published to support evidence of the crystal modifications of ibuprofen. Thus, the mechanisms and consequences of such alterations have yet to be identified for this particular compound.

The objectives of this work were to elucidate the mechanisms of crystal distortion by which ibuprofen is modified during its processing and to investigate these effects on the biopharmaceutical properties of a model formulation.

EXPERIMENTAL

Materials

Ibuprofen USP grade was obtained from the Ethyl Co. (Lot#LH-6-72). Wet granulations containing Fast Flow Lactose (Sheffield lot #59009), Povidone (P.V.P.-GAF lot#G-30223A) and Explotab (Edward Mendell lot#1336) were prepared using purified water. Granule lubrication was achieved using Magnesium stearate (Fisher Scientific Co.). The potassium

monobasic phosphate and sodium hydroxide used for dissolution medium and buffers were obtained from Fisher Scientific. All chemicals were of analytical grade. Ibuprofen standards for spectrophotometry and differential scanning calorimetry (DSC) were provided by the Standard Division of USP Rockville, MD.

Methods

The experimental design consisted of analyzing ibuprofen after dry mixing with excipients, wet massing and tableting. The model formulation, defined in table I was prepared using five process steps presented in table II. The percentage of active were 57, 67, and 77 percent. Mixtures of ibuprofen, the diluent, the binder (6 percent), and the appropriate amount of disintegrant were dry mixed for ten minutes. The powder was wet granulated in a planetary mixer (Kitchen Aid, model K5-A, Hobart) until the end-point, monitored by power consumption, was reached. Flow rate of the granulation liquid remained constant throughout the entire experiment. Granules were dried on a tray at 40 ^{o}C for twelve hours and later mixed with lubricant in a V-blender for ten minutes. Lubricated granules were then compressed into 350 mg tablets using an instrumented F3 single punch press. Three compaction pressures were investigated: low,

intermediate and high (averaging 1.10 and 30 KN respectively).

At the end of the manufacturing steps I, III, IV and V (table II), samples were withdrawn and analyzed by X-Ray powder diffraction and scanning electron microscopy (SEM) photographs. Thermal analysis (DSC) was performed on all samples (Kim et al. 1985) using a Perkin Elmer, series 7 instrumented unit, calibrated with indium and interfaced with a P 7500 E computer. For whole tablets, the electron microscopy photographs were shot at 35 and 80 ^{o} angles on pressed and side surfaces, on horizontal and vertical cross sections of tablets embedded and prepared according to a method described by Hess, (1978).

Crystal Packing

The unit cell of ibuprofen crystal was analyzed using the molecular modeling software. The coordinates of single X-ray reflection data was obtained from the literature (McConnell, 1974) and the molecular arrangement of a crystal lattice, simulated on this program.

Comparative Analysis

In an effort to mimic the effect of processing, ibuprofen and physical mixtures of the formulation were

ground thoroughly for ten minutes in a mortar or melted at a temperature above 80 ^{o}C and recrystallized upon cooling at room temperature (RT). Differential scanning calorimetry was further performed on the samples and their thermal profiles compared to those of pure and formulated ibuprofen. Additionally, ibuprofen hygroscopicity was measured after storage at 35 ^{o}C and 85% relative humidity (RH). Karl Fisher analysis was performed at regular time intervals on 100 mg samples exposed to humidity.

Biopharmaceutical Properties

The dissolution apparatus used a paddle rotating at 50 RPM in a USP phosphate buffer at pH 7.4 and a temperature of 37 ^{o}C. This method using a six vessel dissolution apparatus (Vankel) had been shown to discriminate between various ibuprofen formulations (Romero et al. 1988). An ultra-violet spectrophotometer was used to determine the concentration of ibuprofen at 264 nm in the dissolution fluid. For low ibuprofen concentrations, the percentage dissolved was also calculated from measurements obtained at 220 nm.

Statistical Analysis

All results were analyzed statistically using an analysis of variance at the 99% confidence level to determine differences between enthalpy of fusion and melting ranges. Sums of squares were calculated to perform one degree of freedom comparisons using orthogonal contrasts. These tests allowed the investigation of pure compaction effect on the thermal parameters. A restricted fractional factorial design was used to test the effects of drug disintegrant interactions. The independent variables were amounts of active and the concentration of extragranular disintegrant. All factors had three levels. In the interpretation of the data greatest weight was placed on any effects on dissolution.

RESULTS AND DISCUSSION

Crystal Packing

The single crystal unit cell for the racemate included four molecules: two R(-) and two S(+) isomers, two central hydrogen bonds between the carboxylic functions of dextrorotary and levorotary molecules (Fig. 1). In addition figure 2 shows the juxtaposition of eight crystal unit cells. The hydrogen bonds between cells could be identified. Each intermolecular interaction was shared between four other cells as favored by the preferential positioning of

R(-) and S(+) molecules. Except for the top-left cell retained for baseline comparison, each unit has been cleared of the molecules not involved in the intercellular interactions. The resulting effect is the delimitation of a plane, on which intermolecular distances are most likely to be affected during tangential stress. Thus this eight cell system may explain the observed lattice weakness.

The mass fraction of water obtained by the Karl-Fisher technic, averaged (0.063% + - 0.001) and (0.55% + - 0.004) before and after exposure to humidity respectively. This analysis confirmed that although the moisture increased ten-fold after exposure to 85% relative humidity for 76 hours, it did not exceed 0.55% possibly concentrating at the surface since ibuprofen does not include crystallization water. This amount of moisture, was defined by Alhec and Zografi (Alhec et al. 1990) as plasticization or molecular mobility.

Thermal Analysis

Thermal analysis of ibuprofen indicated that only compaction or grinding of the physical mixture affected the melting point and the heat of fusion (table III). All parameters were compared at the 99% confidence level. Furthermore, the enthalpy of fusion decreased progressively to as low as 18.1 KJ mole during the tablet manufacture. If the assumption that a pure equilibrium exists at the melting

point (Tm) is valid, then the changes are probably indicative of enthalpic modifications (weaker intermolecular interactions) at the crystal surface before compression. then :

$$\Delta G = \Delta H - Tm.\ \Delta S \qquad \text{equation 1}$$

at Tm the melting point, the free energy ΔG should equal zero, with ΔH^f the enthalpy of fusion of the sample, $\Delta\Delta H^f$ the enthalpy loss and ΔTm the melting decrease.

$$\Delta G = 0 \text{ and } \Delta H = Tm.\Delta S \qquad \text{equation 2}$$

Mixing with excipients and processing are the combination factors responsible for the enthalpy drop. Table IV summarizes the statistical analysis of thermodynamic parameters. All enthalpy of fusion were significantly different as determined by the F test (see table IV). The magnitude of the shift depended on the stage of processing. The orthogonal contrasts L1,L2,L3 were found statistically significant. Compression had an effect on the enthalpy of fusion and ibuprofen in lower strength formulation appeared to be less sensitive than in higher strength tablets. The heat of fusion for ibuprofen was less affected in granules than in tablets.

X-Ray Crystallography

In figure 3 the X-Ray diffraction patterns of pure ibuprofen dry granules and ground tablets indicated crystal changes of ibuprofen during pharmaceutical manipulations: dilution with excipients only decreased the intensity of the diffractogram. Wet granulation, however, induced a slight rearrangement of the crystal lattice. At low angles of the spectrum, the 12.2° deflection peak completely disappeared leaving an amorphous region. No further changes were visible on the X-ray diffraction pattern after compaction.

Scanning Electron Microscopy

In order to complement results of the thermal analysis, qualitative observations were performed by SEM. Morphological changes from pure to formulated ibuprofen were visible at the X1000 magnification as shown on figure 4. On the tablets, the crystals are visible but the particle boundaries are not detectable (indicating cold bonding or fusion at the surface). Thus, an ibuprofen network appeared to result from sintering of the ibuprofen crystals during processing. Cross sections of tablets showed a film of PVP (light membrane) covering packs of ibuprofen crystals (dark). Some starch glycolate particles are also visible.

The macroscopic observation of SEM photographs confirmed the crystal disorder suggested by thermal analysis: the wet granulation process induced the lattice fragilization and upon compaction, further crystal disruption occurred with a possible consolidation of the hydrophobic matrix. The disintegration and dissolution analysis confirmed that the ibuprofen network was indeed hydrophobic. When two ibuprofen particles are in contact, within a formulation, thermal properties have already been disturbed: upon compaction, enough mechanical energy is provided to induce cold welding or sintering of the crystalline envelopes of ibuprofen as visualized on these S.E.M photographs.

Effect of Processing

The decrease in Tm averaged a statistically significant 2-3 ^{o}C upon compaction. ΔTm was not proportional to the compression as indicated by paired t tests (fig.5). The reported values of compaction are arithmetic means of upper punch compression forces recorded on the instrumented press. The enthalpy decrease $\Delta\Delta H^{f}$ was defined as the difference between the heat of fusion of pure ibuprofen and the energy of fusion of the formulated ibuprofen. $\Delta\Delta H^{f}$ was indicative of the extent of some crystal modifications. The effects of compaction and low ibuprofen strength on the thermodynamic parameter have been found statistically significant (table

IV). In figure 6 for all strengths studied the enthalpy loss increased and stabilized at a plateau value. Low concentrations of ibuprofen seemed to be less sensitive to mechanical stress. The latter, however, had the highest shift before tableting. These results are conclusive of two opposite effects of a low active excipient ratio during tablet manufacturing. Similar effects had been previously suggested by a study on the pharmaceutical processing of sulfanilamide (Cruaud et al. 1981).

Dissolution Studies

The dissolution profiles of ibuprofen cores also exhibited similar trends, as seen in figures 7-8. Low ibuprofen content led to cores with fast dissolution rates as compared to higher strength dosage forms. Although the data suggested that initial drug release rates might be related to the extent of the ibuprofen network (table V), any correlation was insignificant because of the limited number of points. The crystal modification of ibuprofen created the surface hydrophobic network within the tablet that may be quantified by $\Delta\Delta H^f$. The latter seemed to be the limiting factor of drug release. The dissolution efficiency was optimal for the lowest amount of ibuprofen and the highest concentration of disintegrant (3%) in the 1/3 intragranular

ratio. Formulation with 100% extra or intragranular disintegrant had lower dissolution efficiencies than did formulation combining extra intragranular disintegrant at the same concentration. This might be another evidence of the existence of the hydrophobic network.

CONCLUSIONS

The crystal packing of ibuprofen occurs with a preferential orientation in which a weak plane has been identified as probably responsible for the crystal modifications during compaction.

Pharmaceutical processing does alter the crystal habit of ibuprofen (not its internal structure) in a stepwise manner. We identified three progressive mechanisms:
Mixing with excipients: destabilization and fragilization of intermolecular interactions, wet granulation: distribution of water in the amorphous regions. Both mechanisms account for a drop of 4.1 KJ mole in the enthalpy of fusion and predispose the ibuprofen to cold welding. Compaction: brings the enthalpy decrease to another 6.2 to 8.6 KJ mole and provides enough energy to catalyze sintering as observed on the scanning electron microscope. In addition to lattice rearrangement, the resulting ibuprofen network is hydrophobic and could be the limiting factor of drug dissolution. As a consequence formulators must be extremely

cautious when increasing ibuprofen concentration in tablet formulation.

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Table I: Formulations Used in this Study

Ingredient	Amount in %								
Ibuprofen	57			67			77		
Fast Flow Lactose	36			26			16		
P.V.P.	6			6			6		
Mg Stearate	1			1			1		
% Na Starch Glycolate*	1	2	3	1	2	3	1	2	3
**Details on the the Intragranular ratio*	*1*	*1/2*	*1/3*	*1*	*1/2*	*1/3*	*1*	*1/2*	*1/3*
Purified Water	q.s.			q.s.			q.s.		

All systems contained 1% of intragranular disintegrant

Table II: Experimental Methodology

Processing	Steps	Analysis
Dry Mixing (V-Blender) 10 minutes (Ibuprofen, Lactose, Explotab, PVP)	I	DSC, SEM, X
Wet Granulation (Monitored by power consumption)	II	
12 hour tray drying at 40 C	III	DSC, SEM, X
Mixing (V-Blender) 10 minutes (Granules, Explotab, Lubricant)	IV	DSC, SEM, X
Compaction	V	DSC, SEM, X Dissolution Hardness

DSC: differential scanning calorimetry, SEM: scanning electron microscopy, X: X-ray crystallography,

Table III: Thermal Analysis of Ibuprofen

	M.Pt.(SD) [oC]	ΔH (SD) [KJ/mole]	ΔS [J.G^{-1}.K^{-1}]
IBUPROFEN			
Pure	77.7(0.6)	25.7(0.53)	0.35
Ground	76.8(0.2)	23.7(0.93)	0.33
Melted(*)	76.9(0.4)	23.0(0.91)	0.11
RH(**)	77.2(0.3)	25.6(0.06)	0.35
Physical			
Mixture	77.4 -	21.0 -	0.29
Ground	75.3 -	18.1 -	0.25
Granules	77.3(0.2)	21.9(1.29) [b]	0.30
Tablets	75.2(0.9)	18.2(1.71) [b]	0.25

(*) recrystallized at RT, (**) 76 hours at 85 % RH

(b) statistically significant difference compared to pure Ibuprofen

Table IV: ANALYSIS OF VARIANCE (ENTHALPY OF FUSION)
One degree of Freedom comparisons

SOURCE	DF	SS	MS	F	$F_{0.01,1,42}$
Among Formulations	**9**	**4365.7**	**485.1**	**13.62**	**7.3**
L1 *KN Vs. NoKN*	*1*	–	–	*9.48* *	"
L2 *Low KN VS. High/Reg KN*	*1*	–	–	*42.9* *	"
L3 *A–57% Vs. C–67% or E–77%*	*1*	–	–	*19.8* *	"
Within Formulations	**33**	**1157.3**	**35.6**		

(*) indicates a significant difference (99% confidence level)

Table V: Crystal Modifications and Initial Drug Release Rates

[IBU]	ΔH^f J/g	Release Rate mg/hr
A(57%)	27.2	10.80
C(67%)	36.0	0.66
E(77%)	41.9	0.63

Figure 1: Unit cell of ibuprofen racemate

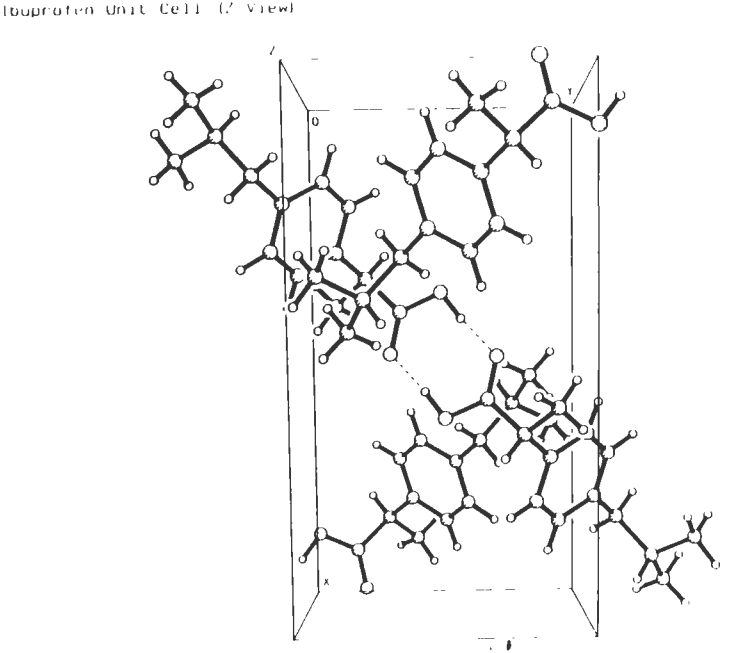


Figure 2: Details of the molecular packing within the ibuprofen crystal lattice

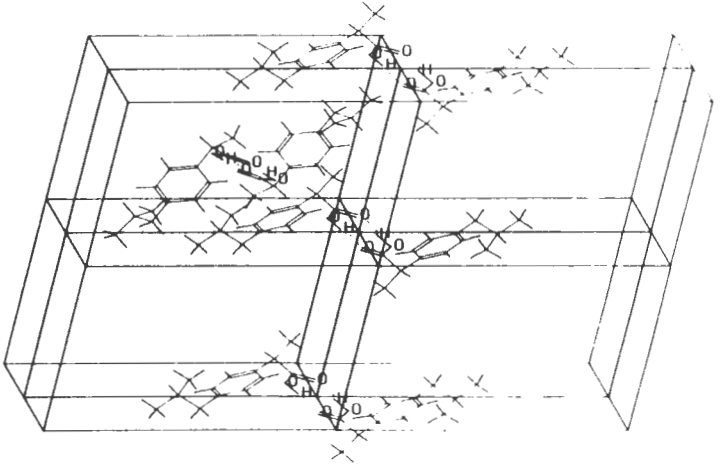


Figure 3: X-ray diffraction patterns of A) Ibuprofen B) Ibuprofen granules and C) Ibuprofen Tablets

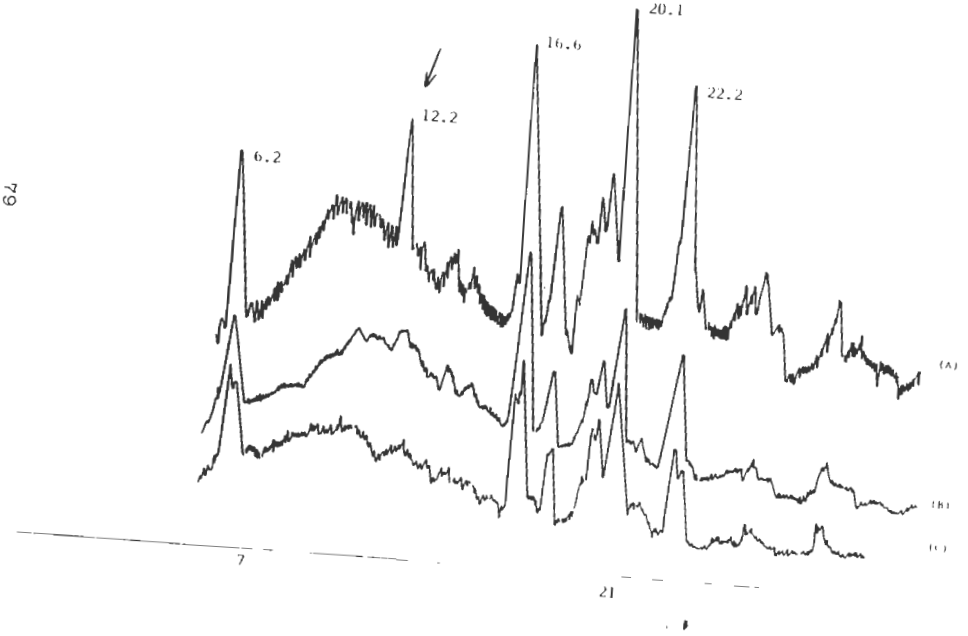


Figure 4: Identification of the ibuprofen network
Electron microscopy photographs of
A) ibuprofen, B) granules, C) tablets:
C1,C2: pressed surface, C3: side walls
C4: vertical cross section

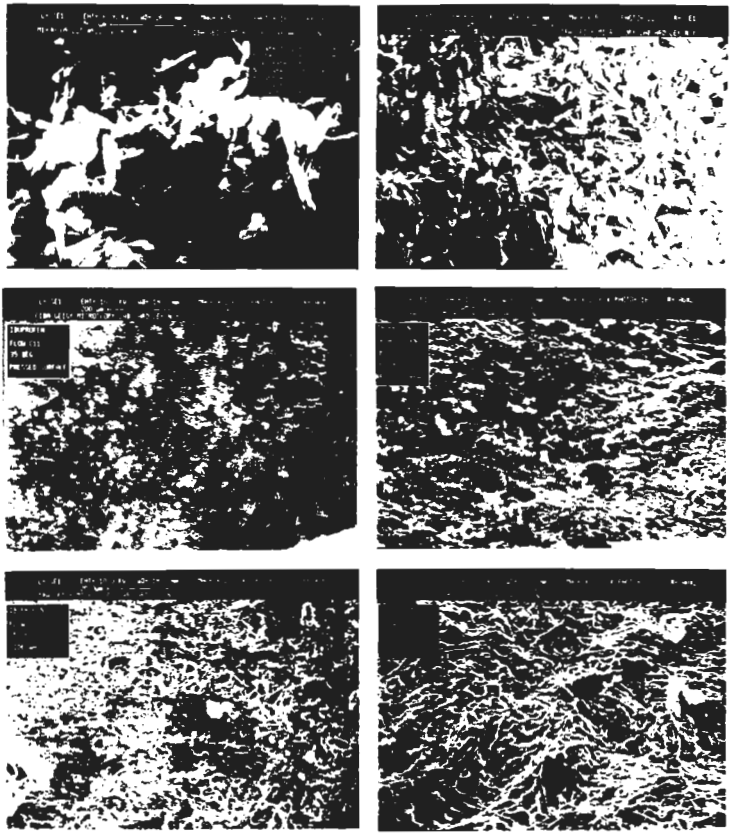


Figure 5: Effect of Compression on the Melting Point

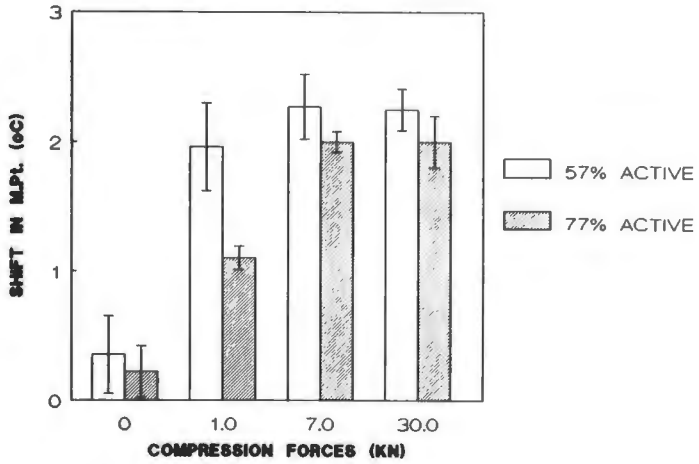


Figure 6: Effect of Compression Forces on the Enthalpy Parameter

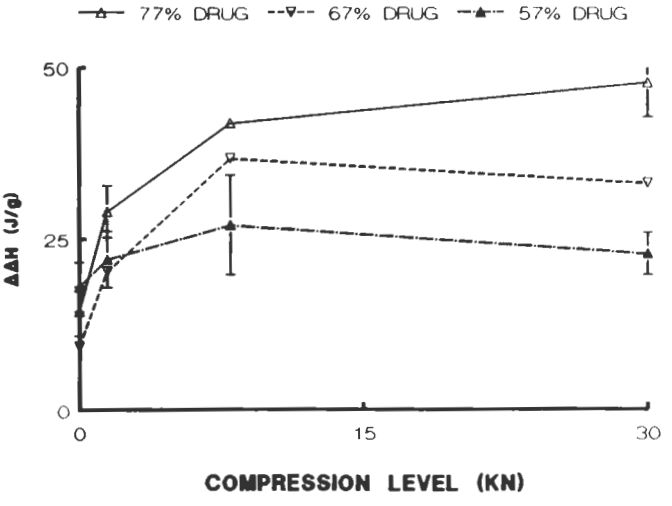


Figure 7: Dissolution of Ibuprofen Cores Disintegrant (0 and 1 intragranular ratio)

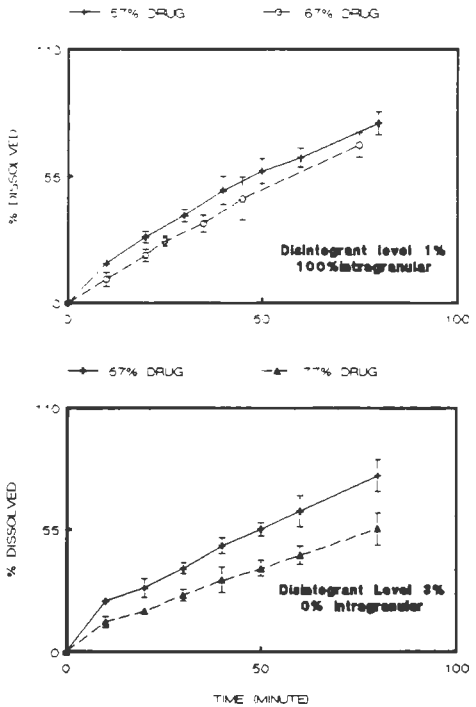
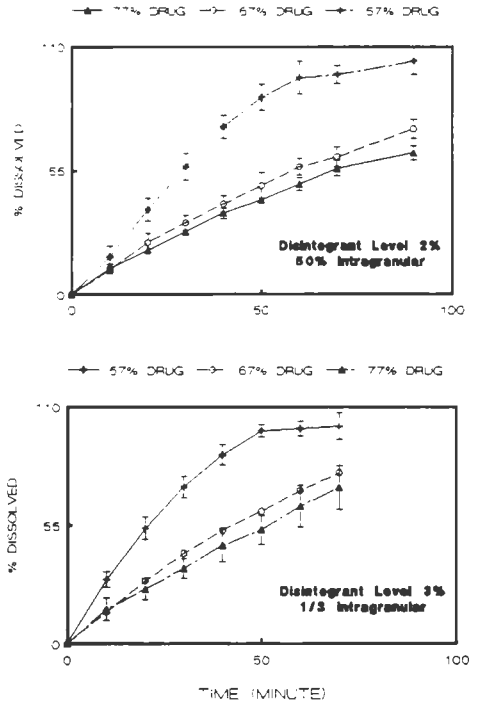


Figure 8: Dissolution of Ibuprofen Cores Disintegrant (1/2 and 1/3 intragranular ratio)



MANUSCRIPT III

AN EVALUATION OF IBUPROFEN BIOINVERSION BY SIMULATION

INTRODUCTION

In an effort to formulate the pharmacologically active enantiomer of ibuprofen into a solid oral dosage form, we examined the literature regarding investigations of stereoselective pharmacokinetics of this arylpropionic acid. Early reports (1) indicated that urinary ibuprofen metabolites were essentially all dextro-rotary (S enantiomer) following administration of the racemate in man. Sensitivity of stereoselective assays and enantiomeric separations have improved and it is generally recognized that there is an enzyme catalyzed inversion of the inactive R enantiomer into the therapeutically active S enantiomer (2,3). Bioinversion of the R to the S enantiomer has been suggested to occur either presystemically (4,5) and or systemically (6). Also, differences in the fraction of R inverted into S have been reported (7,8). To date only four studies have involved the administration of a pure ibuprofen enantiomer to man (6,9). Using the pharmacokinetic model proposed by Jamali et al. (5), we simulated (-)-R- and (+)-S-ibuprofen plasma concentrations following oral administration of (+)-S-ibuprofen, (-)-R-ibuprofen, or the racemate. Simulated and literature values for area under the plasma concentration-time curves (AUC) were used to compare ratios of S:R for different cases of the model and for

comparison of different methods calculating fraction of R inverted to S.

METHODS

Simulations

The one-compartment model proposed by Jamali et al. (5) was used for all simulations of ibuprofen enantiomer plasma concentrations. This model (Fig. 1) assumes first-order processes for absorption and elimination for both R and S enantiomers, as well as for the bioinversion of the R to the S enantiomer. This model appears to have been designed such that total elimination of R, by bioinversion and non-bioinversion routes, should be equal to elimination of S. The literature, however, supports a faster apparent elimination for the R isomer (6-8). The racemate dose was assumed to be 200 mg of (-)-R-ibuprofen and 200 mg of (-)-S-ibuprofen enantiomer. Pharmacokinetic parameters similiar to those used by Jamali et al. (5) were incorporated. Volumes of distribution (Vd) were 10 L for each enantiomer. Absorption rate constants (kar,kas) and elimination rate constants (kr,ks) for both enantiomers were 1.0 and 0.34 hr^{-1}, respectively. The four cases of presystemic (kip) and or systemic (kic) bioinversion reported by Jamali et al. (5) were reproduced as follows: 1) kip=1.5 and kic=0 hr^{-1}, 2) kip=1.0 and kic=0.08 hr^{-1}, 3) kip=0.5 and

kic=0.15hr^{-1}, and 4) kip=0 and kic=0.225 hr^{-1}. Numerical simulation of the model was performed with the Stella simulation software (10), using Euler s method with a time step of 0.01 hr. The apparent terminal elimination rate constants for the R and S ibuprofen enantiomers (App kr and App ks) were estimated from simulated plasma concentrations. The area under the simulated plasma concentration-time profile from time zero to 24 hr after start of dose, AUC(0-24), was calculated for both enantiomers. These simulated AUC's were used to calculate an S R AUC ratio. The AUC's from these simulations were also used to compare different methods of calculation the fraction of the R enantiomer inverted to the S enantiomer following oral administration. In additon, literature values for AUC of R and S (4,6-9,11) were used to calculate an S/R AUC ratio and assess different methods for estimating fractions inverted.

Calculation of Fraction Inverted

For calculations of fraction of ibuporfen inverted in the body, it was assumed that both enantiomers have similiar absorption and disposition parameters. Also, it was assumed that oral administration of racemate was similar to oral administration of equal amounts of S and R ibuprofen enantiomers. These assuptions are recognized as potential limitiations of the calcualtion method but have been used by several authors (5-9, 12, 13).

The relationship assumed was:

(AUC of S after racemate) - (AUC of S after S) - (AUC of S after R) **Equation 1**

where dose of racemate = dose of s - dose of R. and the dose of R and S are equal.

The fraction of R inverted to S (Fr→s) may be approximated by :

Fr→s = (AUC of S after R) (AUC of S after S) **Equation 2**

for equal doses of R and S.

Substitution Equation 1 into Equation 2. additional equations for Fr→s may be derived:

Fr→s = ((AUC of S after racemate)-(AUC of S after S)) (AUC of S after S) **Equation 3**

where dose of recemate = 2*dose of S;and.

Fr→s = (AUC of S after R) ((AUC of S after Racemate)-(AUC of S after R)) **Equation 4**

where dose of racemate = 2*dose of R.

Based on the previous assumptions of identical absorption and disposition for both enantiomers. it may be possible to

approximate fraction inverted in the body following oral administration of the R and S enantiomers, the racemate and S enantiomer, or the racemate and R enantiomer.

In addition one can estimate the contribution of the chiral bioinversion to the plasma levels of the therapeutically active isomer. Rather than calculating the fraction of R converted to S (Equation 2-4), an alternate method of looking at bioinversion is by estimation of the fraction of S which is inverted from R ($F_{s\leftarrow r}$) for a dose of racemate. This may be approximated by the following equation:

$$F_{s\leftarrow r} = \text{(AUC of S after R)} \text{ (AUC of S after racemate)} \qquad \text{Equation 5}$$

where dose of racemate = 2* dose of R(-).

Additional variations of Equation 5 are also possible if one assumes the relationship in Equation 1 to be valid.

RESULTS AND DISCUSSION

Simulated (-)-R- and (-)-S-ibuprofen enantiomer plasma concentration-time profiles and the corresponding S/R plasma concentration ratios appeared to be identical to that reported Jamali et al.(5) for the four different cases of

presystemic and or systemic bioinversion following administration of 400 mg of racemate as 200 mg each of R and S enantiomers. Simulations of the S R AUC ratios, following administration of the racemate, ranged from a value of 4.0 for a presystemic-only bioinversion (Sim #1, Table I) to 1.66 for systemic-only bioinversion (Sim #4, Table I). Simulations of the S R AUC ratios, for administration of 200 mg of the R enantiomer only, ranged from a value of 1.5 for a presystemic-only bioinversion (Sim #5, Table I) to 0.66 for systemic-only bioinversion (Sim #8, Table I). Calculations S R AUC ratios from literature reports of AUC's of R and S enantiomers following administration of the racemate averaged 1.53±0.20 (average±sd), while S R AUC ratios following administration of the R enantiomer were found to be 0.50±0.09 (Table II). Thus, these simulations support a conclusion of systemic bioinversion in man since they appear to be in good agreement with the literature data. Different conclusions by Jamali et al. (4,5), based on use of a plasma concentration S/R ratio, may be due to increased variability in plasma concentrations R and S enantiomers. Since the emphasis of that ratio was placed toward the end of the sampling period when plasma concentrations were relatively low (4,5), a greater error in calculation of the plasma concentration S/R ratio could have arisen from assay variability. It is speculated that calculations S and

R AUC's. for the S R AUC ratio. are not as susceptible to the same degree of such error.

Agreement of simulated S R AUC ratios with literature values does not necessarily support the hypothesis of sytemic bioinversion. since the mode proposed by Jamali et al. (5) may be questioned. The mode (Fig. 1) appears to have been designed to demonstrate similiar elimination for R and S. The sum total of R is (kr-kic)-kic. which equals ks. This creates some confusion since elimination of R by non-bioinversion routes and elimination of S could be comparable. However. elimination of R by processes other than bioinversion was adjusted by the systemic bioinversion rate constant (i.e. kr-kic. Fig. 1). according to the model defined by Jamali et al. (5). It was assumed that this model was derived to demonstrate similiar 'apparent elimination rate constants for both R and S (App kr and App ks. Table I). which holds true for the presystemic-only bioinversion case (Sim #1 and Sim #5. Table. I). However. as the model is admusted toward systemic-only bioinversion. the apparent elimination rate constant for S. determined from simulated plasma concentrations (App ks). decreases. ne suggested modification of the model is not to adjust non-bioinversion elimination of R by the changes in kic. Other model modifications are also possible. such as suggested from the studies by Ahn et al. in the dog (12). from which

it may be speculated that the rate and extent of bioinversion decreases with increasing amounts of (+)-S-ibuprofen. Thus, a model incorporating saturable enzymatic inversion may prove to be a more correct model. We also appreciate the fact that gastro-intestinal membranes of different animal species contain the enzymatic system responsible for the stereospecific inversion. Therefore, presystemic bioinversion, if any, could be formulation dependent.

An assessment of the fraction of R inverted to S ($Fr_{R\to S}$) was based on calculations using Equations 2-4 for simulations and literature values for AUC's of the S enantiomer, following administration of S and R, S and racemate, or R based on the same model (Sim #9, Table I). Using Equation 2, and the results for simulation of the R and S enantiomers, the fraction inverted ranged from 0.6 for presystemic-only simulation to 0.66 for systemic-only simulation. It must be noted that Jamali et al. (5) chose parameters for presystemic and systemic bioinversion which would approximate a fraction inverted of 0.6, which is consistent with the data of Lee et al. (8). Assessment of Geisslinger et al.'s data (7), using Equation 2, indicates the fraction of R inverted to S was 0.48 (Table II), although these autors who reported a fraction inverted of 0.33 did not indicate their exact mathematical operation. Calculations of $Fr_{R\to S}$ using Equation 2 ranged from 0.36 to

0.64 for all literature values of AUC after S for administration of the R and S enantiomers (Table II). Fractions inverted calculated for Equations 3 using literature values ranged form 0.25 to 0.74, while Equation 4 yielded values of Fr-s ranging from 0.51 to 0.96. It is important to note that when AUC data was not available for a particular enantiomer, data from Lee et al. (6) or Cox et al (6) were used after normalization for dose. Since differences were found for literature values when fraction inverted was calculated using Equations 2-4, it might be concluded that the relationship described in Equation 1 is not a rigorous as originally assumed. In the four studies involving administration of (-)-R-ibuprofen, the AUC of S and R averaged 1 2 of the AUC of R after R (49.7%). Using Equation 5, we calculated that after oral administration of the racemic mixture, (+)-S-ibuprofen bioinverted from (-)-R-ibuprofen averaged 38.5% of the total (+)-S-ibuprofen in the systemic circulation.

CONCLUSIONS

The model presented by Jamali et al. (5), for bioinversion of ibuprofen after administration of a racemic mixture, was reviewed and found to be more indicative of systemic bioinversion when the AUC S:R ratio was taken into account and compared to literature values. Results of simulations

with this model demonstrate no diffenece between equations for Fr s. based on AUC of S following administration of R and S. racemate and S. or racemate and R. for the four cases of presystemic and or systemic bioinversion investigated. However. comparison of results of Equations 2-4 using literature data differ such that it does not appear that the relationships assumed for the model hold true under all dosing situations. Calculations Fr- s (Equations 2-4) using literature data averaged 0.52 overall.

We estimated that after racemate oral administration. 1.3 of the total (-)-S-ibuprofen in the plasma is derived from the inversion of (-)-R-ibuprofen. Possibly a 150 mg dose of (+)-S-ibuprofen will be bioequivalent to a 200 mg dose of rac-ibuprofen after oral administration. Whether or not the formulation of the active stereoisomer is a therapeutic improvement can be still argued and other pharmaceuticial characteristics of the drug must be considered.

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Table I

Simulation Parameters and Results for Bioinversion of Ibuprofen,
Based on the Model of Jamali et al. (5)
with Calculations of AUC S/R Ratio and Fraction of R Inverted to S (Fr->s)

Dosing *	Sim #	kip (hr^-1)	kic (hr^-1)	App kr (hr^-1)	App ks (hr^-1)	AUC R (hr*mg/L)	AUC S (hr*mg/L)	AUC S/R Ratio	Fr->s (Eqn.2) using S & R	Fr->s (Eqn.3) using S & Rac	Fr->s (Eqn.4) using R & Rac	Fr->s (Eqn.5) using R & Rac
Racemate	1	1.5	0.0	0.341	0.341	23.52	94.08	4.00	0.60	0.60	0.60	0.38
Racemate	2	1.0	0.08	0.341	0.327	29.40	95.09	3.23	0.62	0.62	0.62	0.38
Racemate	3	0.5	0.15	0.341	0.315	39.20	95.64	2.44	0.63	0.63	0.63	0.39
Racemate	4	0.0	0.225	0.341	0.303	58.80	97.58	1.66	0.66	0.66	0.66	0.40
R only	5	1.5	0.0	0.341	0.341	23.52	35.28	1.50				
R only	6	1.0	0.08	0.341	0.317	29.40	36.30	1.23				
R only	7	0.5	0.15	0.341	0.305	39.20	36.84	0.94				
R only	8	0.0	0.225	0.341	0.296	58.80	38.78	0.66				
S only	9	-	-	-	0.341	-	58.80	-				

* Dose of R = 200 mg
Dose of S = 200 mg
Dose of Racemate = 200 mg of R and S each

Additional Parameters used for all simulations

	R	S	
ka =	1.0	1.0	hr^-1
k =	0.34	0.34	hr^-1
Vd =	10	10	L

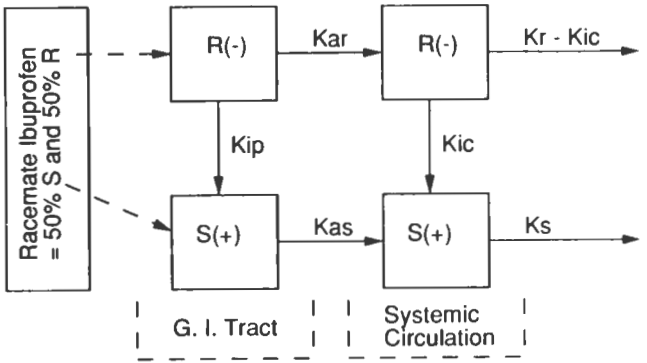
Table II

AUC Values in Man after Administration of Individual Ibuprofen Enantiomers or Racemate
with Calculations of AUC S/R Ratio, Fraction of R Inverted to S (Fr->s), and Fraction of S Inverted From R (Fs<-r)

	Ref #	Route	Dose (mg)	AUC S (hr*mg/L)	AUC R (hr*mg/L)	S/R AUC Ratio	Fr->s (Eqn 2) using S & R	Fr->s (Eqn 3) using S & Rac	Fr->s (Eqn 4) using R & Rac	Fs<-r (Eqn 5) using R & Rac
S Only										
Geisslinger	7	PO	300	67.2	-	-	-	-	-	-
Lee	8	PO	400	93.1	-	-	-	-	-	-
R Only										
Geisslinger	7	PO	300	32.4	57.2	0.57	0.48	-	-	-
Cox	6	PO	300	35.0	63.0	0.56	* 0.50	-	-	-
Cox	6	IV	300	30.0	60.0	0.50	* 0.43	-	-	-
Baille	9	PO	300	25.3	68.0	0.37	* 0.36	-	-	-
Baille	9	PO	304	27.5	68.0	0.40	* 0.39	-	-	-
Lee	8	PO	400	59.3	101.0	0.59	0.64	-	-	-
					Avg =	0.50				
					SD =	0.09				
Racemate										
Geisslinger	7	PO	600	89.8	57.0	1.58	-	0.34	0.56	0.36
Cox	6	PO	600	91.0	61.0	1.49	-	* 0.30	0.63	0.38
Cox	6	IV	600	89.0	59.0	1.51	-	* 0.27	0.51	0.34
Cox	11	PO	400	58.0	42.0	1.38	-	* 0.25	Δ 0.67	Δ 0.40
Cox	11	PO	400	58.0	50.0	1.16	-	* 0.25	Δ 0.67	Δ 0.40
Jamali	4	PO	600	106.9	74.2	1.44	-	* 0.53	• 0.71	• 0.42
Jamali	4	PO	600	121.7	73.9	1.65	-	* 0.74	• 0.58	• 0.37
Jamali	4	PO	600	90.8	56.9	1.60	-	* 0.30	• 0.96	• 0.49
Jamali	4	PO	600	100.2	51.5	1.95	-	* 0.44	• 0.80	• 0.44
Lee	8	PO	800	128.3	82.3	1.56	-	0.38	0.86	0.46
					Avg =	1.53				
					SD =	0.20				
					Average (Overall) =		0.41	0.38	0.70	0.41
					SD (Overall) =		0.10	0.16	0.14	0.05
					Average (True**) =		0.56	0.36	0.64	0.39
					SD (True**) =		0.11	0.03	0.15	0.05
					Avg of Eqns 2-4 =		0.52			
					Avg of Eqns 2-4 (True**) =		0.55			

* compared to Lee data (8) for administration of S, normalized for dose
Δ compared to Cox data (6) for administration of R, normalized for dose
• compared to Lee data (8) for administration of R, normalized for dose
** all parameters used in the equation were obtained from individual studies

Figure 1: Chiral Bioinversion Model Proposed by Jamali et al. (5)



MANUSCRIPT IV

APPROACHES TO STEREOSPECIFIC PREFORMULATION OF IBUPROFEN

Abstract

In an effort to formulate the pharmacologically active ibuprofen isomer ((-)-S-ibuprofen) into a solid oral dosage form, preformulation studies were performed on both the racemate and this stereoisomer of ibuprofen. Results of the respective physical pharmacy profiles were compared to predict the pharmaceutical behavior of the S(-) ibuprofen compound. The enantiomer was more soluble than the racemate in aqueous media but exhibited lower intrinsic dissolution rates, as would be expected from the very small specific surface area. This characterisitic could be a limiting step in the formulation of the optical isomer although less energy was required for the solution of S(+) ibuprofen. On this crystal, there was ten times more moisture layered at the surface and comparative thermal analysis indicated that for both compounds a loss in crystallinity occured upon grinding. Properties in the solid state of S(+) ibuprofen included higher density and excellent flowability as compared to the racemate.

INTRODUCTION

Recently extensive emphasis has been given to the stereospecific nature of drug disposition in the context of

drug development. Regulatory bodies in Europe already recommend the use of stereospecific assays when a racemate is formulated (1). In this case, when the chiral synthesis is possible, the formulation of pure active enantiomers also becomes a priority for research and development. Thus, for drug candidates in the development pipeline stereospecific considerations are critical. For drug products already marketed as racemates, the study of their optical isomers might present a different set of problems (2).

The use of an enantiomer as part of a solution for common pharmaceutical problems is not new. In the early seventies, formulators at Wyeth had described the advantages of using the pure optical isomer of a cytotoxic agent to overcome a solubility problem (3). The differences in physico-chemical properties between racemic and enantiomer have been known and studied for a long time: solid state properties, crystal structures and the effect of isomeric purity on phase solubilities (4-6) have been investigated. Recently the effect of chiral asymmetry on crystal properties was reviewed and it was concluded that for such compounds, biopharmaceutical characteristics must be carefully monitored (7).

Ibuprofen is administered as a racemate and is one of 50 compounds for which detailed stereospecific pharmacokinetics have been reported. Properties in solution and the solid

state have been documented in the literature and conventional preformulation programs have been applied to the study of this antiinflammatory agent (8). It is now generally recognized that formulating ibuprofen is difficult and relies mainly on the expertise of the formulator. Its low solubility in aqueous media at low pHs as well as its poor handling properties contribute to its tedious processing. S(+) ibuprofen, the biologically active isomer of ibuprofen, is now available in large scale quantities through economically viable chemical synthesis and the objectives of this study are to draw a preformulation profile for this enantiomer, investigate the feasibility of a conventional formulation and compare this stereoisomer to the racemate currently used.

EXPERIMENTAL

MATERIALS

Rac-ibuprofen (lot# LH6-72) and (+)-S-ibuprofen (lot# AC-1R) were obtained from the Ethyl Co., Baton Rouge, LA. Monobasic potassium phosphate and sodium hydroxide from the Fisher Scientific Company, were of analytical grade.

METHODS

Analytical: all quantitative determinations in solution were performed using an ultra violet spectrophotometer (Hewlett Packard 8450) at 264 and 220 nanometer wavelengths.

Intrinsic Dissolution Rate (IDR)

The procedure desribed by Woods et al (9) was used to determine the intrinsic dissolution rates. A modified Woods apparatus, designed by Ciba-Geigy researchers (fig. 1) was used. One gram of sample powder was weighted in the die and precompressed at 500 lbs with a Carver hydraulic press. After cleaning the exposed surface the compact was recompressed at 1000 lbs for a dwell time of 5 seconds. The rotating disk assembly was immersed in 500 ml of USP simulated intestinal fluid (SIF) at 37 + - 0.8 ^{o}C and rotated at 100 rpm. The sampling regimen, included 2,5,10,15,20,25,30 and 35 minute time points. Sample volume was 4 cm^3. withdrawn with micro-syringes and replaced with the same volume of SIF at 37^{o}C. All samples were passed through a 0.45 um cellulose acetate filter before analysis. Amounts of drug dissolved were plotted versus time and the slope of the straight line portion was divided by the area of the pellet (A=1.281 cm^2) to yield the intrinsic dissolution rates in $mg.sec^{-1}.cm^{-2}$.

Solubility and Heat of Solution

The method used was the procedure described by Higuchi et al (10). Ibuprofen was added in large excess to buffered solutions in screw-capped vials at various pHs. The tubes were rotated on a labquake shaker in dry ovens for 24 hours to reach saturation (11). The vials were then centrifuged at 2000g for 10 minutes, supernatants withdrawn, filtered, and further analyzed for pH and drug concentrations. Four pH s were studied at temperatures ranging from 25 to 51 ^{o}C.

Solid State Properties

Particle size was measured using a laser light scattering technique (Brinckman Particle Size Analyzer). Surface areas were determined with a Nitrogen adsorption technique and calculated using the B.E.T. equation. This experiment was performed on a Quantasorb instrument. Compressibility and density were evaluated using a procedure proposed by Rees et al (12). About 100 mg of powder were weighted in a volumetric cylinder. Up to 2000 taps were performed on an Erweka instrument. Moisture contents were estimated with a Karl-Fisher technique. Crystal analysis included differential scanning calorimetry to monitor temperatures and enthalpies of fusion on a Perkin-Elmer P7500, all thermal analysis were conducted with a heat flow of 5^{o}C minute:

samples were also analyzed through scanning electron microscopy and X-Ray powder diffraction at the R&D analytical services of Ciba-Geigy in Ardsley, NY.

RESULTS AND DISCUSSIONS

Solid State Properties

Under similar storage conditions (22°C + - 2 and 35% RH) the mass fraction of moisture for the rac-ibuprofen averaged 0.065 + - 0.013% where (+)-S-ibuprofen exhibited 0.34 + - 0.24% of water corresponding to ten times more moisture for this enantiomer. After careful analysis of X-Ray diffraction patterns of dried and humidity exposed ibuprofen samples. there was no change in the crystallinity of both powders upon removal of water. It was speculated that the moisture essentially sticks to the crystal surface. Both compounds had a very low water content but according to Ahelc et al (13) they still require special attention because the water distributes only in amorphous regions. X-ray powder diffraction patterns indicated different crystal structure for the racemate and its enantiomer with very different deflection peaks especially at low angles of the spectrum. Ten minutes grinding resulted in a loss of crystallinity for both powders with a decrease in deflection peaks intensity (fig. 2). For ground S(+) ibuprofen. some peaks were more intense at low angles of the spectrum (11-15). This difference might be

due to the decrease in particle size but indicated a modification of the crystal nature. The thermodynamic parameters, presented in table 1 confirmed these conclusions. The endotherm temperature was 20°C lower for the S(-) enantiomer and the enthalpy of fusion averaged 35 J g less than the racemate. Upon grinding there was a decrease in the enthalpy of fusion for the ibuprofens proportional to entropy changes. There was no modifications in the melting points in either case confirming no rearrangement of the internal lattice(s). Nevertheless, although the internal structure might not have been modified, upon grinding there was a decrease in the particle size of (+)-S-ibuprofen and the compound became difficult to handle as a result of flowability loss.

Based on the monolayer gas adsorption theory we calculated the true surface area of the two compounds using the B.E.T. equation. The B.E.T. values for racemate and the S(-) isomer were respectively 3.4 10^{-1} (0.01) and 2.8 10^{-3} (1.9 10^{-4}) m^2/gram indicating a specific surface area more than 100 times smaller for the enantiomer. Although it has been found that BET values vary between sources (14), the amplitude of this difference might be a potential problem of the formulation specifically in the dissolution/bioavailability behavior. The particle size with a mean ranging from 83 to 149 um was very large compared to 5-38 um for the racemate (14) and was responsible for the

excellent flowability of the bulk material. The scanning electron microscopy observations in fig.3 indeed confirmed the descriptive analysis and the unusual nature of the crystal surface of (+)-S-ibuprofen. The optical isomer existed as large boxy needles consisting of the crystal unit. All particles had a very smooth surface.

Bulk and tapped densities averaged 34 and 56 % respectively and were similar to the racemate analyzed. The compressibility flowability as evaluated by plotting Log Vo V against the number of taps (fig.4), where Vo is the initial volume and V the tapped volume, was consistently above the 0.1 assymptote for the racemate and considered poor (12) as compared to the S(-) enantiomer. Thus the optical isomer might be a good candidate for direct compression.

Dissolution Kinetics

Because of the unique dissolution characterisitics of each compound, the rate plots presented in fig.5 had different slopes, and consequently different intrinsic dissolution rates. Surprinsingly the IDR for S(+) ibuprofen averaging 8.1 $ug.sec^{-1}.cm^{-2}$ was smaller than the IDR of the racemate with a mean at 11.6 $ug.sec^{-1}.cm^{-2}$. It appears that under our experimental conditions, the dissolution rate of the enantiomer was limited by its very small surface area (rather than enhanced by its high solubility). In addition,

aged compacts at Room Temperature for 3 days and further analyzed under the same conditions had lower intrinsic dissolution rates IDR than original disks immediately analyzed after manufacture (fig.6).

Heat of Solution

The solubility of the two ibuprofens was determined at different temperatures in various buffers. The van't Hoff equation relates the solubility in mole fraction or mole percent to the inverse of the absolute temperature of an ideal solution:

$$-\text{Log } Xi = (\Delta Hf\ RT) + \text{constant}$$

where Xi is the ibuprofen concentration in mole fraction. ΔHf the heat of solution. R the perfect gas constant and T the absolute temperature in degree Kelvin. The Van't Hoff plots for rac-ibuprofen (fig.7) and (+)-S-ibuprofen (fig.8) yield heats of solution at different pH's presented in table 2. The slopes varied with pH indicating that the heat absorbed by the systems during dissolution varied with the extent of ionization. The energy of solubilization decreased with ionized species and at pH 7.7, the heat of solution became slightly exothermic for both ibuprofens. This result

confirmed that when ionization was the principal factor in dissolution (at high pHs) this phenomena actually released energy. At this level of lower [H+] concentrations, S(-) ibuprofen consistently exhibited lower heat of solutions than its racemate form showing that the enantiomer is more soluble in aqueous media. For example the aqueous solubility of (-)-S-ibuprofen in a pH 7.7 phosphate buffer at 37 ^{C}C was 6.0 mg ml compared to 5.0 mg ml for the racemate. Under our experimental settings, at pH 4.5 (pKa of ibuprofen) the concentrations were most variable and yield close to zero slopes for both ibuprofens.

CONCLUSIONS

The physical pharmacy profiles of S(+) ibuprofen and its racemate form were compared. Thermal analysis indicated that the optical isomer existed as a different crystal form exhibiting different solid state properties which could be of concern for the formulator. Particle size was increased and the flowability was improved. The enantiomer existed as large boxy crystals with unusually low surface area. This might be a major limitation for an oral solid formulation. In fact the intrinsic dissolution rate of this compound was found smaller than the IDR of the rac-ibuprofen which was not predicted from the solubility data and not previously documented in the literature. Solubility determinations

revealed that (-)-S-ibuprofen was more soluble in aqueous media at pHs higher than 4.5 but not to the extent anticipated from a review of the stereochemical literature. Also at pH 7.7 heats of solution were slightly exothermic for both ibuprofens indicating that at this pH the solubilization process released some energy. It has been argued from a pharmacokinetic pharmacodynamic stand point that formulating S(+) ibuprofen might be a therapeutic improvement (9). however, considering these elements of physical pharmacy special attention should be given to the formulation, in order to overcome the potential problems of dissolution, low melting levels and compatibility. Thus, S(+) ibuprofen might be readily absorbed through the jejunum but its dissolution characteristics could be a rate limiting step of bioavailability for an oral solid dosage form. If lower doses are required, it is anticipated that S(-) ibuprofen could be a good candidate for direct compression. In summary, we believe that the formulation of (+)-S-ibuprofen is certainly achievable provided the unique characteristics of this drug are kept in mind. There are significant differences between this enantiomer and the racemate and the potential advantages (therapeutic and biopharmaceutical) of the S(+) drug might be considerable.

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Table I : THERMAL ANALYSIS OF IBUPROFEN			
"As Received"	Racemate	S(+)	R(-)
MELTING RANGE	75-77 oC	53-55 °C	53-55 °C
ENTHALPY OF FUSION	135.1 J/G	86.8 J/G	87.0 J/G
"Ground"			
MELTING POINT	75.3 C	55.1 C	
ENTHALPY OF FUSION	115.2 J/G	82.3 J/G	

TABLE II: HEAT OF SOLUTION FOR (+)-S AND RAC-IBUPROFEN

	pH	ΔH (KJ.MOLE^{-1} . $^{\circ}$K^{-1})
RAC-IBUPROFEN	1.3	32.2
	4.5	-0.3
	6.0	38.8
	7.7	-5.2
(+)-S-IBUPROFEN	1.3	51.5
	4.5	-0.04
	6.0	29.9
	7.7	-16.4

Figure 1

Cross-Section of the Modified Woods Rotating Apparatus

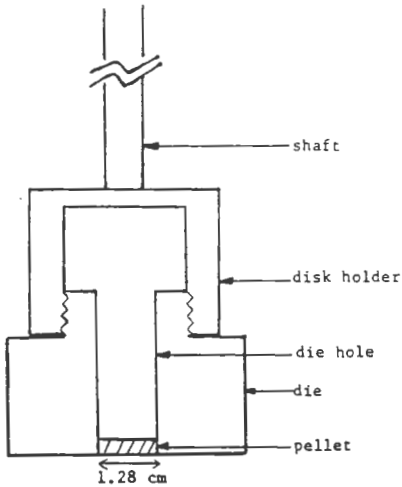


Figure 2

Powder X-Ray Diffraction Patterns: Effect of Grinding
(A): S(+) ibuprofen "ground", (B): S(+) ibuprofen "as received",(C): Rac-ibuprofen "as received"

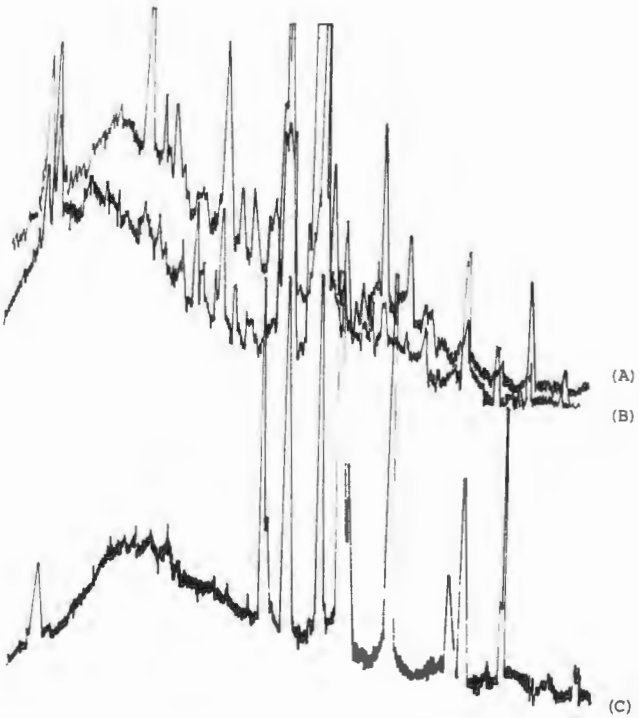


Figure 3

Scanning Electron Microscopy Photographs of S(+) Ibuprofen

50, 250, 500 and 1000X Magnification

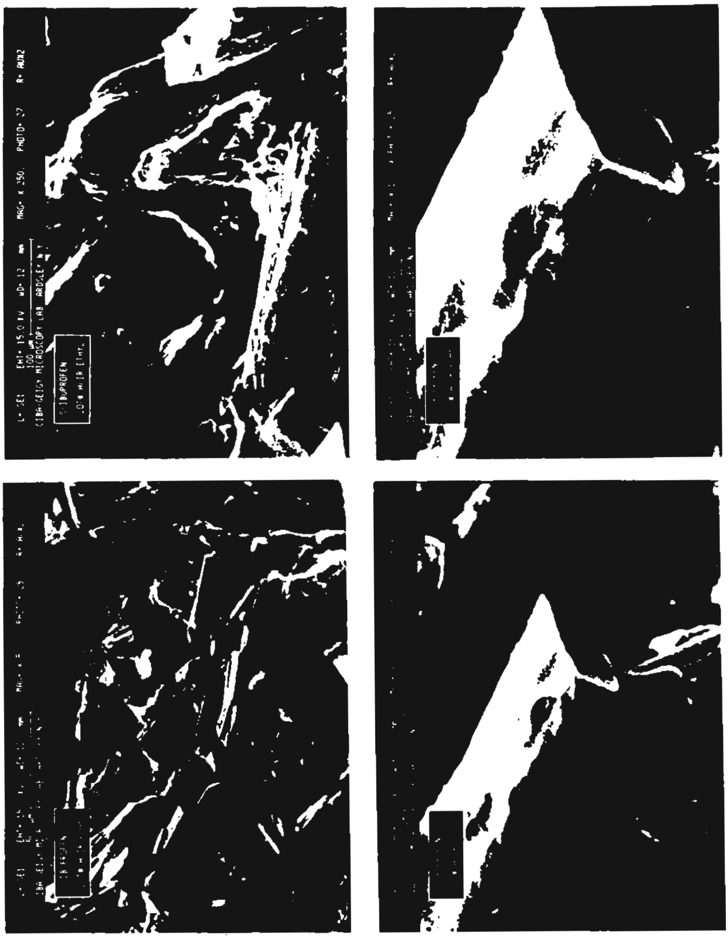


Figure 4
Log Vo/V Versus No of Taps

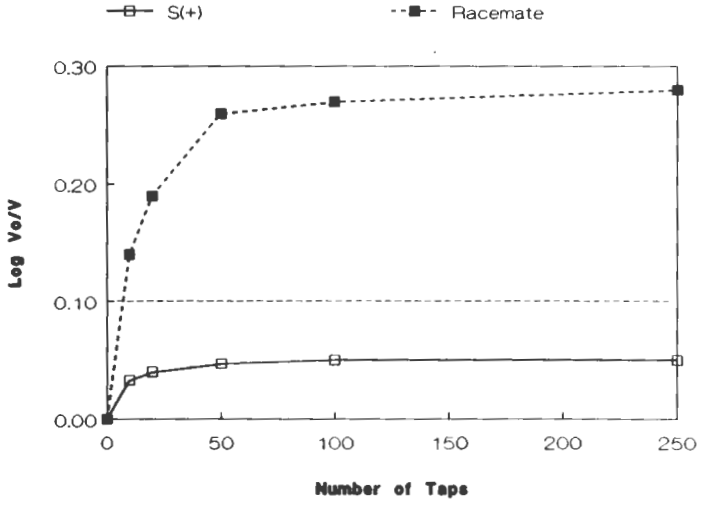


Figure 5: Intrinsic Dissolution Modified Woods Apparatus

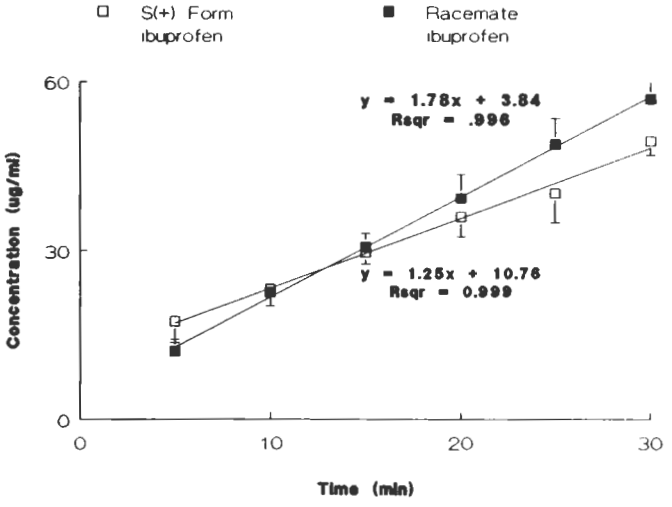


Figure 6: Dissolution of S(+) ibuprofen
Effect of Aging

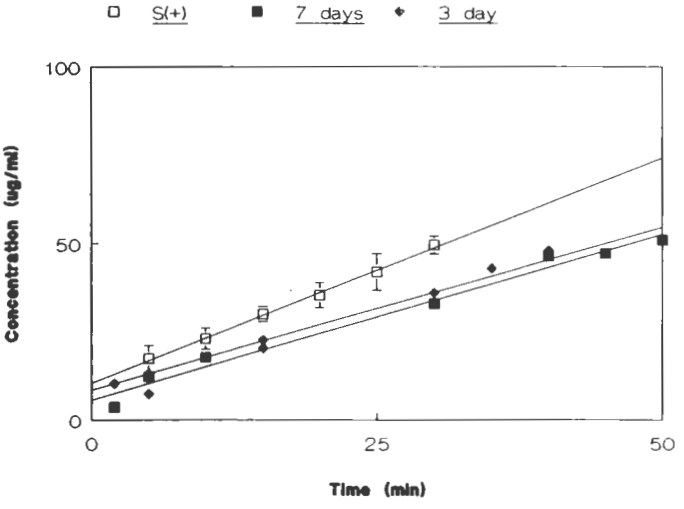


Figure 7
Vant'Hoff Plots [Rac-ibuprofen]

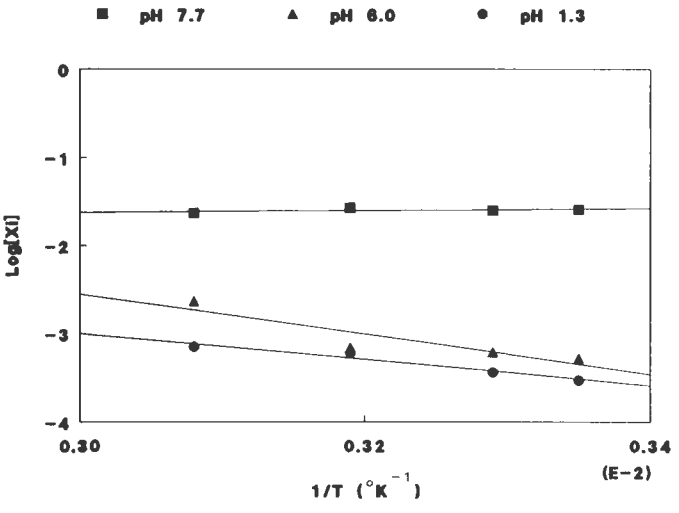
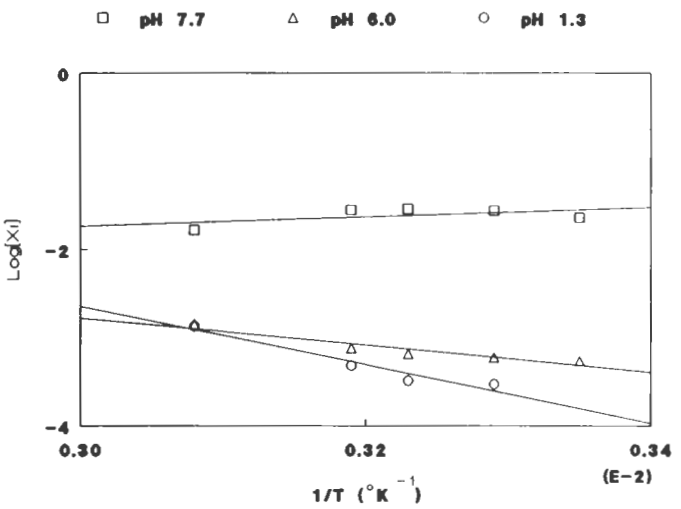


Figure 8
Vant'Hoff Plots [(+)-S-ibuprofen]



MANUSCRIPT V

STEREOCHEMICAL ASPECTS OF THE MOLECULAR PHARMACEUTICS OF IBUPROFEN

ABSTRACT

Thermal analysis, thermodynamics of solution and molecular modeling of (+)-S-ibuprofen and rac-ibuprofen gave information on how heterochiral or homochiral interactions would affect the processing of ibuprofen. The study confirmed that rac-ibuprofen exists as a true racemate with a 10% eutectic pure enantiomer composition. Both the racemate and the (+) isomer crystal unit cells include four molecules and crystallize in the $P2_1$ c and $P2_1$ space groups respectively. Thus the intermolecular forces were different between the crystals. As a consequence the (+) enantiomer lattice was more fragile but only slightly more soluble than the racemate in aqueous media. The solid-state structure contributions to solubility were very different between the two crystals ($\Delta H^S_{S(+)}$= 51.1 and ΔH^S_{rac}= 32.2 in KJ mole) but the standard free energy of solution were found to be comparable for both compounds.

INTRODUCTION

It is generally recognized that (+)-S-ibuprofen is the enantiomer of ibuprofen inhibiting the prostaglandin synthetase (1-2). Stereospecific and conformational characteristics of this isomer are required for interaction

with the cell receptors responsible for the therapeutic antiinflammatory activity. Another consequence of chirality could be the differences between the crystal habit of the two separate isomers and the racemate. We now report a continuation of our studies in this area (3).

We have previously reported higher solubility, lower melting point and smaller intrinsic dissolution rates for the (-) isomer compared to the racemate form (3). The combination of tests used in this study is, we believe, essential when one is investigating a chiral compound or an optically pure isomer. For example, the thermal behavior of sobrerol and diastereoisomers was reported (4) and the crystal structures of cytostatic agents (stereoisomers and mixtures) were elucidated (5), both in support of formulation efforts. In another study the thermodynamic functions of solution of non steroidal antiinflammatory agents, including ibuprofen have been thoroughly studied by Pecci et al (6) to determine the contribution of the solid state structures in promoting solubility. The physical characteristics can be related to the molecular packing of the crystals under study (7).

The combination of all these tests and the comparative analysis is a unique approach to the formulation of ibuprofen enantiomers that has been largely overlooked before the stereospecific synthesis became economically viable.

EXPERIMENTAL

Materials

Racemic and S(+) ibuprofen were supplied by the Ethyl Corp. (Baton Rouge, LA). Methanol from the Fisher Scientific Co. was used for ibuprofen recrystallization and was of analytical grade. Potassium phosphate monobasic and sodium hydroxide were obtained from the Malinckrodt and J.T. Baker chemical companies, respectively.

Methods

Thermal Analysis: Ibuprofen mixtures containing various enantiomeric proportions were prepared by slow recrystallization at 42.6 ^{o}F from methanol and after melting. Thermal analysis were performed on (-)-S-ibuprofen [S(-)], (-)-R-ibuprofen [R(-)], rac-ibuprofen [Rac] and mixtures using a differential scanning calorimeter [DSC] from Perkin Elmer, series 7. The heating rate was set at 5 ^{o}C/minute under nitrogen flushing. Thermal endotherms were integrated to obtain thermodynamic functions used for the phase-diagrams. Theoretical solid-liquid equilibrium curves were drawn using the Prigofine-Defay equation (eq.1) for the racemate completely dissolved in the melt and the Schroeder Van-Laar

equation (eq.3) for the simple eutectic formation (9). Thus, eutectic temperature and compositions could be determined:

$$Ln4x(1-x) = 2\ \Delta H^{rac}\ (R\cdot(1\ T^{rac} - 1\ T^{f}))\quad \text{equation 1}$$

$$Lnx = \Delta H^{S}\ (R\cdot(1\ T^{S} - 1\ T^{f}))\quad \text{equation 2}$$

where x is the mole fraction of the more abundant enantiomer in the mixture, whose melting ends at T^{f} (^{o}K) : ΔH^{S} and ΔH^{rac} are the enthalpy of fusion of the pure S(-) and the racemic form respectively: Tm^{S} and Tm^{rac} are the corresponding melting points and R is the gas constant at 1.987 $cal.mol^{-1}.{}^{o}K^{-1}$.

Crystal Analysis: Single crystal X-ray diffraction was performed on small crystals of (+)-S-ibuprofen from the bulk compound. Reflection data was obtained from Ethyl Co. and processed on a molecular modeling program (Shelxtl^{r}). Crystal data is given in Table I. The structure was solved in the space group $P2_1$ c . Analysis of the unit cell allowed the identification of the molecular packing and hydrogen bonds network within the monoclinic crystal. Similar information on the racemate was obtained from the literature (10) and compared to the newly obtained molecular packing data of the (+) enantiomer.

Solubility: Excess amounts of both compounds were suspended in 0.05M aqueous buffered solutions at pH 1.3. At

this pH. the drug is essentially unionized since the ibuprofen pKa is believed to be in the range of 4.6 to 5.2. Screw cap vials were rotated on a Labshaker for 24 hours at temperatures ranging from 25 ^{O} to 52 ^{O}C in walk-in ovens. Quantitative analysis was performed using ultra violet spectrophotometry at selected wavelengths (220 and 264 nm). The thermodynamic functions of solubilities were evaluated as follows:

The chemical potential of a solute (s) in equilibrium with its pure form (s) may be written as:

$$\mu = \mu - R \cdot T \cdot \mathrm{Ln}\ x \qquad \text{equation 3}$$

The variation of the solubility expressed in x^{w} (mole fraction in the solution) can be integrated to :

$$\mathrm{Ln}\ x^{w} = \mathrm{Cstant} + (-\Delta H^{O} \quad R) \cdot (1/T) \qquad \text{equation 4}$$

and experimental data can be analyzed by plotting Ln of the solubility versus 1/T. The free energy of solubility at a given temperature can be obtained from :

$$\Delta G^{O} = -R \cdot T \cdot \mathrm{Ln}\ x^{w} \qquad \text{equation 5}$$

and the entropy of solution is derived from the third law of thermodynamics:

$$\Delta G^{C} = \Delta H^{O} - T \cdot \Delta S^{O} \qquad \text{equation 6}$$

All thermodynamic parameters were analyzed to compare the racemate and S(+) ibuprofens. The contribution of ibuprofen stereochemistry (solid state structures) to solubility, was investigated . In a recent study, thermodynamic functions were used to predict and separate the roles of solid-state structures from solute-solvent interactions in promoting the solubility of a solid nonelectrolyte (6).

RESULTS AND DISCUSSION

Thermal Behavior

Thermodynamic functions for both compounds are reported in Table II. Melting parameters (Tm and ΔH) obtained from the thermograms in figures 1-2, were used in equations 1 and 2 to obtain the phase-diagram in figure 3 (10). Experimental data were in good agreement with the theoretical lines indicating the fusion of the Eutectic at about T^{eu}= 321 ^{O}K and a eutectic composition of 10.0 % on each side. As previously anticipated (3,10) the thermal analysis confirmed that ibuprofen naturally occurs as a unique racemic compound. A

Peterson "i" ratio of 1.77 was calculated from equation 7 (9):

$$\text{"i"} = (Tm^{rac} - Tm^{eu})(Tm^{s} - Tm^{eu}) \qquad \text{equation 7}$$

where Tm^{eu} is the eutectic temperature as determined experimentally and from the phase diagram.

Although somewhat arbitrary in character, this ratio clearly indicated that ibuprofen has a strong tendency to crystallize as a true racemate. The melting point of both optical isomers was 20 to 22 ^{o}C lower than the racemate form - and eutectics were very close to the edges of the diagram making any enantioselective resolution by crystallization impossible. The test of the Prigofine-Defay equation was performed a posteriori and the straight line in figure 4 confirmed the model.

Crystal Packing

Perspective drawings of the molecular packing in the crystals of (+)-S-ibuprofen are given in figures 5a-5c. (+)-S-ibuprofen is more water soluble than the racemate (3) and it is of interest to seek the basis for the differing solubilities or thermal behavior in terms of intermolecular attractions forces. Although having the same number of molecules, S(+) crystals have a totally different unit cell

than the racemate (Fig.5a). The array of S molecules involved in homochiral interactions probably spreads the mechanical stability strength of the crystal (Fig.5b). The preferential molecular arrangement in the $P2_1$ plan exhibit some of the acid groups "face-up' and others 'face down' so that all the layers of molecules are interconnected with pairs of hydrogen bonds to carboxyl groups. Assuming that the top layer of the crystal is one crystal surface is different for the two compounds. Thus in the case of (-)-S-ibuprofen there are more exposed carboxyls and less hydrophobic layers. There are several points of interest: first the greater number of crystallographically independant molecules in the S crystals: secondly there are no obvious relationships between molecular packing in the lattice of racemate and enantiomers. Finally, the structural data reflects different intermolecular environments. In order to pack together, molecules of the same chirality had to be somewhat flexed in order to meet the space requirements of the lattice. A qualitative measure was the superposition of two (+)-S-ibuprofen molecules involved in the same hydrogen bond which clearly demonstrate the torsion (Fig.5c). We hypothesize that further crystal elasticity or fragility would result from the packing.

Solubility

The solubility of crystalline solid is determined by the free energy changes from the solid state to a sclution. As indicated in table II. ΔG^{o} at 25 ^{o}C is slightly higher for the racemate than for its S(-) isomer which may account for the differing solubilities. Similar conclusions could be drawn from the analysis of fusion parameters. The enthalpy and entropy contributions to water solubility as revealed by the thermodynamic functions in table II. are very different suggesting that solid state structures are responsible for these differences.

CONCLUSIONS

It was confirmed that rac-ibuprofen naturally occurs as a racemic compound (10) with a eutectic temperature approaching 320 ^{o}K. This behavior although quite conventional, has some serious implications in the formulation of the biologically active stereoisomer. Heterochiral interactions formed preferentially. Thus, both qualitatively and quantitatively, the intermolecular network of interactions in crystal unit cells of the racemate significantly exceeds that existing within cells of the pure enantiomer and can also reasonably account for the differing solubilities, thermal behavior and further processing characteristics. The

literature indicates that in some instances, when the melting point of pure stereoisomers is substantially lower, these optical isomers are several fold more soluble than the corresponding racemate (5,8). In this case, the S isomer was only slightly more water soluble than rac-ibuprofen. We attribute this phenomena to the molecular arrangement in the lattice of (+)-S-ibuprofen. Solid-state structure contributions (ΔH) to solubility were different between the (+) isomer and the racemate. At pH 1.3 the entropy effect (ΔS) counterbalanced this effect and standard free energy were almost equivalent for the two crystals. These results confirmed the low specific surface area and the slow intrinsic dissolution rate of (+)-S-ibuprofen. In addition, the crystal lattice exhibited potentials for mechanical instability if perturbed by components of high hydrogen bonding affinities.

Acknowledgements: We are indebted to Ciba-Geigy Corporation for the award of a fellowship and to Dr. Lukas, Director of P.P.T. for his supervision during the course of this project. We thank Dr D. Bauer, Ethyl Corp. for providing us with single crystal X-ray diffraction data.

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Table I: Crystal Data for (+)-S-Ibuprofen and Rac-ibuprofen

	(+)-S-ibuprofen	Rac-ibuprofen
Formula	C13H18O2	C13H18O2
Molecul.Weight	206.3 grams	206.3 grams
Crystal System	Monoclinic	Monoclinic
Space Group	P21/c	P21
a(Å)	12.46	14.67
b(Å)	8.03	7.88
c(Å)	13.53	10.73
α(Å)	-	-
β(Å)	112.95	99.36
γ(Å)	-	-
# of molecules in the cell	4	4
Density (g.cm)	1.098	
CuKα Radiation		

Table II: Thermodynamic Functions of Melting and Solubility

Ibuprofen	Rac	(+)-S	(-)-R
Melting			
Tm Melting Point (°K)	349	327	327
ΔH Enthalpy ($KJ.mole^{-1}$)	25.5	17.9	17.9
ΔS Entropy ($J.mole^{-1}.°K^{-1}$)	73.2	54.8	54.8
Solution			
ΔH ($KJ.mole^{-1}$)	32.2	51.5	-
ΔS ($J.mole^{-1}.°K^{-1}$)	6.7	73.4	-
ΔG_t ($KJ.mole^{-1}$)	30.2	29.6	-

Figure 1
Typical DSC Endotherms
A) pure rac-Ibuprofen and B) pure (+)-S-Ibuprofen

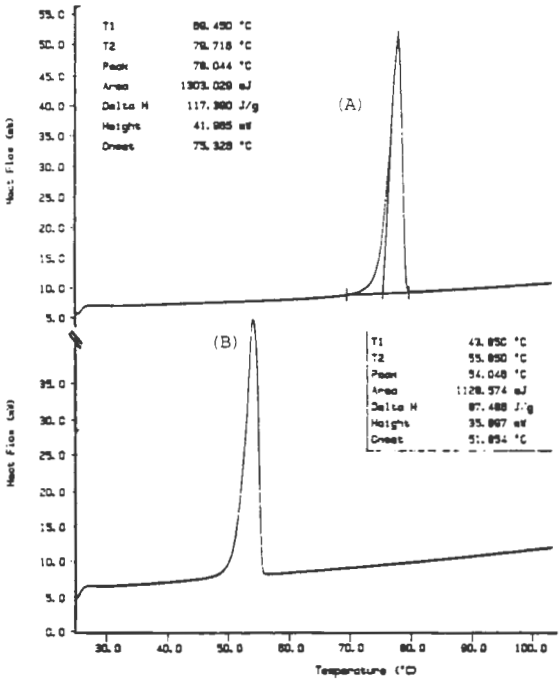


Figure 2
DSC Thermograms of Ibuprofen
Different Enantiomeric Composition

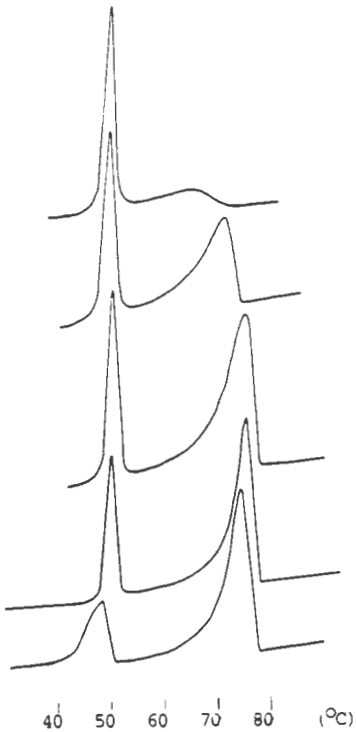


Figure 3
Phase-Diagram of Ibuprofen
Determination of Enantiomeric Composition

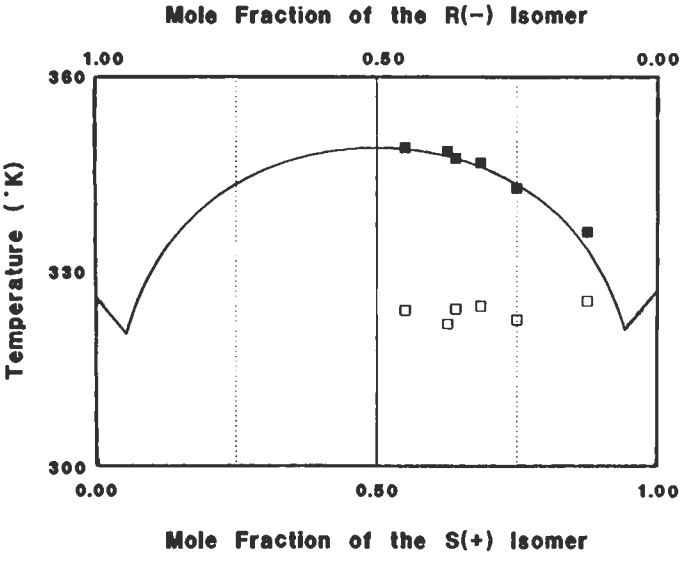


Figure 4
Test of the Prigofine–Defay Equation

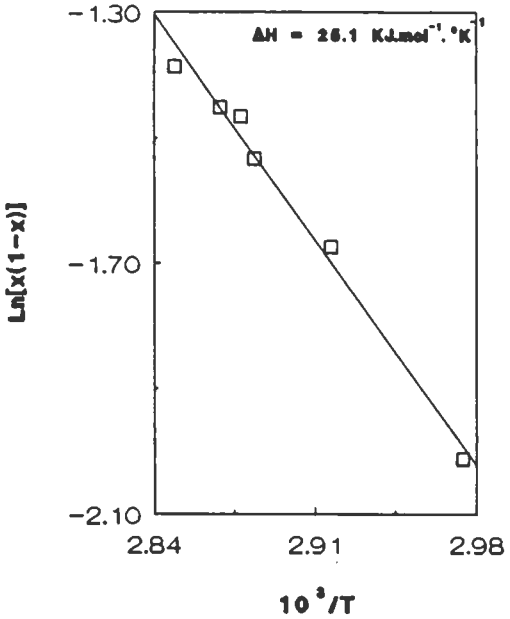


Figure 5a
Crystal Unit Cell of (+)-S-Ibuprofen

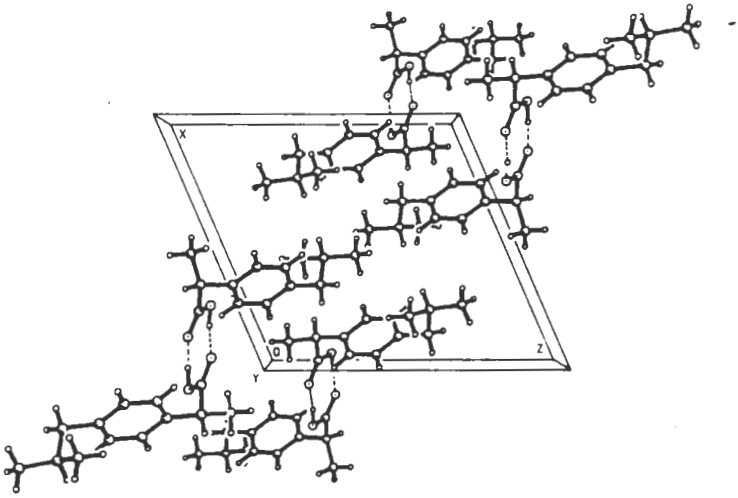


Figure 8b
Crystal Lattice of (+)-S-Ibuprofen

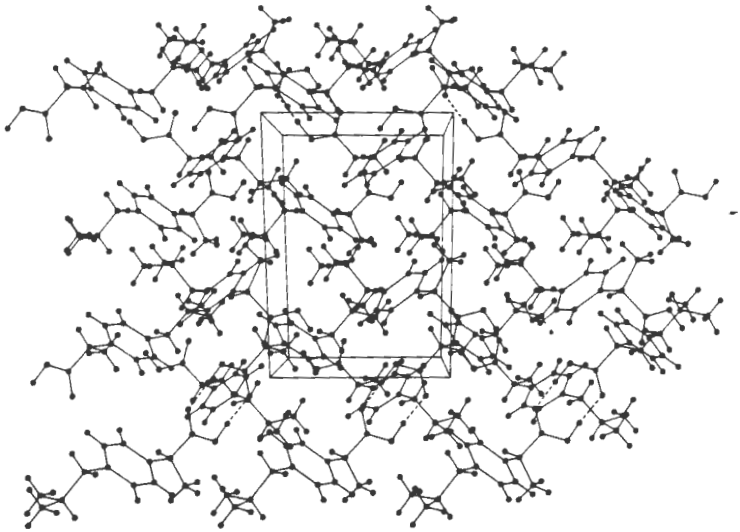
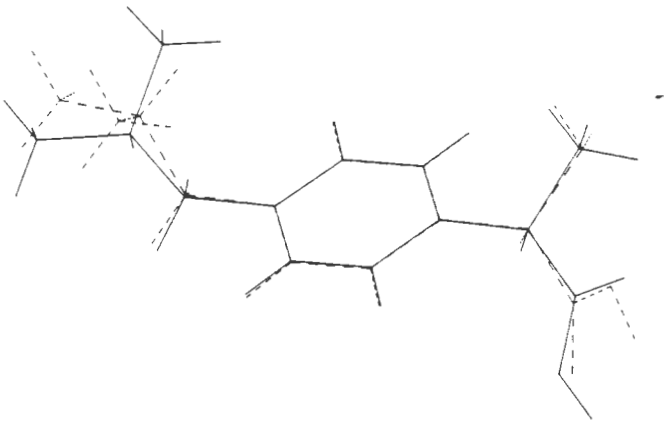


Figure 5c
Superposition of (+)-S-ibuprofen molecules involved in the same hydrogen bond



MANUSCRIPT VI

FORMULATION OF THE BIOLOGICALLY ACTIVE STEREOISOMER OF IBUPROFEN

ABSTRACT

As part of an effort to formulate the biologically active stereoisomer of ibuprofen, the effects of pharmaceutical processing on rac-ibuprofen and (+)-S-ibuprofen crystals were compared. This comparative analysis is a unique concept used in stereoselective formulation. It was not found possible formulate the (+) isomer using wet granulation, however direct compression appeared most promising. Tablets so formulated showed rapid dissolution and other tablet properties were fully acceptable. Mixing with excipients decreased the enthalpy of fusion of ibuprofen and compaction induced low temperature eutectics indicated by DSC endotherms. Stress storage of the (-)-S-ibuprofen seriously affected the handling properties of the dry formulations.

INTRODUCTION

With the exception of Naproxen, all profens currently used as non-steroidal antiinflammatory agents are marketed as racemates (1). For most of these drug substances the dextro-rotary or S(+) optical isomer seems to be responsible for the therapeutic activity, that is the stereospecific inhibition of cyclooxygenase. In addition, various pharmacokinetic reports have been published suggesting that

for some of these aryl propionic acids. bioinversion of the inactive enantiomer into the active isomer S(-) take place in vivo. by enzymatic mechanisms (2). For ibuprofen. as much as 33% of the S(-) form could result from this biotransformation. Thus a racemate (containing 50% of R(-)) could yield 2·3 of active ibuprofen in the systemic circulation (3).

Unfortunately. this pharmacokinetic rationale is only one of the factors which must be considered for the successful formulation of the pure enantiomer in a drug delivery system. Previous investigations by Romero et al (4) have shown that the presently available (+)-S-ibuprofen has a relatively small specific surface area which might be somewhat of an impediment to bioavailability (although solubility was found higher than the racemate). Some handling properties such as flowability were greatly improved while compatibility screening indicated substantial crystal distortion under processing (low temperature eutectic). The molecular packing structure in the crystal lattice was elucidated (5) and it was concluded that (+)-S-ibuprofen exists as a totally different crystal than the racemate form. Analysis of homochiral interactions also revealed that the enantiomer crystal might be fragile and more susceptible to distortion.

The study of a pure enantiomer as an answer for a pharmaceutical problem is not new in process development (6) but the analysis comparing the relationships between molecular

aspects of crystal stereochemistry and biopharmaceutical performances appears to be a new approach to stereospecific drug formulation. Recommendations on the processing of (+)-S-ibuprofen were formulated based on this study and previous reports.

EXPERIMENTAL

Materials

Ibuprofens (racemate and S isomer) were obtained from the Ethyl Corporation (Baton Rouge, LA). Fast flow lactose (Sheffield), polyvinylpirrolidone (GAF) and Explotab (Edward Mendell) were selected as diluent, binder and disintegrant respectively. Simulated intestinal fluid was prepared using potassium phosphate monobasic (Fisher Scientific), distilled water and the pH adjusted to 7.4 with sodium hydroxide (Malinkrodt).

Methods

A formulation program was undertaken, including a compatibility screening, tablet manufacture and analysis of biopharmaceutical properties.

As in previous investigation (7), emphasis was given to the study of crystal modifications during processing and

their effects on the performance of the final product for both ibuprofens.

Thermal and Compatibility Studies

Calorimetry was the analytical tool selected for these studies. A Perkin Elmer Series 7 thermal unit was used. A P7500E computer, interfaced with the system, allowing data aquisition and integration of the thermal endotherms. Systems of increasing concentrations of (+)-S-ibuprofen and excipients were mixed at room temperature for 24 hours on a Labshaker rotating at 40 RPM. Table I summarizes the experimental design. Samples were withdrawn, tested for differential scanning calorimetry (DSC) profiles on a Perkin Elmer differential scanning calorimeter and stability after exposure to the following stress conditions in humidity chambers: 37 ^{o}C at 85 % relative humidity (RH), and 50^{o}C at 75% RH for one and seven days. Macroscopic observations were also recorded. Tablets made of 67% (-)-S-ibuprofen and excipients were ground and analyzed for endotherms to assess crystal modification.

Attempts made to formulate the (+)-S-isomer into tablets by wet granulation were unsuccessful. It was found impossible to dry the granules at temperatures between 30 and 40^{o}C. A direct compression formulation was developed using the same excipients used for the racemate and compared to

tablets having the same rac-ibuprofen concentration. Tablets of rac-ibuprofen were prepared according to a formulation design already validated (6). Dry mixtures of 67% (-)-S-ibuprofen, 23% Fast flow lactose, 6% binder and 3% disintegrant (Explotab) were prepared using a V-blender rotating at 30 RPM for ten minutes and further lubricated with 1% magnesium stearate for five minutes. A Carver hydraulic press was used to produce 350 mg tablets at 2500 lbs compression force for a 15 second dwell time. Three regions of the compaction spectrum (low, intermediate and high) were also investigated for crystal distortion. The experimental protocol included measurement of disintegration time, hardness, dissolution profiles and thermal analysis.

When possible statistical analysis was performed at the 95% confidence level either using a paired t test or ANOVA to compare (+)-S-ibuprofen and Rac-ibuprofen powders.

RESULTS AND DISCUSSION

DSC profiles of ibuprofen mixtures were integrated for melting points, enthalpy of fusion and heat capacity. Mixing with excipients had an influence on the thermodynamic parameters of fusion largely due to increasing amounts of impurities (table II) and the extent of surface crystal distortion appeared inversely proportional to the ibuprofen concentration. Similar observations were made for the

racemate. The mixtures became more difficult to handle with increasing concentrations of (+)-S-ibuprofen. Samples of the formulations, kept at different temperatures and relative humidities ranging from 35° to 50°C and 35 to 85% respectively were withdrawn at different time intervals. After only 24 hours under the above storage conditions it was impossible to handle any mixtures which had been stored at 50°C, 37°C and 75% RH. All high strength miniformulations had "melted". After one week of storage at room temperature the thermal analysis revealed that eutectic formation lowering the melting point and heat of fusion (table III), apparently made the ibuprofen mechanically unstable.

It appeared that S(+) had a greater tendency to form eutectics than the racemate with the selected pharmaceutical excipients. The crystal distortion is further facilitated by stressful mixing (e.g. planetary mixer). This observation is in agreement with the previous analysis of molecular arrangements in the crystal unit and the peripherical localization of the intermolecular "Hydrogen" bond network of the (+) enantiomer (7).

Biopharmaceutical analysis was performed on the 350 mg tablets. Conventional rac-ibuprofen formulations (using Wet Granulation) were compared to (+)-S-ibuprofen (Directly Compressible) formulation. Disintegration times averaged 8'(4') for the S(+). These tablets did not erode as did the corresponding racemate tablets averaging 53'(6'). instead

they disintegrated rather quickly and became a paste sticking to the grid. The mean hardness for these tablets was 30(19) N.

Using a procedure testing for the extent of crystal modifications in solid dosage forms (7) thermodynamic parameters of ground tablets were analyzed to compare (+)-S-ibuprofen to the racemate. Results of the calorimetric analysis are presented in table IV and figure 1. Thermal endotherms of mixtures after compaction indicated a "clear" eutectic (first endotherm) at lower temperature. The eutectic also appeared after three days of mixing and was not proportional to the compaction level, as was previously found for the racemate (7). As predicted from molecular modeling the manifestation of lattice rearrangement upon compaction are different between (+)-S-ibuprofen and the racemate.

Although higher intrinsic dissolution rates had been found for the racemate (partly due to its small particle size), the dissolution for 67% (-)-S-ibuprofen from the directly compressible formulation was faster than for the racemate (Table V). The time for 50% dissolution of (+)-S-ibuprofen was three times smaller (figure 2) and at twenty minutes 58.2(12)% (average(SD)) of (+)-S isomer had dissolved whereas only 22.8(4) % of rac-ibuprofen was released. It is speculated that the presence of excipients and crystal

distortion might be beneficial in enhancing the dissolution of the S isomer.

CONCLUSIONS

The results of this study were in agreement with a preformulation profile (6) reporting that (-)-S-ibuprofen crystal particles are very "fragile" and loose their handling properties when stressed or ground. Therefore further processing will require a gentle mixing. Crystal arrays of (-) and (-) molecules in racemic crystals are totally different than molecules in the lattice of (-)-S-ibuprofen (7). As a consequence, crystal distortion was significantly different between the two compounds. In addition to modification of the crystal habit, the S isomer exhibited a eutectic upon compaction. The dissolution of (-)-S-ibuprofen from the dosage forms was faster when compared to the racemate. It is hypothesized that the particle size decreased upon compression and or the excipients improved the dissolution. Another result of the thermal analysis indicated that as observed for the racemate, excipients protected the ibuprofen crystal from further distortion. Lower strength formulations had decreased heat of fusion but improved mechanical properties.

In conclusion a directly compressible formulation of the S(-) isomer is a feasible alternative to the conventional

wet granulation formulation currently used for racibuprofen. Based on the analysis of the stereoisomer at the molecular level, suggestions for future work can be formulated. Research for neutral excipients including flow properties and the effect of storage under various conditions could bring practical answers. Mixing (time and techniques) appear to be critical. It is important to preserve the S crystal intact before tabletting. Finally, the effect of storage on the biopharmaceutical properties of the tablets should be studied. There was eutectic formation resulting from compaction.

Acknowledgements: We greatly appreciate the support provided by Ciba-Geigy Corporation and the input of Dr. G. Lukas Director of Pharmaceutics and Pharmaceutical technology.

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7. Monitoring Crystal Modification in Systems Containing Ibuprofen.

A.J. Romero, L. Savastano and C.T. Rhodes

submitted for publication

Table I: Experimental Design						
Formulation	A[a]	B[a]	C[a]	D[*]	E[a]	
(+)-S-ibuprofen	17	37	57	67	77	%
Binder (PVP)	6	6	6	6	6	%
Diluent (F.F.Lactose)	73	53	33	23	13	%
Disintegrant (Explotab)	3	3	3	3	3	%
Lubricant (Mg.St.)	-	-	-	1	-	%

*. Tabletted at several compression forces (ranging from 600 to 15000 lbs)
a. subjected to stress storage

Table II: Thermal Analysis of (+)-S Formulation (24 hr mixing)					
Thermodynamic Functions	Pure ibuprofen	Formulations: A	B	C	E
Melting Point (Tm in ^{o}K)	327[a]	325.5[a]	325.8[a]	326.4[a]	326.4[a]
Enthalpy of Fusion (KJ/mole) average +/- (SD)	18.3 (0.2)	11.6 (1.1)	14.6 (1.6)	16.0 (1.1)	16.5 (1.0)

a. Relative standard deviation (RSD) less or equal than 0.05

Table III: Thermal Analysis of (+)-S Formulation (One week storage at RT)					
Thermodynamic Functions	Pure ibuprofen	Formulations: A	B	C	E
Melting Point (Tm in °K)	327[a]	324.2	325.0	325.3	325.5
Enthalpy of Fusion (KJ/mole)	18.3 +/- 0.2	10.5	12.7	16.2	15.4

a. Relative standard deviation (RSD) less or equal than 0.05

Table IV: Thermal Analysis of (+)-S-ibuprofen Tablets (67%)
Effect of Compaction

Compression Force (KN)	^{1}Tm (°K)	$^{1}\Delta H$ (KJ/mole)	^{2}Tm (°K)	$^{2}\Delta H$ (KJ/mole)
0.0[a]	-	-	327.8	18.3
0.0[b]	-	-	325.7	17.3
0.0[c]	314.5	1.79	325.4	16.8
2.7	313.9	0.66	325.1	16.9
5.3	318.1	0.92	324.7	16.2
10.7	314.3	0.53	325.7	17.7
22.2	314.1	0.53	325.9	18.6
26.7	313.8	0.68	325.4	17.5
35.6	314.5	0.35	326.2	19.0
44.5	314.0	0.41	325.7	18.5

a. Pure (+)-S-ibuprofen "as is"
b. 67% (+)-S-ibuprofen formulation after ten minutes of mixing
c. 67% (+)-S-ibuprofen formulation after three days of mixing

Table V: Biopharmaceutical Properties of Ibuprofen Tablets				
	Average +/- (SD)			
	Time for 50% (Minutes)	% Dissolved in 20 Min.	Hardness (KN)	Disintegration Time (Minutes)
(+)-S-ibuprofen	17	58.2(12)	30.0(19)	8(4)
Rac-ibuprofen	53	22.8(4)	22.2(5)	53(6)

Figure 1: Crystal Distortion in Tablets
Comparison of DSC Endotherms

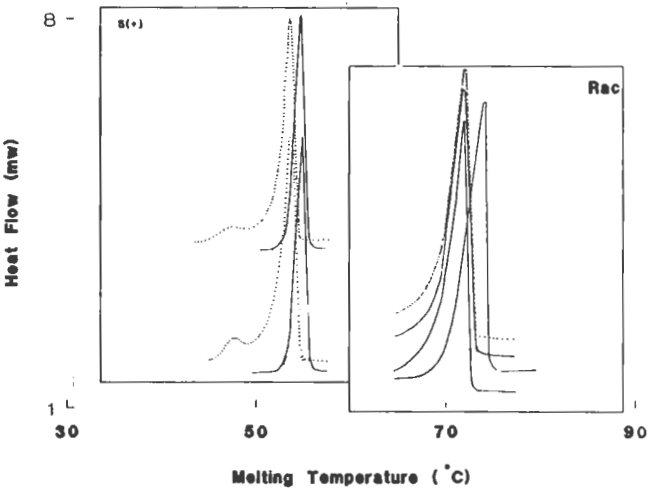
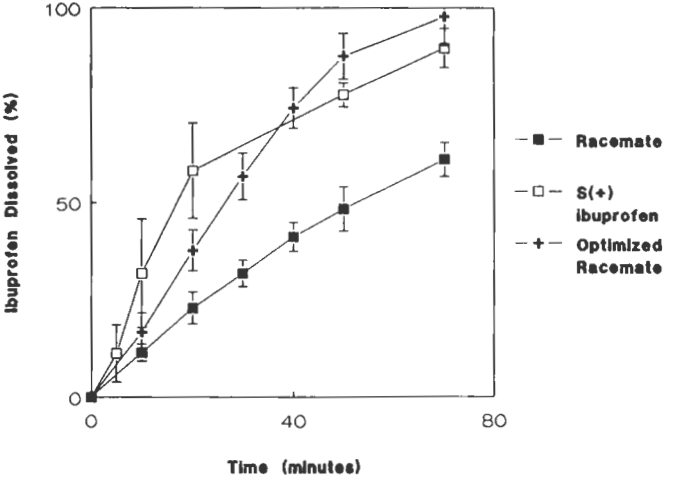


Figure 2: Dissolution of Ibuprofen Cores Comparison of S(+) and Racemate



CONCLUSIONS

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

In the early stages of this study, I defined tests and methods that made possible the identification of crystal properties that affect the processing of ibuprofen. It was shown that different sources of ibuprofen, all meeting the United States Pharmacopeia compendial standards, had very variable crystal characteristics. These differences had a dramatic effect upon the selection of processing parameters and not all the sources of ibuprofen can be regarded as interchangeable. This report is one of the first promoting the use of different standard operating procedures (SOP's) for sources from different suppliers of ibuprofen. Also, given the five sources of this NSAID agent, it is conceivable that it might be advantagous to make USP standards more rigorous. I have no reason to doubt that other drugs may also require this adjustment of the formulation process if they are obtained from different manufacturers or different synthetic routes.

This study underlines the importance of crystal engineering and strict physical pharmacy profiles in drug development. I was able to demonstrate, with relative confidence that sintering is the molecular mechanism by which ibuprofen transformation is achieved during formulation. A resulting hydrophobic network within tablets was the cause

of many formulation problems with this compound. Several analytical tools usually reserved to other fields of pharmaceutical research (e.g. SEM, DSC, molecular modeling) proved extremely useful and should be incorporated in regular formulation preformulation activities especially when single isomers are being considered as replacement for racemic drugs.

The same methodologies were applied to the analysis of the (-)-S-ibuprofen. It is well established that drug substances with a chiral center may exhibit pharmacologically-active and inactive stereoisomers. When this is the case, there might be indeed a powerful argument to replace racemate with the single pure enantiomer. The rational for this change has to be carefully reviewed.

It is believed that this thesis is one of the first report to demonstrate that in addition to biological differences (previously publicized), rac-ibuprofen and (+)-S-ibuprofen are essentially different in terms of processing and formulation. Both quantitatively and qualitatively differences in homo and heterochiral interactions can account for a certain degree of the differences in mechanical properties and solubility. For example, direct compression is impossible and wet granulation is the only way to formulate the racemate, whereas the reverse may be true for the S isomer. Some characteristics of the racemate must be kept

in mind but I can assess with confidence that the (+) S-ibuprofen is a totally independent crystal that should be formulated as a new drug substance.

The comparative analysis of molecular pharmaceutics of the racemate and stereoisomers revealed a useful tool for stereospecific drug development. Enantiomers should be considered as early as possible as new candidates for formulation. It may well be that conclusions for ibuprofen could be applied to others chiral compounds and in view of the above factors, I strongly recommend that all changes from racemate to pure isomers be subjected to extensive molecular scrutiny in early preformulation program, using the approach that was designed.

My results suggest, however, that more studies on the effect of stress storage of (+)-S-ibuprofen and its formulations should be conducted. This compound is very fragile. Formulators must proceed with extreme caution as it is more likely that (+)-S-ibuprofen will sublime even at room temperature. Other studies are currently under way to refine the model of stereospecific bioinversion in man, adding a feed back inhibition from the S isomer. Such studies could involve the administration of different enantiomeric composition in a rat model and the monitoring of systemic plasma concentrations of S and R ibuprofen. A faster absorption of (+)-S-ibuprofen has been observed when this isomer

is administered within the racemate form than alone. Optimizing ibuprofen therapy could lead to administering a small amount of (-)-R-ibuprofen. In this thesis it was found that chiral bioinversion occur essentially systemically. The results suggested that enantiomeric AUC ratios were extremely powerful in studying realistic ibuprofen pharmacokinetics.

The work reported in this dissertation has demonstrated that careful attention has to be directed to crystal characteristics and physico-chemical properties of raw materials. Consequently USP compendial standards should be more rigorous. The results clearly indicate that a profound change of the approach to formulating drug substances is necessary using combinations of new testing methods readily available. This concept is in my opinion essential when the drug substance includes a chiral center and developing pure isomers might be a possibility.

SECTION III

MANUSCRIPT VII

USE AND LIMITATIONS OF THE TRUNCATED AREA UNDER THE CURVE IN BIOEQUIVALENCE TESTING

ABSTRACT

Computer simulations were used to evaluate the truncated area under the plasma level-time curves (AUC_t) as indicators of the bioequivalence between test and reference products. Plasma concentrations were simulated from one and two compartment open models using first order absorption rate constants (Ka) and bioavailability (F) ranging respectively from 45 to 200% and 60 to 140% of the reference values. The pharmacokinetic parameters were selected to cover a wide range of disposition rate constants (0.01-0.79 $hr.^{-1}$). The area under the blood level-time curves was calculated using the trapezoidal rule at each time point (t) according to a conventional sampling regimen. The extent of absorption (AUC_{inf}) was calculated, using integrals of the general blood equations. The ratios of AUC_t: test to reference and AUC_t to AUC_{inf} were determined. For most simulations, the ratios changed very little between the end of the absorption period, the last time point and the time infinity. AUC_{tmax} was not a good parameter to compare the bioavailability of two drug products. Three groups of different mathematical behavior were identified, in which bioequivalence determination might present some problems when using a single AUC. Several truncated AUC ratios, however, could provide meaningful information on absorption

rates for bioequivalence testing. In our study the AUC_{80} was consistently a good indicator of bioequivalence.

INTRODUCTION

Since the seventies, the rate of discovery and development of new therapeutic entities has been decreasing while the number of major drugs going off patent has been increasing (1). As a result there has been a steady expansion of the generic market (2). This phenomena was enhanced by the Drug Price Competition and Patent Restoration Act which expedited the approval of generic drugs. Under this act, the therapeutic equivalency of generic products may be assessed on the basis of a bioequivalence test. The fundamental assumption being that once in the general circulation, the same active drug undergoes the same disposition and metabolism independent of the dosage forms. Therefore, drug products showing "similar" bioavailability could be termed bioequivalent according to specifications provided by the Food and Drug Administration (FDA).

Bioavailability has been defined as the rate and extent at which a drug ingredient is absorbed from the drug formulation and becomes available to the site of action (3). Details on the experimental design and statistical techniques for these bioavailability studies have been described extensively elsewhere (4-5) and for some drugs the Division

of Bioequivalence at the FDA provides guidelines for the required in-vivo bioequivalence study (6). In our report, the statistical issues, although intimately related to bioequivalence inferences, will not be discussed. The ratios of area under blood level profiles for reference and test products will be analyzed.

To date, although no cases of bioinequivalence between approved drug products have been documented, there are some reports showing concerns on the therapeutic efficiency of generic products (7-11). In a recent analysis, however, the FDA found that in 80% of 224 bioequivalence studies, the difference of area under the plasma level-time curves for the tests to reference products averaged 3.5% ± 5% (12). For such products, the natural intersubject variability is more likely to affect the pharmacodynamic response of drugs, than differences in bioavailability (extent and rate of absorption). While AUC_{inf} provides complete information on the ultimate extent of absorption, Cmax (maximum peak concentration) and Tmax (time to the peak) are dependant on the sampling regimen. Therefore, the confidence interval for those parameters is often wide and the regulatory agency gives less weight to the variation of these parameters (12). Thus, there is a need for alternative parameters to provide more reliable information on the rate of absorption (15). Two recent reports suggested that more emphasis should be placed on truncated area under the blood profiles (AUC_t or

AUC_{XX}, where t is the sampling time corresponding to XX per cent of the AUC_{inf}, in the determination of bioequivalence (16,23). Many bioequivalence studies compared AUC_{tlast} (tlast = time of last measurable concentration) (17-19), but only recently, few used the truncated area AUC_t (22) or addressed the pharmacokinetic relevance of this parameter (20,21,23). In Japan, statistical testing on AUC_{tlast} instead of AUC_{inf} is required to assess bioequivalence between two products (19). The issue of the last sampling time remains a question mark. The approach required by the FDA is that AUC's should be calculated from plasma levels which have fallen to at least 10% of the peak concentration. This method is useful but empirical. In essence, there is no simple rule of thumb governing the principles of bioequivalence testing.

The objectives of this computer simulation are to investigate the use and limitations of truncated areas in bioequivalence studies and to stress the pharmacokinetic relevance of this parameter. We hope to define rational limitations and boundaries in the practical use of incremental AUC's. Our goals are to demonstrate that ratios of truncated AUC's can be used with good reliability in bioequivalence testing as they conform to both the statistical appropriateness and the pharmacokinetic/pharmacodynamic relevance.

METHODS

Plasma levels were simulated from one and two compartment open model equations using first order elimination constants (KE) ranging from 0.04 to 0.2 hr^{-1} and sets of alpha (α), beta (β) from 0.17 to 0.79 and 0.01 to 0.17 hr^{-1} respectively. For each KE and each set of α,β the pharmacokinetic parameters characterizing the dosage forms, bioavailability (F) and rate of absorption (Ka), were ranged from 60% to 140% and 45% to 200% of the reference product values. All conditions are reported in table I and II. All necessary nomenclature is given in the appendix. It is well known that AUC_{inf} remains constant with varying rates of absorption and we were interested in observing the behavior of truncated areas in the various combinations. The one compartment open model (figure 1, equation 1) and the two compartment open model (24) with elimination from the central compartment, (figure 2, equation 2) were used in these simulations.

$$Ct = (F.D.Ka\ V).(e^{-KEt} - e^{-Kat}) \qquad \text{eq.1}$$

$$Ct = \frac{Ka.F.D.(K21-\alpha)}{V.(Ka-\alpha).(\beta-\alpha)} . [e^{-\alpha t}] +$$

$$\frac{Ka.F.D.(K21-\beta)}{V.(Ka-\beta).(\alpha-\beta)} . [e^{-\beta t}] +$$

$$\frac{Ka \cdot F \cdot D \cdot (K21 - Ka)}{V \cdot (\alpha - Ka) \cdot (\beta - Ka)} \cdot [e^{-Kat}] \qquad \text{eq. 2}$$

The disposition parameters for a drug, were assumed to be independent of the dosage form and for each KE or set of (α,β), the pharmacokinetic parameters of the dosage forms. F and Ka were varied. The reference products that we arbitrarily designated for comparison purposes had the same F, Ka over the KE or α,β ranges.

Simulation algorithms were coded in Pascal and TurboPascal and compiled on IBM Personal Computers. Most of the equations we used were derived from mono, bi and triexponential traditional relationships between concentration and time. Calculations of AUC ratios (AUC_{test} AUC_{ref}) depended on the time available for absorption T (20, 25), where T was the end of the absorption period. Derivations and calculations for one and two model compartments are given in Appendix 2.

T, the time for 99% of the absorption to occur, can be estimated from the first order process using the Wagner-Nelson method (22) where the fraction unabsorbed is obtained with :

$$T = 4.606 \; Ka \qquad \text{eq. 3}$$

T ranged from 1.6 to 5.8 hours. It has been suggested that AUC should be calculated at least through 3.5 half

lives for accuracy (24). However, it appeared that AUC_t at t=2T is usually within a few percent of the AUC_{inf} (17, 24).

For the two compartment study, the procedure was similar except the classic triexponential function (equation 2) describing an open two compartment model. If we maintain our assumption that V, K21, D, α and β remain constant and independent of time, we can express the extent of absorption as

$$At = \int Ctdt \qquad \text{eq.4}$$

Using the ranges of pharmacokinetic parameters reported and reference values from table III, area under the curves were calculated using the trapezoidal rule (AUC_t) and equations previously defined (AUC_{inf}) : the area at t = infinity was

$$A = F.D/V.KE \qquad \text{eq.5}$$

for one compartment and

$$A = AT + [\ CT/\alpha + CT\ \beta\ ] \qquad \text{eq.6}$$

for two compartments

AUC ratios (test/ref) were than evaluated. Time points such as t= T, 3 2T and 2T, 3T were given special attention. This study is not exhaustive in any means because we had to use a limited set of parameters. Nevertheless, this report presents an approach in the potential use of truncated area in bioequivalence determination.

RESULTS AND DISCUSSION

Area under the curve ratios have been discussed by Wagner (24) and Loo and Riegelman (25) who proposed several mathematical relations for percent of drug absorbed, independent of the model. Incomplete availability can influence the absorption process and true absorption rate constants cannot be calculated with complete accuracy from plasma levels (27). Furthermore, when the absorption is the limiting factor, flip-flop phenomena may occur (Ka<KE) and AUC_{inf} cannot be correctly estimated (28). In other instances, when both zero and first order kinetic model describe the absorption process (29), the rate of absorption is very difficult to estimate with accuracy. For bioequivalence testing, however, the extent and rate of absorption, as measured from blood concentrations, by comparing using AUCs, Tmax and Cmax do not fall within the previous limitations. In this study, the Ka's are apparent absorption rate constants and represent also immediate release characteristics of the dosage forms being tested. We fully understand the limitations of the simulations since the pharmacokinetic models apply to situations where the formulation behavior is the limiting factor of drug absorption.

In many bioequivalence studies, an analysis of variance is conducted on pharmacokinetic parameters such as C_t or AUC_t and the classical hypotheses are tested:

H_0: Test = Reference or $\Phi = 0$

H_a: Test ≠ Reference or $\Phi \neq 0$, where Φ is a measure of the difference in bioavailability. This test is not consistent with the definition of bioequivalence because the power of the test is aimed at testing H_a not the null hypothesis H_0. The current acceptance criteria for bioequivalence is that the true AUC or AUC_{inf} of the test formulation should be within 20% of the reference mean:

$$AUC_{test} - AUC_{ref} \leq |0.20| (AUC_{ref})$$

which can be written as:

$$[AUC_{test} - AUC_{ref}] / AUC_{ref} \leq |0.2$$

$$[AUC_{test} / AUC_{ref}] - 1 \leq |0.2| \text{ thus}$$

$$0.8 \leq AUC_{test} / AUC_{ref} \leq 1.2$$

Therefore the AUC ratios are compatible with the nature of bioequivalence testing.

In a previous investigation (16) three cases of bioequivalence testing were simulated. In all simulations the truncated area under the curve (AUC_t), accounting for 60% of

the AUC_{inf}. resulted in making the tests more sensitive to bioavailability differences. It was also anticipated that the ratios of AUC_{tmax} could be of critical importance for the determination of bioequivalence as it is intimately related to the rate of absorption. Unfortunately, we found that for high bioavailability (F), and low rate of absorption (Ka) (50 to 75% of the reference value), the AUC_{tmax} still falls within the acceptable range 80-120% . In other cases, the AUC_{tmax} ratio did not indicate equivalence for bioequivalent products. The AUC_{tmax} ratios are not reliable indicators of the absorption rate as they failed to indicate equivalence in instances when Ka and relative bioavailability were actually within the acceptable 20% of the reference value (Table IV). In the bioavailability profiles illustrated in figure 3, drug product B having a Ka of 2.0 (143% of the reference value) with a relative bioavailability of 70%, and drug product C having a Ka of 0.8 (50% of the reference value) with a relative bioavailability of 130%, were compared to the reference formulation A.

All truncated AUC ratios identified the inequivalence except when approaching tmax for which the AUC ratios indicated false bioequivalence as marked by the bold vertical line. Furthermore, the calculation of this parameter is very sensitive to the sampling regimen and we do not recommend its use in bioequivalence comparison.

In this project, more than 700 simulations were performed and analyzed, although only significant figures are presented in this report. The discussion is divided into three scenarios which we found critical in bioequivalence testing: 1)the relative bioavailability (F) is outside the acceptable range of 80 to 120% , 2) the relative bioavailability is within the allowed interval with Ka ranging from 50 to 200% of the reference value, and 3) at the boundaries of bioequivalence F=80% and F=120%. In each case the effects of unacceptable performances for the rate of absorption were carefully monitored.

Case 1: when Ka and F of the test product were both outside the range of 80 to 120% of the reference values, 94% of the time the truncated AUC detected the bioinequivalence at all time points (tables V and VI). In particular situations, when the bioavailability is above 120% and the absorption rate is below 80%, the effect of the truncated estimate for the extent of absorption is counterbalanced by those two parameters and the ratio of truncated areas indicated false bioequivalence. As KE or α, β increase, more absorption is included in the truncated AUC (30) and larger portions of AUC_{inf} are sensitive to absorption rates. In figure 4, the high relative bioavailability (F) compensates for the slow rate of bioavailability (Ka=57%) and AUC ratios indicate false bioequivalence. This situation shows that a product

with unacceptably high extent of absorption and unacceptably low rate of absorption can be found bioequivalent using certain truncated estimates.

Case 2: when the relative bioavailability is within acceptable ranges and the Ka varies from 50 to 200% of the reference product. 74% of the AUC_t ratios (RAT.XX) showed bioequivalence for only 30% of truly bioequivalent case products, indicating that truncated areas are more sensitive to the extent than the rate of absorption as shown in table V. Nevertheless, the sensitivity of the AUC_t ratios to the rate of absorption is proportional to the disposition phases. It is known that changes in elimination affect pharmacokinetic parameters of one and two compartment models (31) and this study confirmed that as a consequence, such changes also influence bioequivalence determination.

Case 3: at the boundaries, when F=80%, the truncated AUC_{20} is only discriminating when absorption rates are low. When F=120% and Ka is more than 20% of the acceptable limit, AUC_{20} is sensitive to the high rate of absorption. In both cases, if you increase the disposition rate constants, larger portions of the the AUC_{inf} become discriminating and show inequivalence. The percentage of the AUC_{inf} indicative of true bioinequivalence due to the absorption rate, varies with the elimination phases. Figure 5 illustrates two examples of minimum AUC ratios required to demonstrate true

bioinequivalence. for formulations exhibiting low absorption rate.

Particular cases where the AUC ratios showed bioinequivalence for products with extent and rates of absorption within the acceptable range were critical. For all cases except a slow elimination rate constants of 0.01 hrs^{-1}. when F=120% and the absorption rate constants were high but within the acceptable 100 to 120% of the reference values. the truncated AUC's for 20 to 50% of the AUC_{inf} indicated consistently false inequivalence (Table VII). In our study. the truncated ratio of AUC accounting for 80% of the AUC_{inf} did indicate bioequivalence consistently throughout these disposition situations.

Another limitation when relative bioavialability (F) ranged from F=100 to 120% in which AUC and Ka ranged from 67 to 196.4% the truncated area ratios did not indicate bioinequivalence at any time (t) or for any proportional section of the AUCinf. The same limitations apply to the use of AUC_{inf} in the bioequivalence comparison since AUC_{inf} is independent of the absorption rate. Within this interval. the truncated AUC was completely dependent on the extent of bioavailability.

In all cases, when F was 80 to 120% of the reference value, 98% of the time AUC_{80} was also within the same range confirming what had been previously reported (21) on the similarity of truncated and infinity AUC ratios. Truncated

ratios for early portions of the AUC_{inf} were smaller than the relative bioavailability (figure 6) when the absorption rate constant was below the reference value 1.4 hrs^{-1} for the test products. Conversely, when Ka was greater than the reference value, early truncated area ratios were larger than the relative bioavailability (figure 7). The cut-off point or time where the truncated AUC ratios became equal to the relative extent of absorption was related to the absorption rate constants and exhibited a U-shaped curve in figure 8, which indicated that these critical times or percentages of AUC_{inf} were high for low absorption rate products. decreased as Ka increased and increased again when the rate of absorption was above 100% of the reference value. The slope of AUC ratio Vs. time, in early portions of the plasma profile, was proportional to rate and extent of absorption. This parameter could be an additional estimator to compare absorption kinetics in bioequivalence study and further investigations are needed on this topic. In our study, the cut-off point ranged from 35 to 85% for acceptable formulations (table VIII). For perfectly bioequivalent products, this point could theoretically be 0. The time corresponding to these percentages depends on the elimination characteristics, (figure 9). Furthermore, if the MEC or MIC [Minimum Efficient Concentration or Minimum Inhibitory Concentration] can be estimated accurately, it has been argued that the comparison of truncated AUC's for bioequivalence purposes

has also a pharmacodynamic relevance (31), since the AUC_{xx} ratios compare portions of the plasma levels significant for the therapeutic window (figure 10).

CONCLUSIONS

A first criticism of the emphasis given to AUC_{inf} in bioequivalence testing was the fact that bioequivalence is aimed at comparing the absorption characteristics of two dosage forms. It is well known that AUC_{inf} is not dependent on the rate of absorption Ka, thus only extent of absorption could be compared with accuracy.

We feel that extrapolation of the AUC_{tlast} to infinity could bring unnecessary variations not representative of realistic differences or equivalences between a reference and a test product. On one hand, if the extrapolated area is large, the true difference in extent of absorption may become proportionally insignificant. On the other hand, the extrapolation is calculated from the last points, usually in the most variable analytical region and this might introduce differences between truly bioequivalent products. In most cases the AUC_{inf} ratios and AUC_{80} ratios for our simulations did not vary significantly indicating that 80% of the AUC_{inf} was a good indicator of the extent of bioavailability. AUC_{tmax} was not consistently representative of AUC_{inf} or rate of absorption. We do not recommend the use of AUC_{tmax}

alone to compare bioavalailability because extent and rate of absorption outside acceptable ranges can in some cases balance each other.

The proportion of the AUC_{inf} required for sensitivity in bioequivalence testing depends on the combination of elimination rate constants and the absorption rate constant. In all cases, the AUC ratios at early times overestimated relative bioavailability for low absorption rates of test products, and underestimated for high absorption rates before reaching the true relative bioavailability value. Important failures of the truncated areas occurred where the relative bioavailability was high and the rate of absorption was lower than the reference value as well as cases where acceptable low F and low Ka led to false inequivalence as a result of a counterbalancing effect. As or KE increased over 0.01 $hr.^{-1}$, larger portions of the AUC_{inf} were necessary to assess bioinequivalence. These simulations suggested that 80% of the AUC_{inf} was substantially reliable in the range of our study for determining the relative extent of absorption which had more influence on bioequivalence than the rate of absorption. Although truncated AUC ratios have to be further investigated (as indicators of absorption rates), we feel that examining truncated areas provides additional and qualitative information that strengthen bioequivalence testing.

APPENDIX 1: Nomenclature

D : Dose

V : Volume of distribution

Ka : First order absorption constant in $hr.^{-1}$

KE : First order elimination rate constant in $hr.^{-1}$

: Distribution rate constant

: Elimination rate constant

At : Area under the curve at time t

Ct : Blood concentration at time t

AT : Area under the curve at the end of the absorption period

CT : Plasma concentration at " " " " "

AUC_i : Area Under the Curve

AUC_{ref} : Area under the Curve for the reference product

AUC_{test} : Area under the curve for the test product

Ka%: Ratio of Ka test to reference in percentage

F% : Ratio of bioavailability test to reference in percentage

RATXX Ratio of truncated AUC's at t corresponding to XX% of AUC_{inf} reference

i= inf : at time infinity

i= tmax: at time of the peak

i= t : at time t

APPENDIX 2: Calculations

For the one compartment model if t ≤ T. blood levels are given by eq.1 and the extent of absorption can be integrated and written as (21, 22):

$$At = \frac{F.D}{V.KE.(Ka-KE)} (KEe^{-Kat} - Kae^{-KEt} - Ka - KE) \qquad \text{eq. 1}$$

where At is the area under the blood level-time curves from 0 to time t. The following ratios can be derived (21) where Ka' and F' indicate the pharmacokinetic parameters for the reference product:

$$\frac{At}{At'} = \frac{F \,.\, (Ka' - KE).(Kae^{-KET} - KEe^{-Kat})}{F'.\, (Ka - KE).(Ka'e^{-KEt} - KEe^{-Ka't})} \qquad \text{eq. 2}$$

If t > T the plasma levels can be described (17) by:

$$Ct = CT.e^{-KE(t-T)} \qquad \text{eq. 3}$$

where CT is the blood concentration at time T. thus the AUC at time t can be written (21) as :

$$At = AT - Ct.(1 - e^{-KE(t-T)}).1\ KE \qquad \text{eq. 4}$$

with AT. the area under the curve from time 0 to T. The ratios can therefore be calculated (21):

$$\frac{At}{AT'} = \frac{AT + (1-e^{-KE(t-T)}).Ct\ KE}{At' + (1-e^{-KE(t-T')}).Ct\ KE} \qquad \text{eq. 5}$$

For the <u>two compartment model</u> if t ≤ T.

therefore. At can be derived

$$At = \frac{Ka.F.D.(K21-\alpha)}{V.(Ka-\alpha).(\beta-\alpha)} \cdot \frac{1}{\alpha} \cdot [1-e^{-\alpha t}] +$$

$$\frac{Ka.F.D.(K21-\beta)}{V.(Ka-\beta).(\alpha-\beta)} \cdot \frac{1}{\beta} \cdot [1-e^{-\beta t}] +$$

$$\frac{F.D.(K21-Ka)}{V.(\alpha-Ka).(\beta-Ka)} \cdot [1-e^{-Kat}] \qquad \text{eq.6}$$

The area under the curve at t infinity can be derived from equation 9 and calculated using the pharmacokinetic parameters independent of time:

$$A = \frac{Ka.F.D.(K21-\alpha)}{V.(Ka-\alpha).(\beta-\alpha)} \cdot \frac{1}{\alpha} +$$

$$\frac{Ka \cdot F \cdot D \cdot (K21-\beta)}{V \cdot (Ka-\beta) \cdot (\alpha-\beta)} \cdot \frac{1}{\beta} +$$

$$\frac{F \cdot D \cdot (K21-Ka)}{V \cdot (\alpha-Ka) \cdot (\beta-Ka)} \qquad \text{eq.7}$$

The ratio At At* can also be estimated easily. On the other hand, if t<T, the blood levels can be expressed as:

$$Ct = CT \cdot (e^{-\alpha(t-T)} - e^{-\beta(t-T)}) \qquad \text{eq.8}$$

therefore, At the area under the curve at time t can be integrated and written as:

$$At = AT - [1-e^{-\alpha(t-T)}] \cdot CT/\alpha + [1 - e^{-\beta(t-T)}] \cdot CT/\beta \qquad \text{eq.9}$$

The area at t = infinity is derived in equation 10

$$A = AT + [\ CT/\alpha + CT/\beta\ ] \qquad \text{eq.10}$$

and the ratios can be calculated from At At*:

$$\frac{At}{---} = \frac{AT + [1-e^{-\alpha(t-T)}] \cdot CT/\alpha + [1-e^{-\beta(t-T)}] \cdot CT/\beta}{---}$$

eq.11

$$At^* \quad AT^* + [1-e^{-\alpha(t-T)}] \cdot CT^*/\alpha + [1-e^{-\beta(t-T)}] \cdot CT^*/\beta$$

with $T' = 4.606 Ka'$ and $T = 4.606 Ka$. In this particular case. AT and AT' are calculated from equation 9 and CT. CT' from

equation 2 for t=T.

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Table I : One compartment model simulations Parameters

Variable Parameters		
Elimination rate constant KE	1/hr	0.04, 0.08, 0.12, 0.16, 0.2
Absorption rate constant Ka	1/hr	0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9
Absorption Period T	hrs.	5.74, 5.12, 4.60, 4,18, 3.84, 3.4 3.30, 3.06, 2.88, 2.70, 2.56, 2.4
Relative bioavailability		0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3
Constant Parameters		
Volume of distribution Vd	l	10
Dose D	mg	1000

Sampling Regimen in hours: 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 5.0, 6.0, 8.0, 10, 12, 16, 48, 72, 96, 120

Table II : Two Compartment Model Simulations Parameters			
Variable Parameters			
Disposition Rate Constants	α	1/hr	0. 17, 0.32,.0.47, 0.63, 0.79
	β	1/hr	0.01, 0.09, 0.17
Absorption Rate Constant	Ka	1/hr	0.65, 0.95, 1.4, 2.0, 2.75
Absorption Period	T	hrs	7.08, 4.84, 2.30, 2.30, 1.68
Relative Bioavailability	F		0.6, 0.8, 1.0, 1.2, 1.4
Constant Parameters			
Volume distribution	V	l	10
Dose	D	mg	1000
Sampling Regimen:	1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 10, 12, 15, 24, 48, 72, 96, 120, 144, 168, 192, 216 (hours)		

Table III : Pharmacokinetic Parameters of the Reference Products			
One compartment model			
First Order Absorption Rate	Ka :	1.4	1/hr.
Bioavailability,	F :	1.0	
Two compartment model			
First Order Absorption Rate	Ka :	1.4	1/hr.
Disposition	K21:	0.2	1/hr.
Bioavailability	F :	1.0	

Table IV: Pharmacokinetic Relevance of AUCtmax				
Ka%	F%	AUCtmax Ratio	AUC48 Ratio	AUC120 ratio
One Compartment Model				
94.0	0.8	0.78	0.80	0.80
87.5	0.8	0.76	0.80	0.80
50.0	1.3	0.94	1.30	1.30
75.0	1.3	1.16	1.30	1.30
Two Compartment Model				
142.8	0.7	0.81	-	0.69
50.0	1.3	0.87	-	1.30
78.5	1.3	1.15	-	1.30
85.7	0.8	0.74	-	0.80

Table V : One Compartment model, AUC ratios for bioinequivalence								
KE	F%	KA%	RAT.20	RAT.30	RAT.40	RAT.50	RAT.60	RAT.80
0.04	70	57	0.63	0.66	0.67	0.68	0.68	0.68
	70	143	0.73	0.71	0.71	0.71	0.70	0.70
	130	57	1.17	1.23	1.25	1.29	1.39	1.30
	130	143	1.35	1.32	1.32	1.31	1.31	1.30
0.20	70	57	0.50	0.56	0.58	0.61	0.63	0.68
	70	143	0.81	0.77	0.75	0.74	0.72	0.71
	130	57	0.94	1.03	1.07	1.13	1.17	1.27
	130	143	1.51	1.43	1.40	1.37	1.34	1.31

RAT.XX : AUC ratios (test to reference) at XX% of the AUC infinity

Table VI : Bioinequivalent Products-AUC Ratios Twocompartment Model				
F%	60		140	
KA%	46	196	46	196
RAT20	0.585	0.600	1.35	1.40
RAT30	0.590	0.600	1.37	1.40
RAT40	0.593	0.600	1.38	1.40
RAT50	0.596	0.600	1.39	1.40
RAT60	0.597	0.598	1.39	1.40
RAT70	0.598	0.599	1.40	1.40
RAT80	0.599	0.600	1.40	1.40

Table VII : False Inequivalence
(for F = 120% and Ka = 114%)

	RAT20	RAT40	RAT60	RAT80	MODEL
Ke					
0.04	1.22	1.22	1.21	1.20	O
0.12	1.24	1.24	1.22	1.20	N
0.20	1.27	1.25	1.24	1.21	E
β					
0.01	1.20	1.20	1.19	1.19	T
0.03	1.27	--	--	1.19	W
0.09	1.28	1.21	1.19	1.19	O
0.17	1.30	1.27	1.22	1.19	

Table VIII: Truncated AUC Ratios equating Relative Bioavailability Time and Corresponding % of AUCinf

One Compartment KE (1/hr)	0.04		0.12		0.20	
Ka (1/hr)	1.2	1.7	1.2	1.7	1.2	1.7
F=0.7						
Time (hr.)	16	12	24	24	24	24
% AUCinf	46	36	61	61	61	61
F=1.0						
Time (hr.)	14	14	15	15	15	15
% AUCinf	74	74	84	84	84	84
F=1.3						
Time (hr.)	13	13	14	13	14	14
% AUCinf	84	84	89	84	89	89

Two Compartment β (1/hr)	0.01		0.09		0.17	
Ka (1/hr)	0.95	2.00	0.95	2.00	0.95	2.00
F=0.6						
Time (hr.)	24	10	12	7	7	6
% AUCinf	32	13	70	49	82	75
F=1.0						
Time (hr.)	36	12	15	8	15	9
% AUCinf	39	16	78	54	87	65
F=1.4						
Time (hr.)	48	24	15	9	9	5
% AUCinf	52	32	78	59	90	65

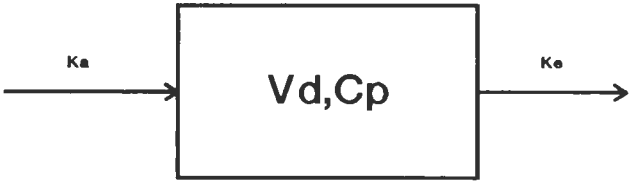


Figure 1
One Compartment Model

Figure 2: Two Compartment Model

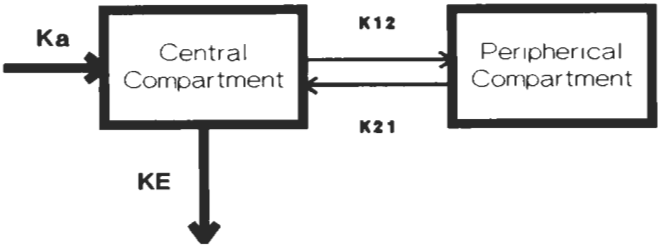


Figure 3a

Pharmacokinetic Relevance of AUCtmax

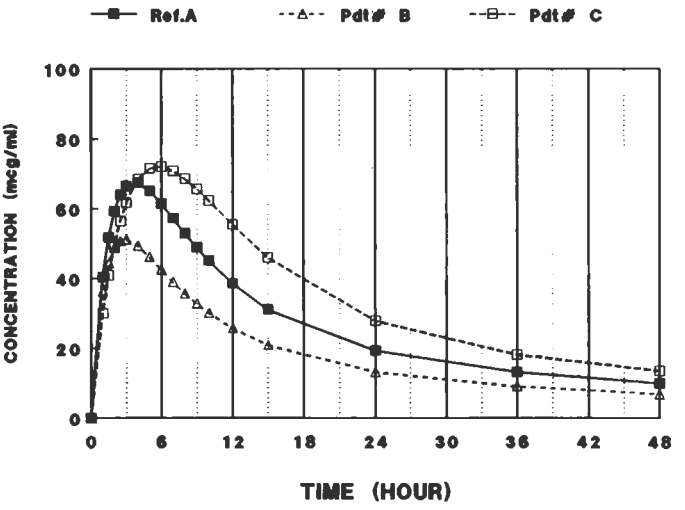


Figure 3b

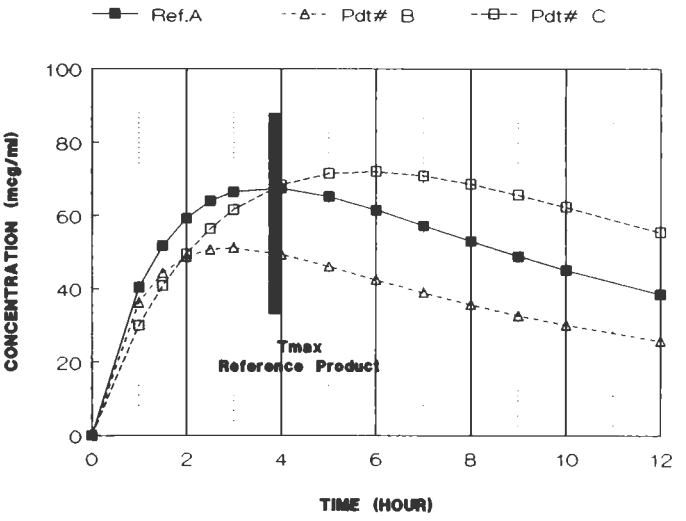

Ref.A
Pdt# B
Pdt# C
100
80
60
40
20
0
CONCENTRATION (mcg/ml)
Tmax
Reference Product
0
2
4
6
8
10
12
TIME (HOUR)

Figure 4a: One Compartment Model
% of AUCinf Reference

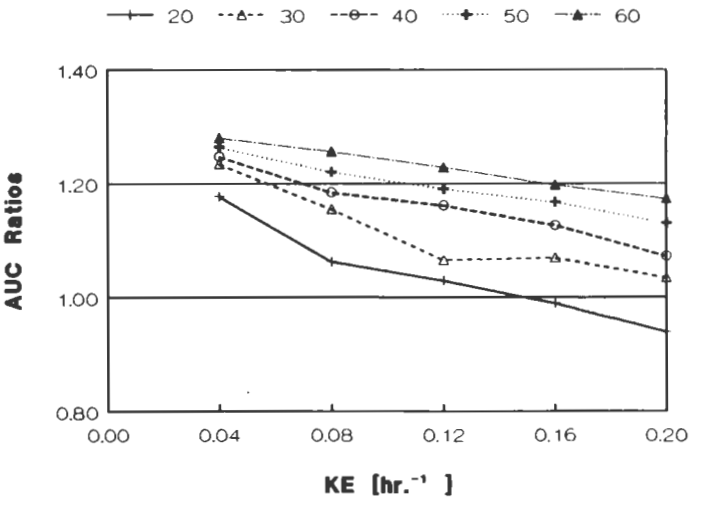


Figure 4b: Two Compartment Model
% AUCinf Reference

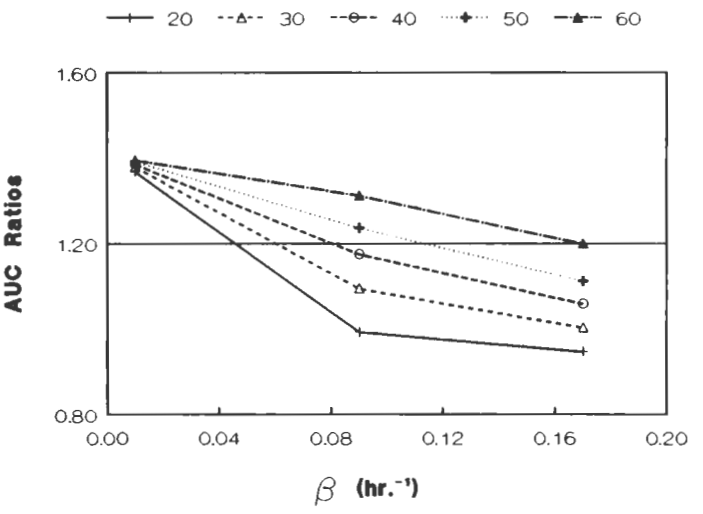


Figure 5: % of AUCinf required to show Bioinequivalence (F=120%)

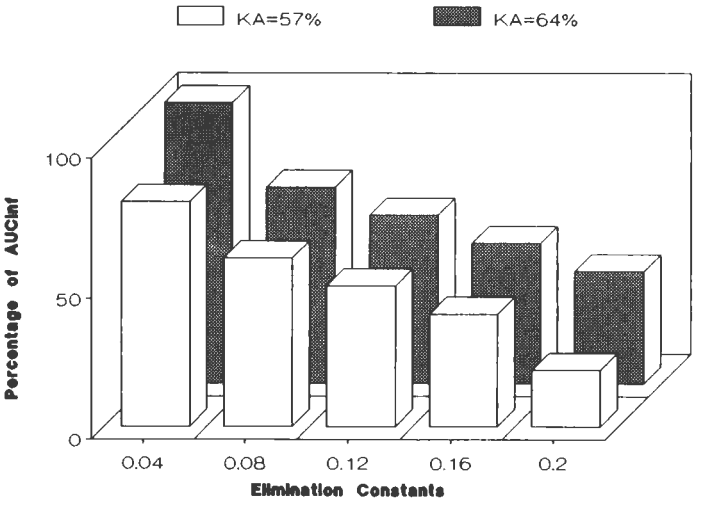


Figure 6
AUC Ratios Vs. Time (Slow Absorption)

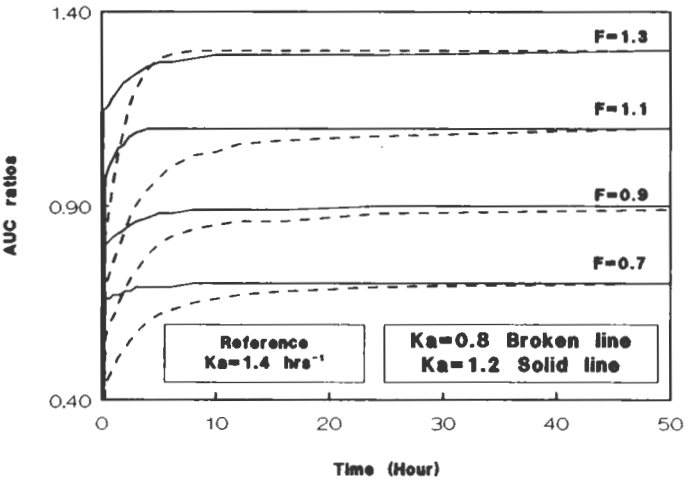


Figure 7
AUC Ratios Vs. Time (Fast Absorption)

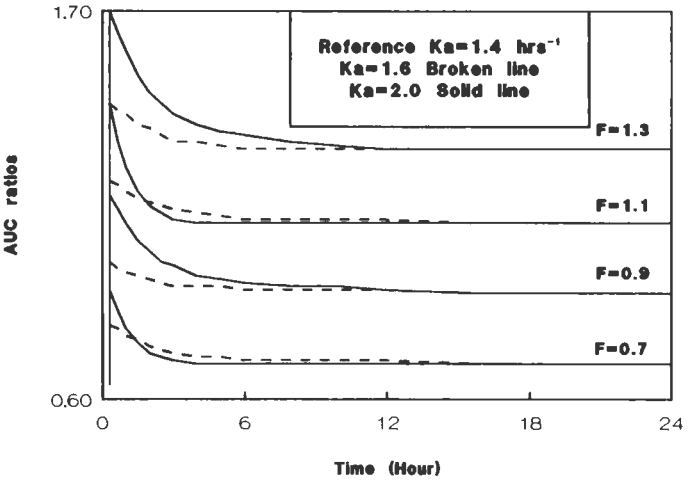


Figure 8: Percentage of AUCinf when AUC ratio = (F)

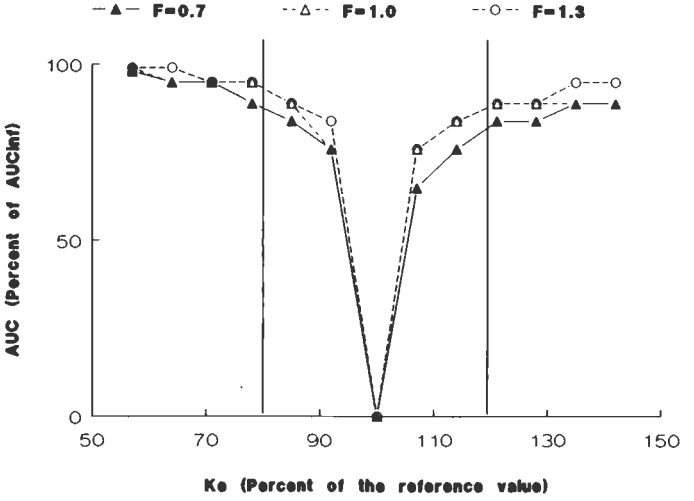
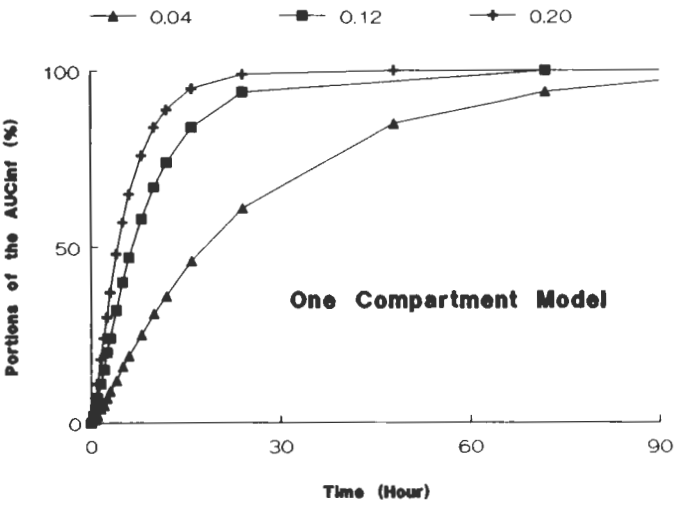


Figure 9a: % of AUCinf observed at any time after dosing – Effect of KE



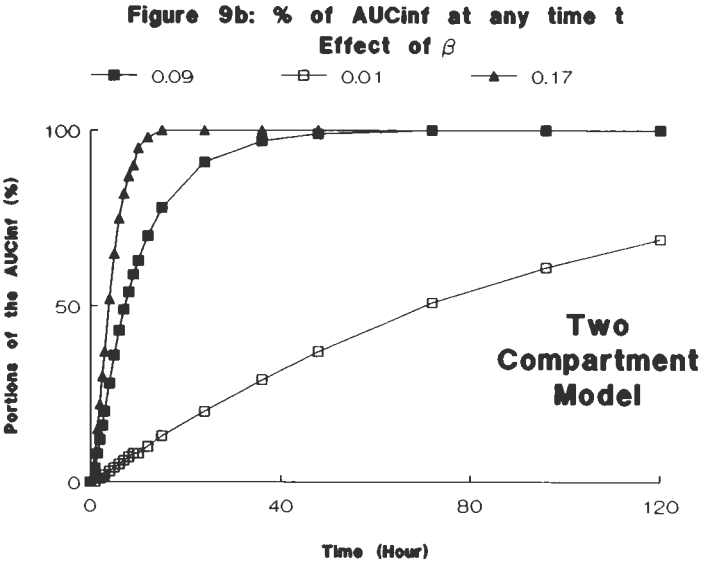
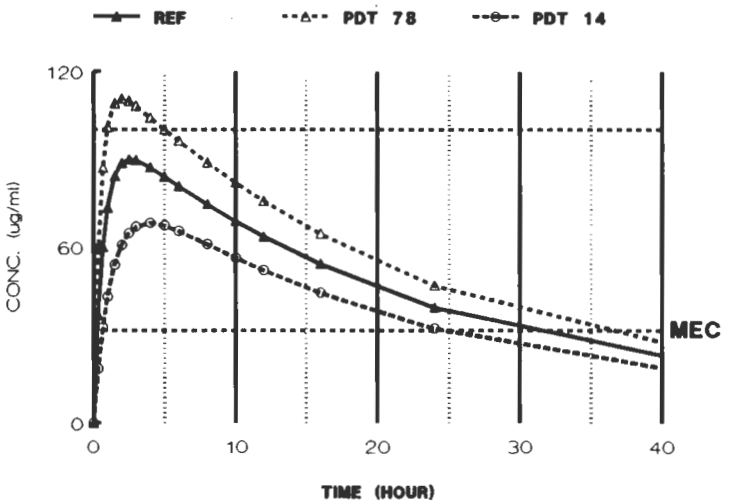


Figure 9b: % of AUCinf at any time t
Effect of β
0.09
0.01
0.17
100
50
0
Portions of the AUCinf (%)
0
40
80
120
Time (Hour)
Two
Compartment
Model

Figure 10: Therapeutic Window Boundaries of Bioequivalence



SECTION IV

APPENDIX A

In early stages of the wet granulation experiment, a power meter or wattmeter, was connected between the power and our planetary mixer in order to validate the end-point determination. A plotter was wired to the meter to record the variation in the power needed to rotate the paddle within the granulation. Thus if the courant can be assumed to be constant, one has a fair approximation of the torque or resistance to movement of the mass being granulated.

The apparatus was improved and the wattmeter was connected with a interface and an IBM PC. A data acquisition software averaged and reduced the noise thus allowing the recording of clear variation wattage. Figure 1 is a typical power consumption trace for ibuprofen formulations without the computerized interface.

Figure 2 is a calibration curve used in the dissolution experiment. Ibuprofen concentration were measured by ultra violet spectrophotometry at 264 nm.

Figure 1: Typical Power Consumption Profile of Ibuprofen Formulation C131

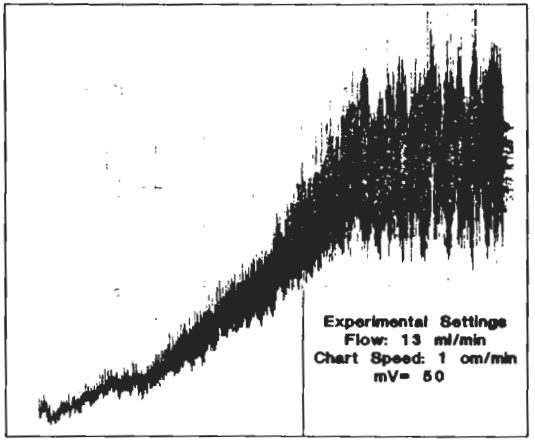
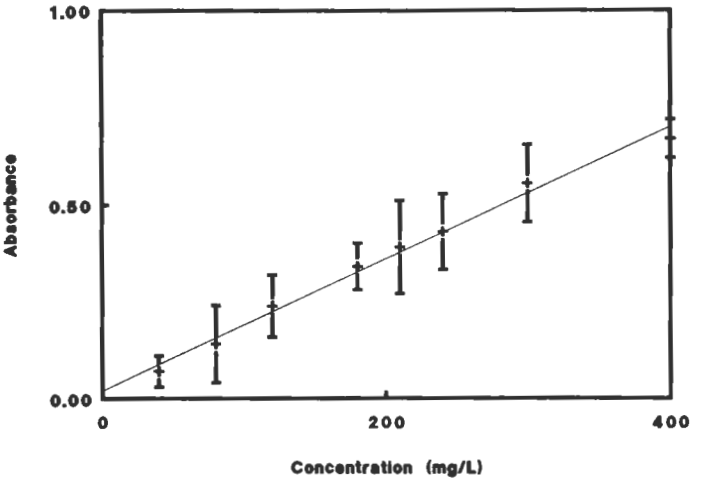


Figure 2:
IBUPROFEN CALIBRATION CURVE AT 264 nm



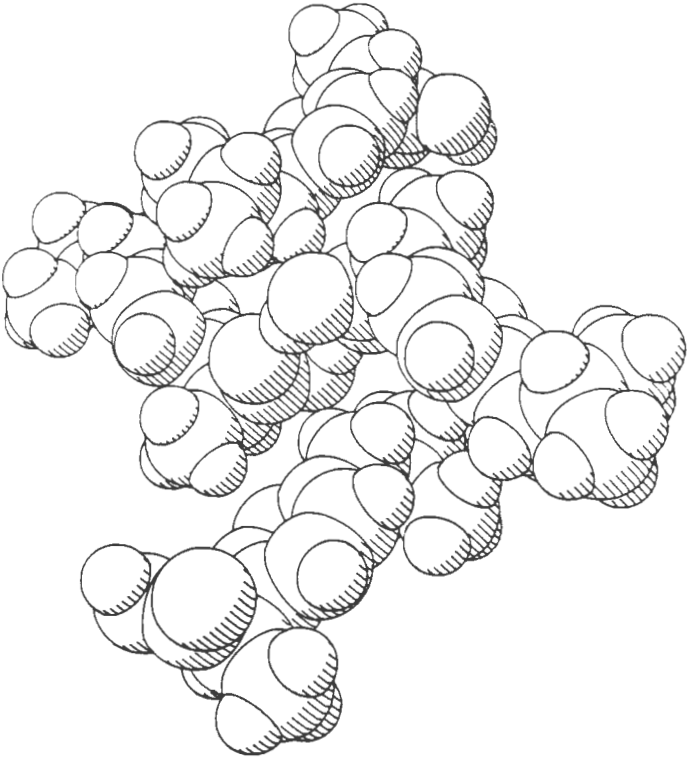
APPENDIX B

X-RAY CRYSTALLOGRAPHY AND MOLECULAR MODELING

Single X-ray diffraction data of rac ibuprofen and (+)-S-ibuprofen were used with the "macromodel" software to examine the 3D molecular packing in the crystal lattice (figures 1 to 5).

From single crystal coordinates it was a simple matter to generate powder X-ray diffractograms. Thus it was possible to predict and validate the single powder X-ray crystallography (figure 6). however. the intensities were dependant on how randomly the crystal is oriented in the x-ray beam. Therefore. only the position of the peaks could be predicted not the optical purity.

Figure 1: Molecular Packing in the crystal Lattice of Rac-ibuprofen



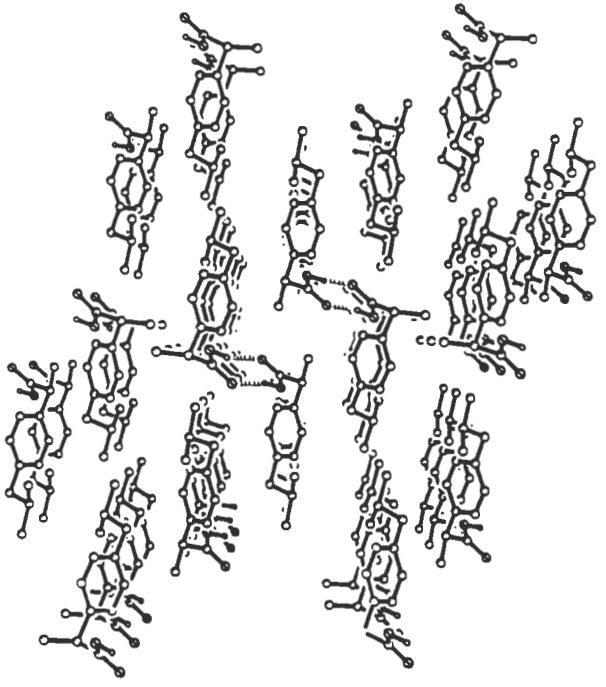


Figure 2: Representation of the (+)-Ibuprofen crystal lattice from a different angle

Figure 3: Arrays of (+)-S and (-)-R Ibuprofen Molecules in the crystal of rac-Ibuprofen (Dash lines are hydrgen bonds)

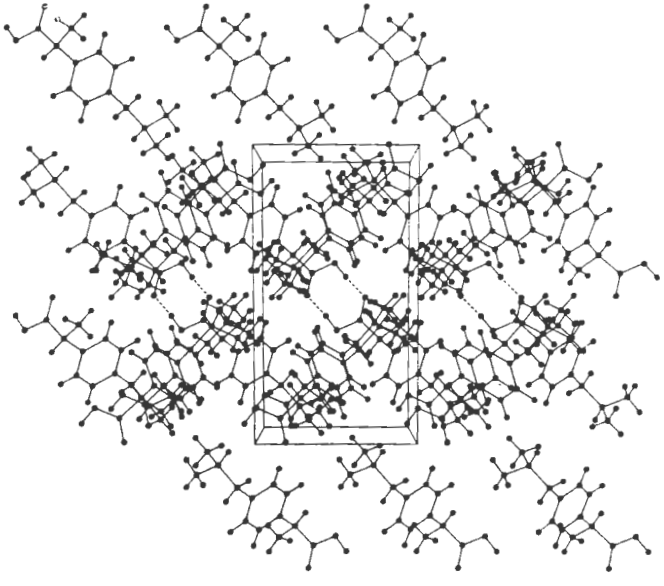
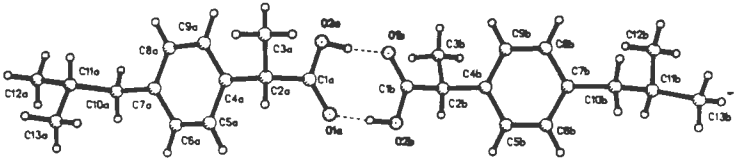


Figure 4: Details of the homochiral hydrogen bonds in the (+)-S-Ibuprofen crystal lattice



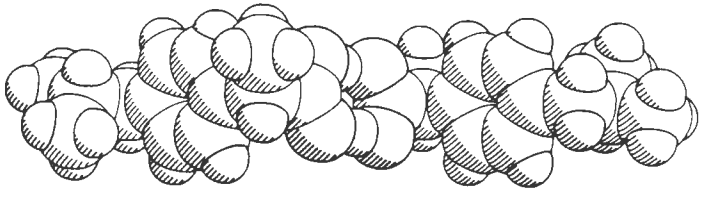


Figure 5: Identification of the molecular torsion
Superposition of 5 molecules A and B
involved in the same hydrogen bond

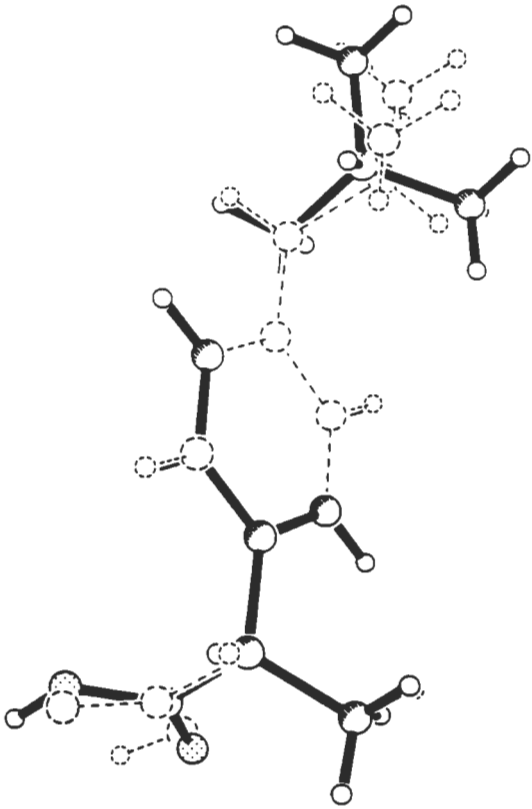
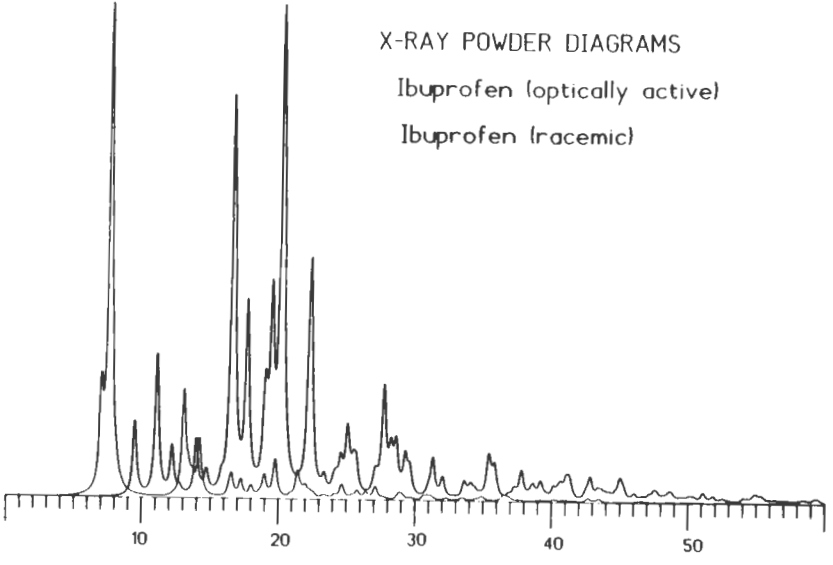


Figure 6: Theoretical X-ray Diffractograms



APPENDIX C

THERMAL ANALYSIS: VALIDATION OF THE EXPERIMENTAL DESIGN

Differential Scanning Calorimetry Procedures

DSC scans of all ingredients of the formulations including ibuprofen, were performed (Figures 1 to). Qualitative assessment of all melting characteristics were evaluated to check if the thermal behavior of excipients will affect the fusion of the pure active drug in the range of 70 to 79 ^{o}C and 45 to 60 ^{o}C. Physical mixtures were also tested for endotherms and compared to pure ibuprofen. From all the thermograms analyzed, there was no evidence of adverse interference in the range of our study.

When necessary, the fusion parameters (Tm andΔ H) were recalculated from the indium standard using the following equations:

$$K = \frac{\Delta Hf \cdot Mc}{Ac} \qquad \text{equation 1}$$

$$H_S^f = \frac{K \cdot M \cdot As}{Ms} \qquad \text{equation 2}$$

with K: the calibration constant; Mc: the mass of Indium; Ac: the area under the thermogram for the calibrant; As: the peak area of the sample; M: the MW of the sample

Purity Determination:

Using a differential scanning calorimetry method, it was possible to validate the enantiomeric purity of the (+,-S-ibuprofen. This thermal approach has been described in the Thermal Analysis Newsletter #3, and #5 by A.P Gray and edited by Perkin-Elmer, Norwalk Conn. The method consisted of calculating the amount of impurities from the melting point depression

$$\Delta T = To - Tm \qquad \text{equation 3}$$

and using the Van't Hoff equation:

$$To-Tm = RTo^2 \cdot X_2 \quad \Delta H^f \qquad \text{equation 4}$$

with To : Absolute melting temperature of the sample

Tm : the experimental fusion temperature

ΔH : the enthalpy of fusion in cal mole

R : the molar gas constant: $1.987 cal.mole^{-1}.{}^{o}K^{-1}$

X2 : the mole frcation of impurity

The fraction melted can be calcuated from

$$F = (To-Tm)/(To-Ts) \qquad \text{equation 5}$$

and $Ts = To - (Tc-Tm) . 1 F$ equation 6

with Ts: the melting point of the sample for a given fraction melted.

F : Fraction melted

From a DSC scan of the pure indium, the angle α was determined (figure 16) and used in figure 17 to obtain true values of melting. The corresponding fraction melted were calculated.

Ts was plotted against 1 F in figure 18. The intercept To and the slope were derived from the straight line and used in equation 4 to produce the amount of impurities. In (+)-S-ibuprofen, X2 the mole fraction of impurities ranged from 0.0081 to 0.0115. Thus the purity of this compound ranged from 98.85 to 99.18 %. It is not known if the impurities responsible for Lattice deffects might be in part (-)-R-ibuprofen molecules. Enantiomeric purity was also investigated with phase diagrams in manuscript V.

Figure 1: Typical DSC thermogram of Ethyl ibuprofen

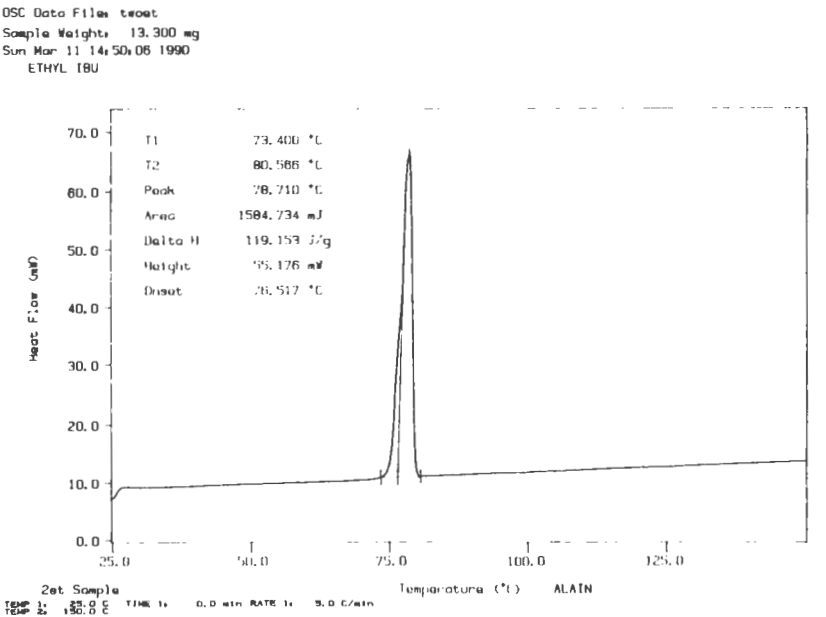


Figure 2: DSC Scan of Polyvinyl Pirrolidone (Povidone) in the temperature range of interest

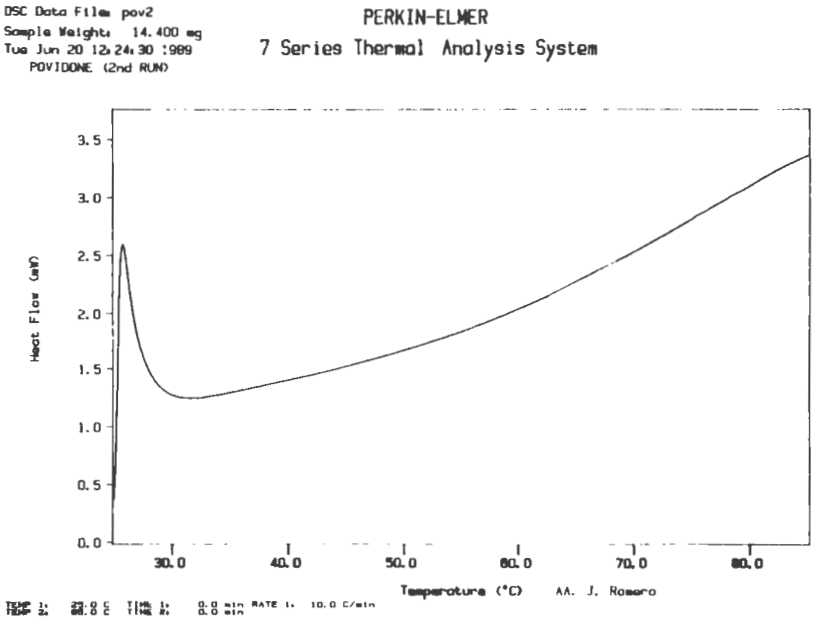


Figure 3: DSC Scan of Na Starch Glycolate (Explotab) in the temperature range of interest

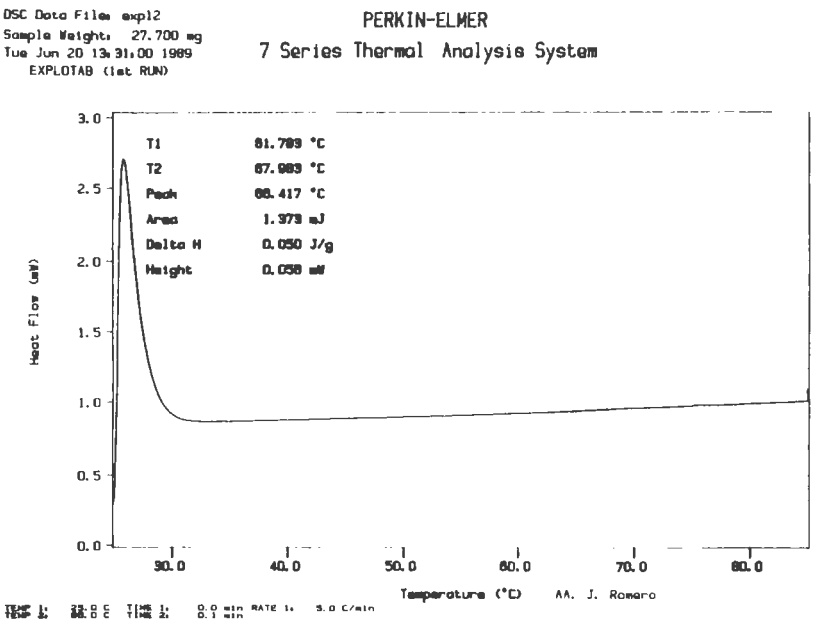


Figure 4: DSC Scan of Lactose (Fast Flow Lactose) in the temperature range of interest

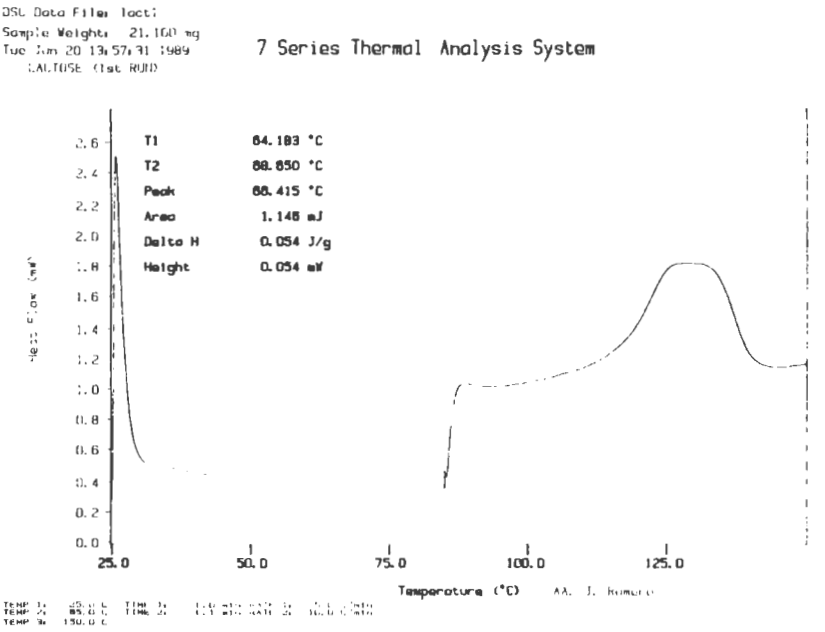


Figure 5: Example of a typical endotherm of Ethyl E131;

An 77% ibuprofen formulation with 1% of

disintegrant 1/3 intrgranular

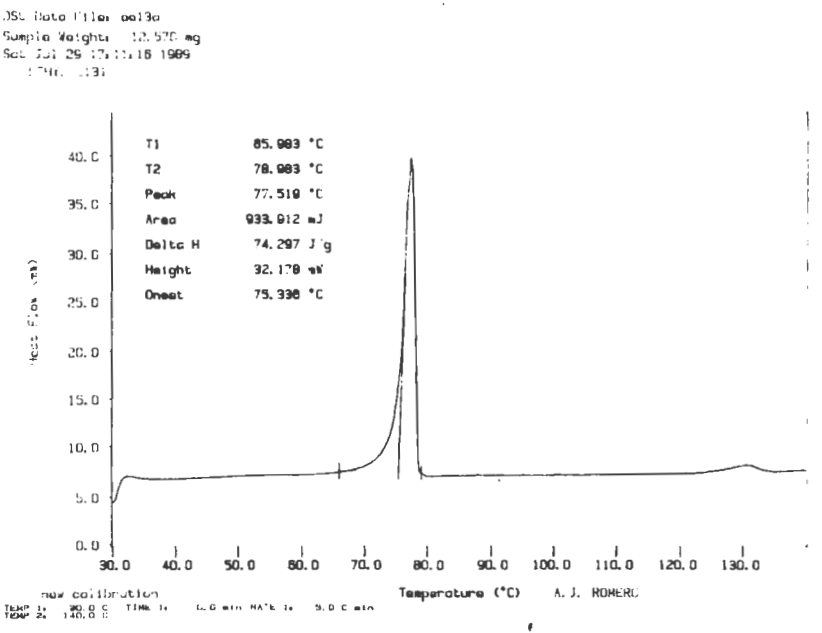


Figure 6: Example of a typical endotherm of a ground ibuprofen tablet obtained at regular compaction

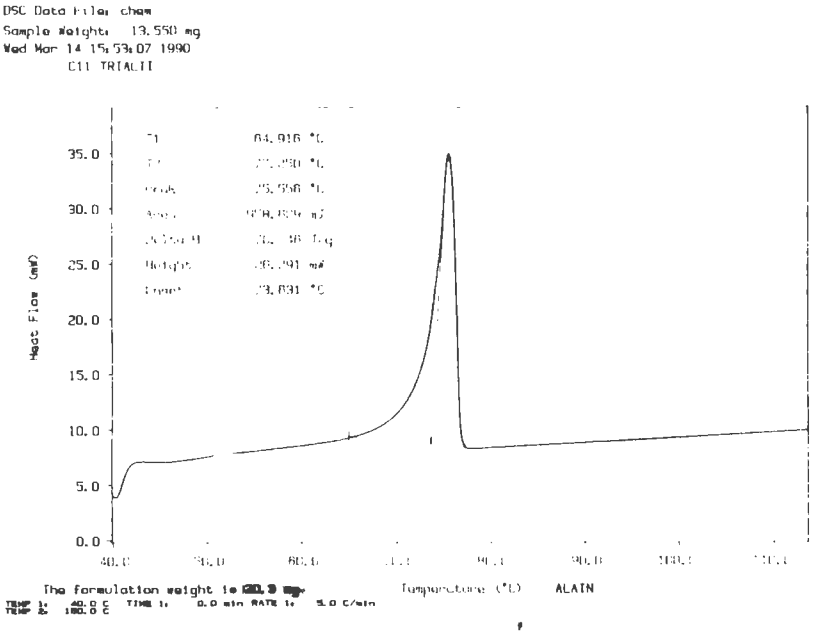


Figure 7: Example of a typical endotherm of a ground physical mixture (before wet granulation)

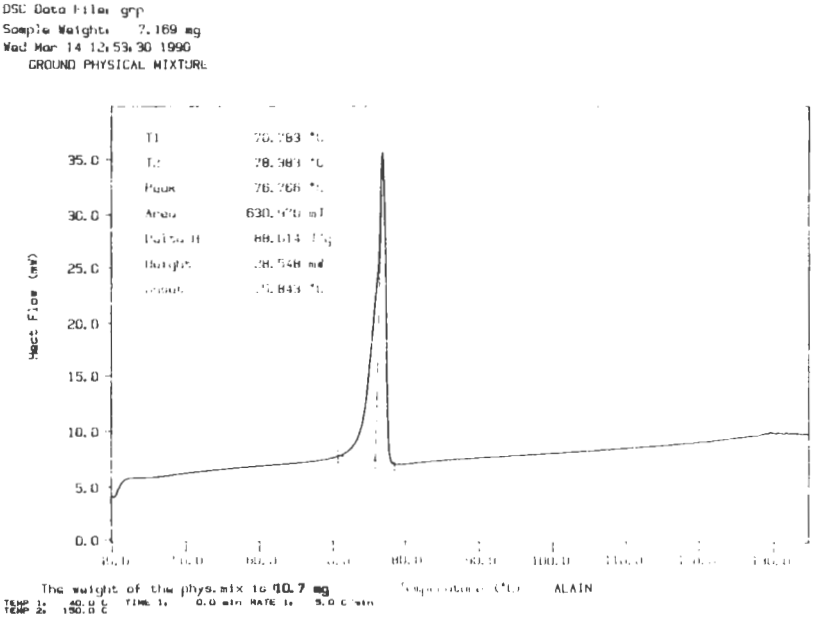


Figure 8: Typical Endotherm of (+)-S-ibuprofen

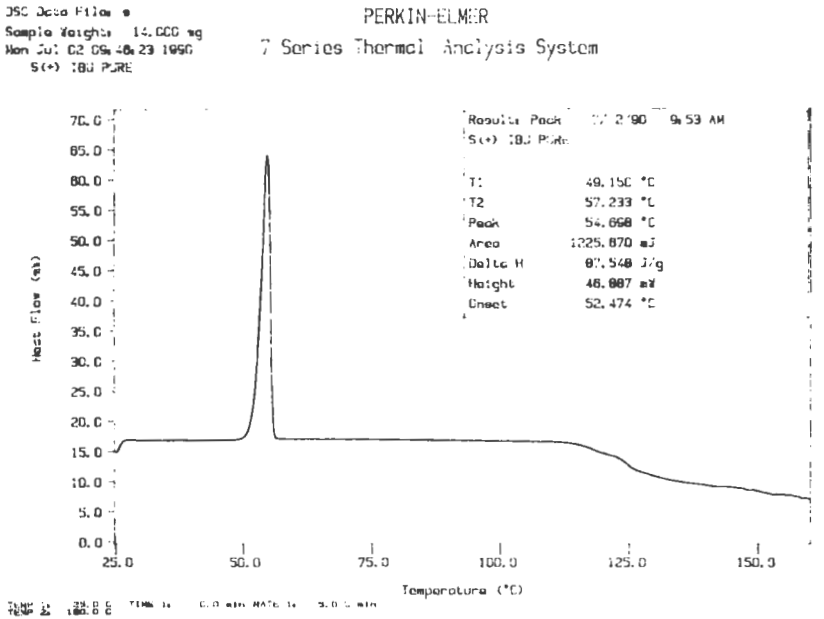


Figure 9: Endotherms of (+)-S-ibuprofen and rac-ibuprofen recrystallized at 4°C from methanol liquors.

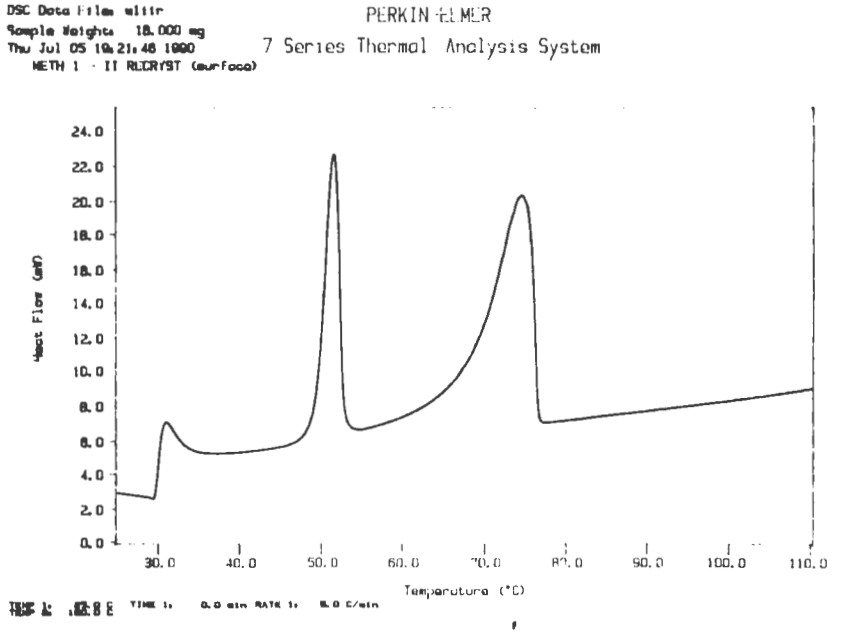


Figure 10: Thermogram of a Sand racemate mixture (25- 75) melted and recrystallized at 4 ^{o}C

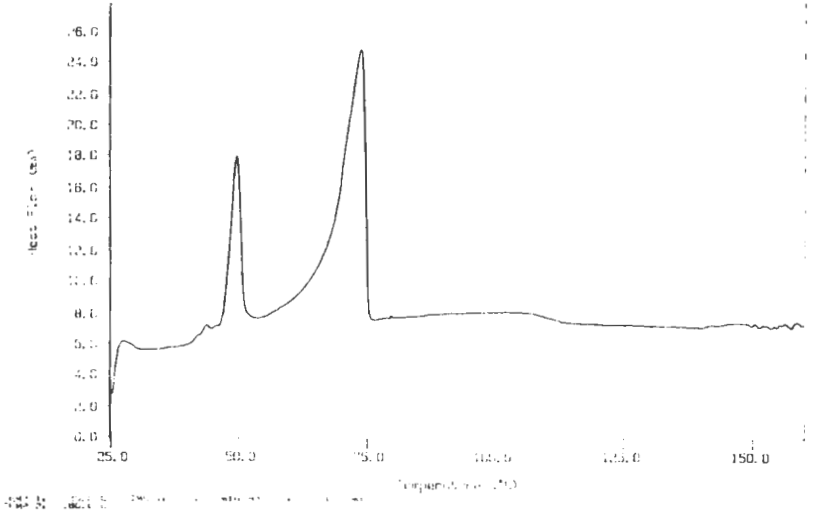


Figure 11: Example of S and racemate physical mixtures

[(75-25%)-24 hours-Labshaker-Room Temperature]

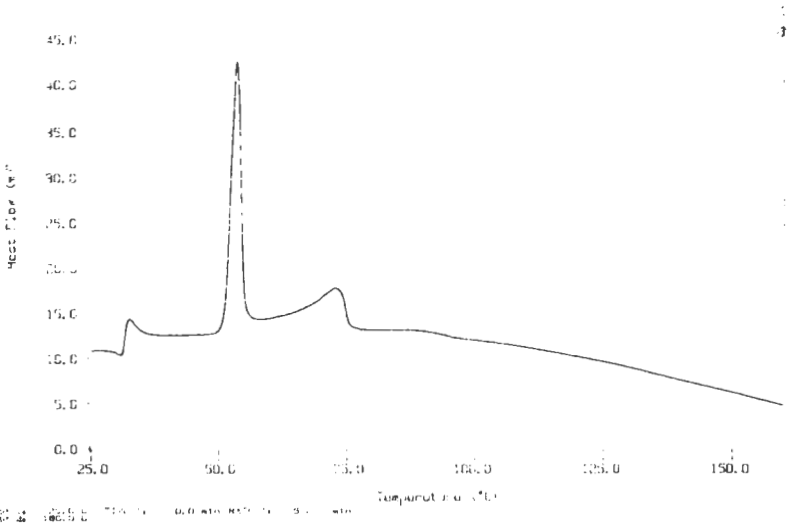


Figure 12: Thermogram of a directly compressible (+)-S-ibuprofen formulation [24 hours of mixing]

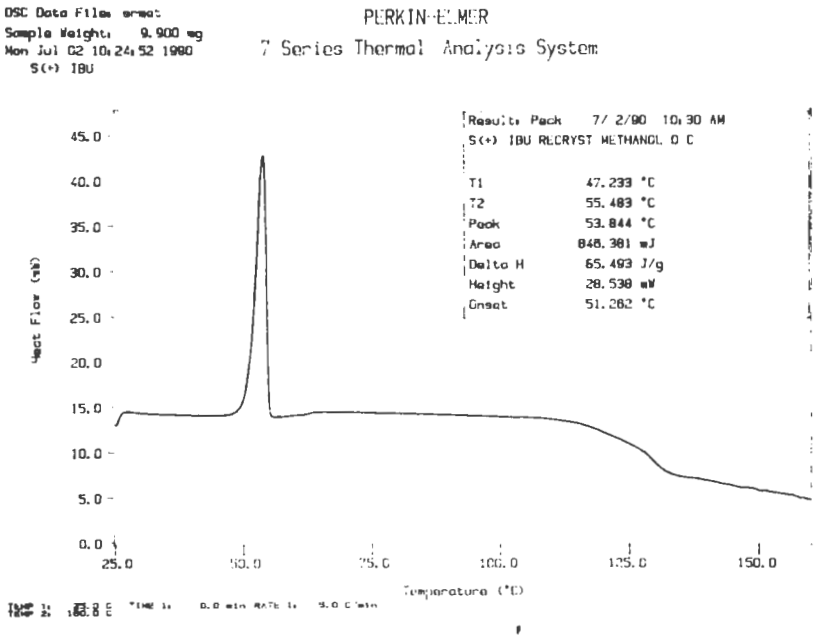


Figure 13: Thermogram of a directly compressible (+)-S- ibuprofen formulation [72 hours of mixing]

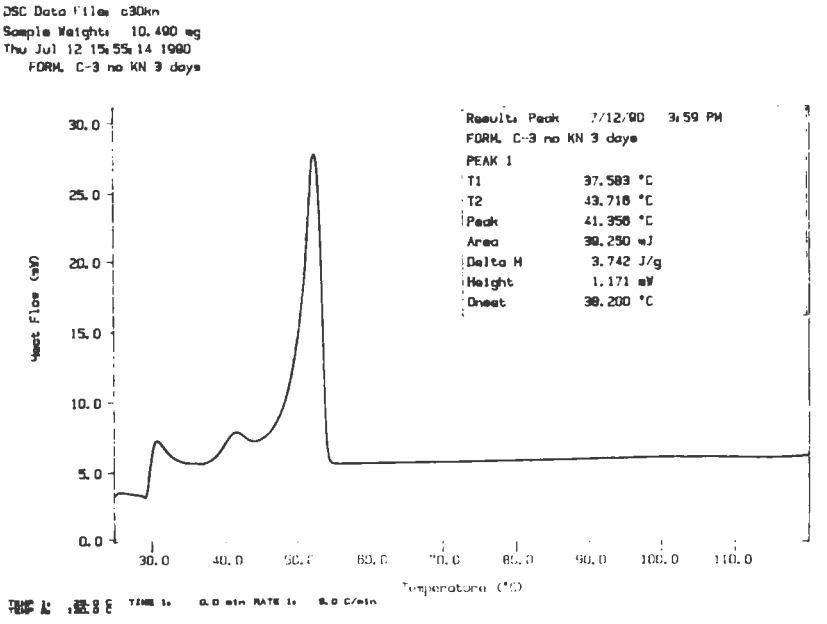


Figure 14: Thermogram of a ground (+)-S-ibuprofen tablet obtained at 600 lbs

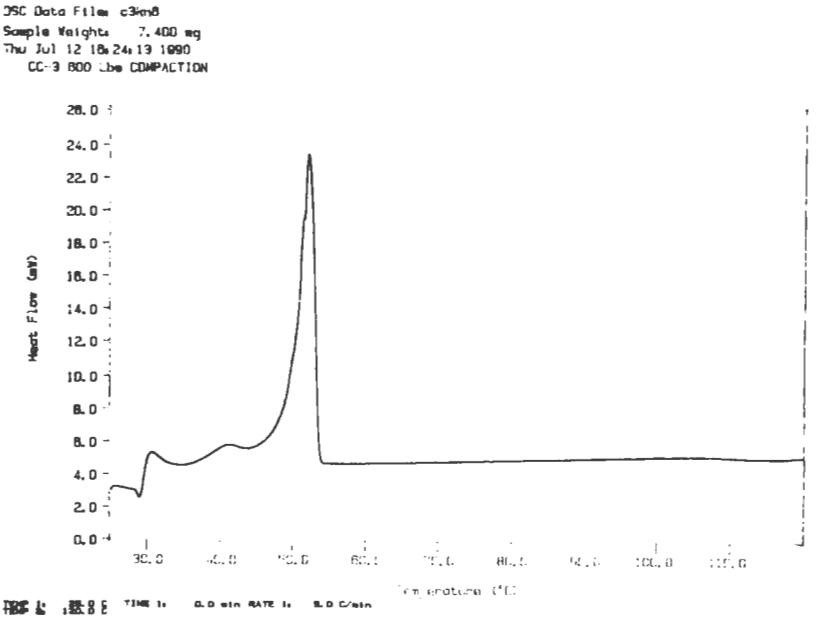


Figure 15: Thermogram of a ground (+)-S-ibuprofen tablet obtained at 1200 lbs

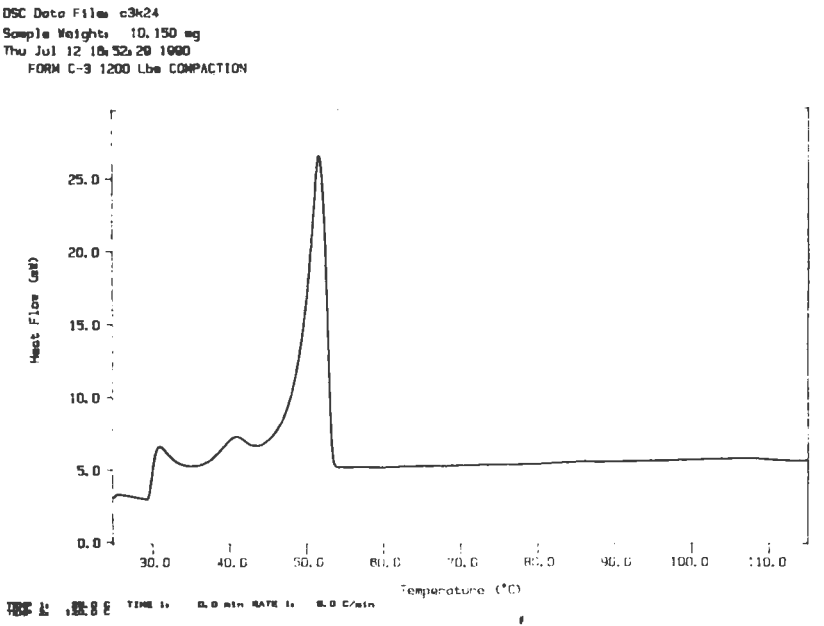


Figure 16: High Purity Indium Melt

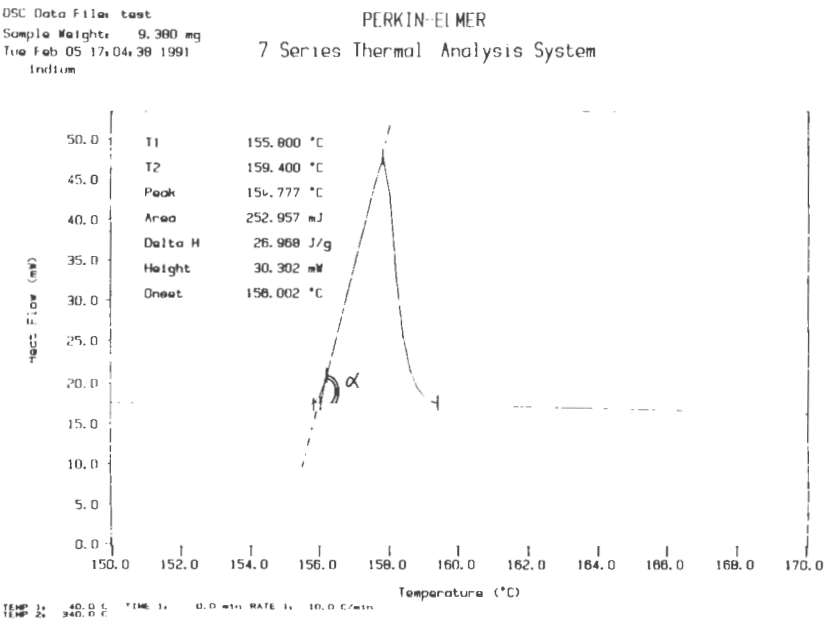


Figure 17: DSC Scan of Ibuprofen

(+)-S-ibuprofen Purity Run

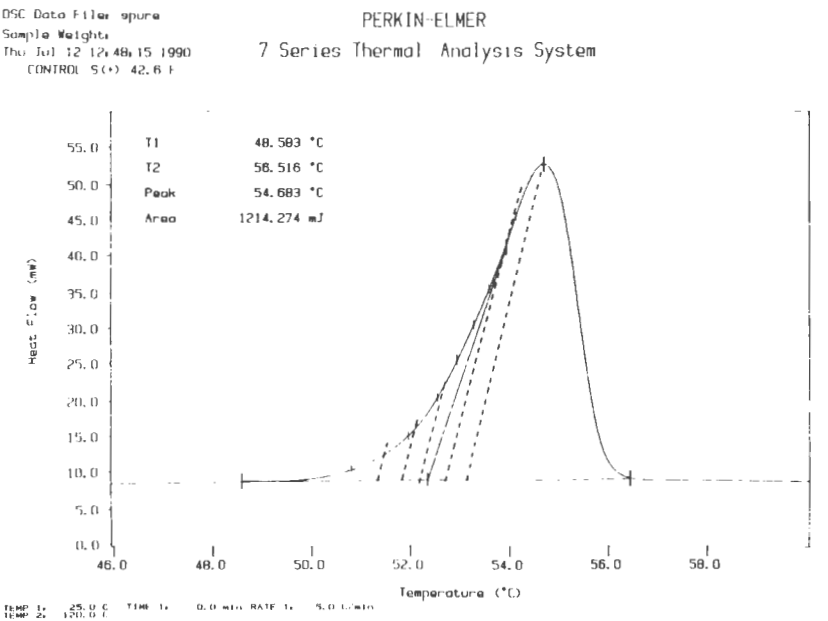
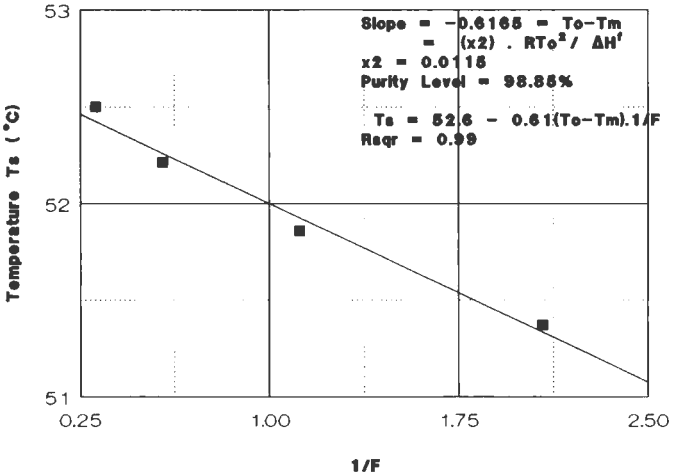


Figure 18
(+)-S-Ibuprofen Purity Determination



APPENDIX D

EXPERIMENTAL COMPLEMENT OF MANUSCRIPT II

In this work other experiments were performed to complement the results. However I found some of it incomplete or not statistically powerful. Therefore, in agreement with the authors it was decided not to incorporate these data in the manuscript for publication. Some of the data is graphically presented in figures 1 and 2 or tabulated (table I).

Figure 1:
Disintegrant Time of IBU formulation

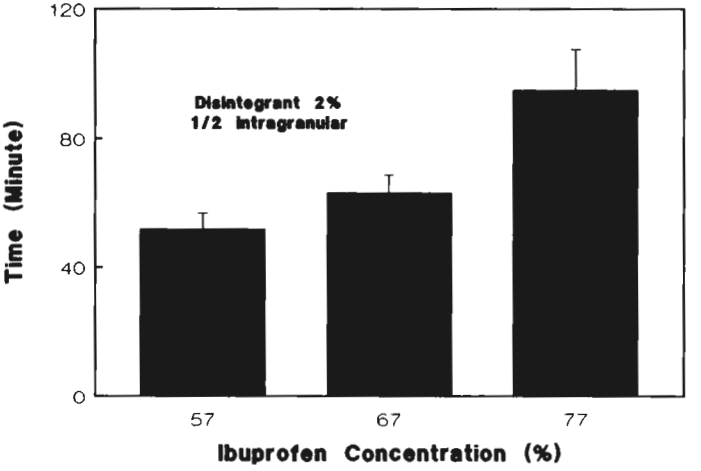


Figure 2: Effect of Compression on Hardness

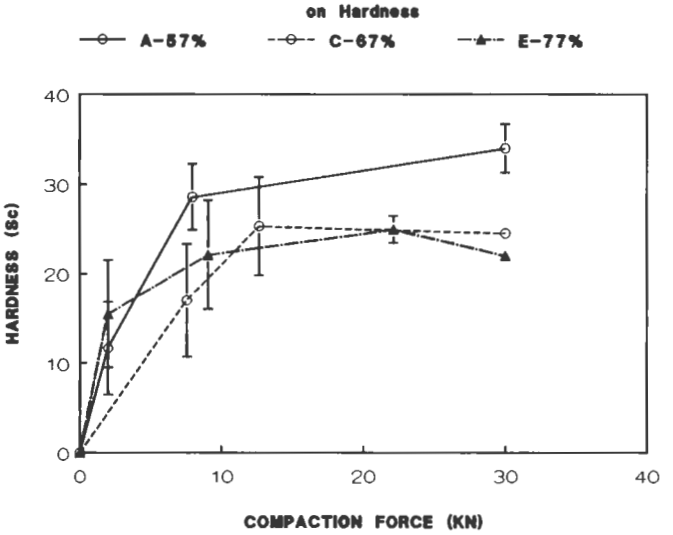


Table I: Pure ibuprofen Compacted: Thermal Analysis

Compaction Level in KN	$\Delta H'$ (J/g)	Tm (°C)
Mean (SD)		
0	127.1	77.5
1(1)	127.8	75.3
8(3)	124.5	74.7
30(3)	125.0	75.5

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