Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Acetylsalicylic Acid

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ABSTRACT: A biowaiver monograph for acetylsalicylic acid (ASA) is presented. Literature and experimental data indicate that ASA is a highly soluble and highly permeable drug, leading to assignment of this active pharmaceutical ingredient (API) to Class I of the Biopharmaceutics Classification System (BCS). Limited bioequivalence (BE) studies reported in the literature indicate that products that have been tested are bioequivalent. Most of the excipients used in products with a marketing authorization in Europe are not considered to have an impact on gastrointestinal motility or permeability. Furthermore, ASA has a wide therapeutic index. Thus, the risks to the patient that might occur if a nonbioequivalent product were to be incorrectly deemed bioequivalent according to the biowaiver procedure appear to be minimal. As a result, the BCS-based biowaiver procedure can be recommended for approval of new formulations of solid oral dosage forms containing ASA as the only API, including both multisource and reformulated products, under the following conditions: (1) excipients are chosen from those used in ASA products already registered in International Conference on Harmonization and associated countries and (2) the dissolution profiles of the test and the comparator products comply with the BE guidance. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:2653–2667, 2012

Keywords: acetylsalicylic acid (ASA); absorption; bioavailability; bioequivalence; Biopharmaceutics Classification System; permeability; solubility; stability

INTRODUCTION

A biowaiver monograph of acetylsalicylic acid (ASA) based on literature data and additional solubility and dissolution experimental data is presented. In biowaiver monographs, the risks of assessing bioequivalence (BE) for a specific active pharmaceutical ingredient (API), including both reformulated products and new multisource drug products, based on *in vitro* rather than *in vivo* studies are evaluated under consideration of the biopharmaceutical and clinical properties of the API in question. On the basis

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of this evaluation, a recommendation is made as to whether a Biopharmaceutics Classification System (BCS)-based biowaiver approval for the new formulation is advisable or not. Guidances for reaching the decision to recommend or advise against a biowaiver decision have been published by the World Health Organization (WHO),[1] the European Medical Agency (EMA),[2] and the US Food and Drug Administration (FDA),[3] and these are taken into consideration in generating biowaiver monographs. Biowaiver monographs have already been published for a variety of APIs and are available online at the website of the International Pharmaceutical Federation (FIP) (URL: http://www.fip.org/www/index.php? id = 642).

This biowaiver monograph pertains to drug products containing ASA as the only API and not to combination drug products.

METHODS

Literature Search

A literature search was performed via Google and PubMed using keywords, including BCS, ASA, polymorphs, hydrate, absolute bioavailability, fraction absorbed (FA), pharmacokinetics, BE, mucosa, and hydrolysis.

Solubility Determination of ASA

In order to determine the solubility of ASA, buffers were prepared at four pH values: a 0.1 N hydrochloric acid (HCl) at pH 1.0, a 50 mM sodium phosphate buffer at pH 3.5 (which is equal to the p*K*a of ASA), a 50 mM sodium acetate buffer at pH 4.5, and a 50 mM sodium phosphate buffer at pH 6.8. Preliminary experiments were carried out to estimate the solubility and, based on these results, an appropriate excess amount of ASA was added to volumetric flasks containing 250 mL of the HCl or buffer solution. The flasks were shaken in an incubator shaker at 37°C. The shaking time was 45 min for the 0.1 N HCl solutions, 20 min for the pH 3.5 buffer solutions, and 15 min for pH 4.5 and 6.8 buffer solutions. The shaking time was selected to ensure that no more than 2% of the dissolved ASA was hydrolyzed to salicylic acid (SA). Triplicate sample preparations were carried out for the solubility testing at all pH levels. The pH values were measured before and after the dissolution of ASA.

For the solubility studies, the following high-performance liquid chromatography (HPLC) method was used to measure the solution concentrations. The system comprised a Phenominex Luna C18 column (Phenomenex, Torrance, California), a mobile phase consisting of methanol and phosphate buffer at pH 2.4, a photo diode array detector, a sample chamber in which the temperature is controlled at 4°C to minimize the hydrolysis of ASA while waiting to be injected, and a 4 min run time for each sample injection. All samples were filtered through a 0.45 μm pore size syringe filter and diluted with solvent as necessary. The method adequately separated the ASA and SA and could precisely and accurately quantitate the amount of both ASA and SA in solutions.[4]

Stability-Indicating Dissolution Test of ASA Tablets

Dissolution of aspirin tablets (batch no: BTA9T21; Bayer Vital GmbH, Leverkusen, Germany) was performed in simulated gastric fluid (SGFsp), pH 1.2, and simulated intestinal fluid (SIFsp), pH 6.8, using a DT 80 paddle apparatus (Erweka, Heusenstamm, Germany). The tablets were tested in 500 mL medium (freshly degassed), maintained at 37 ± 0.5°C and stirred at 75 rpm using United States Pharmacopeia (USP) apparatus 2. Samples were withdrawn at 15 and 30 min when tested in SGFsp and at 15, 30, and 60 min in SIFsp. The withdrawn samples were immediately diluted with acetonitrile (ACN) and analyzed by an HPLC method based on that of Kees et al.[4] An R-18e LichroCART® (125 × 4 mm^2, 5 μm; Merck KGaA, Darmstadt, Germany) column was used as a stationary phase. The mobile phase constituted of water, ACN, and 85% orthophosphoric acid (740:180:0.9). The flow rate was set at 1.8 mL/min and injection volume was 20 μL. ASA and SA were simultaneously detected, and concentrations were calculated from calibration curves of aspirin and SA, which were prepared by diluting stock solutions (prepared in ACN) to obtain concentration ranges of 30–200 μg/mL and 3–20 μg/mL, respectively. Peak areas were measured at 237 nm using an ultraviolet detector and the concentrations of ASA and SA were determined by extrapolating the corresponding peak areas from the respective calibration curves.

GENERAL CHARACTERISTICS

Name: Aspirin (BAN);[5] ASA; 2-acetoxybenzoic acid. The structure is shown in Figure 1.

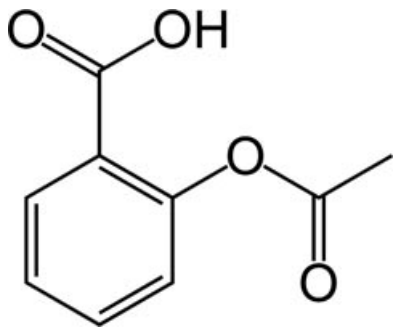

Figure 1. Chemical structure of acetylsalicylic acid.

Chemical formula: $C_9H_8O_4$
Molecular weight: 180.157 g/mol

Therapeutic Indications, Dosing Recommendations and Therapeutic Index

Review of the literature reveals that ASA is one of the most commonly used salicylate drugs. Salicylates belong to the pharmaceutical class of nonsteroidal anti-inflammatory drugs. Furthermore, ASA is used therapeutically as an anticoagulant, fibrinolytic agent, platelet aggregation inhibitor, and cyclooxygenase (COX) inhibitor.

Acetylsalicylic acid has analgesic, anti-inflammatory, and antipyretic properties. It is used in adults for the relief of mild to moderate pain such as headache, dysmenorrhea, myalgias, and dental pain. ASA is rapidly metabolized *in vivo* into the principal active metabolite, SA. ASA is also used in the management of pain and inflammation in acute and chronic rheumatic disorders such as rheumatoid arthritis, juvenile idiopathic arthritis, osteoarthritis, and ankylosing spondylitis. In the treatment of minor febrile conditions, such as colds or influenza, ASA can reduce temperature and relieve headache and joint and muscle pains. The pharmacological activities of ASA and SA in the treatment of pain cannot be segregated from a practical point of view.

The usual oral dose of ASA as an analgesic and antipyretic is 300–1000 mg as a single dose, repeated every 4–8 h according to clinical needs.[6] The maximum daily dose is 3–4 g.

Acetylsalicylic acid is also used for its antiplatelet activity in the initial treatment of cardiovascular disorders such as angina pectoris and myocardial infarction and for the prevention of cardiovascular events in patients at risk, noting that only ASA, and not SA, has an irreversible platelet effect. Other related uses include the treatment and prevention of cerebrovascular disorders such as stroke.

Adverse events observed during treatment with ASA are generally mild in nature and reversible by dose reduction or discontinuation of treatment.[6] They include upper and lower gastrointestinal (GI) tract disorders (dyspepsia, GI, and abdominal pain, etc.), increased risk of bleeding (perioperative hemorrhage, hematoma, epistaxis, etc.), hypersensitivity reactions (asthma syndrome, rash, edema, etc.), transient hepatic impairment with increase in liver transaminase (very rare), dizziness, and tinnitus.[7] Indications for ASA therapy in children are extremely limited because of the risk of Reye's syndrome, but include juvenile idiopathic arthritis and Still's disease.[5]

Ingestion of more than 10 g of ASA may result in intoxication owing to disruption of acid–base homeostasis.[6] Effects of overdose include tinnitus, abdominal pain, hypokalemia, hypoglycemia, pyrexia, hyperventilation, dysrhythmia, hypotension, hallucination, renal failure, confusion, seizure, coma, and even death.[8] A dose of 25–30 g can cause the death of an adult when no countermeasures are taken.[9]

Overall, the therapeutic range of ASA is not considered to be narrow.[10]

PHYSICOCHEMICAL PROPERTIES

Salts, Esters, Polymorphs, and Hydrates

Acetylsalicylic acid is usually given orally as the free acid, but various salts also exist. These include aluminum, calcium, and sodium salts as well as lysine salts, the latter of which are intended for intravenous administration. Furthermore, esters of ASA (alkyl- or aralkyl-substituted ASA such as methyl, ethyl, allyl, or benzyl acetylsalicylate) have been applied for the topical treatment of inflammation. In this monograph, only products containing ASA as the free acid are considered. Hydrates of ASA have not been described in the literature, whereas as many as six polymorphs (obtained by recrystallization from different solvents and temperatures) have been reported.[11–13] The commercial ASA, which demonstrates high dissolution characteristics, consists of crushed, irregular shaped crystals.[11,13] The polymorph of the commercial ASA has not been reported in the open literature.

Partition Coefficient

Log *P* is the *n*-octanol–water partition coefficient: log $P_{ASA} = 1.18$.[14] clog*P* is a partition coefficient calculated for uncharged molecules by using the clog*P* programme from BioByte Corporation (Claremont, California). Kasim et al.[14] reported a ClogP_{ASA} of 1.02, calculated using three different fragmentation methods based on atomic contributions to lipophilicity.

p*K*a

Acetylsalicylic acid is a weak acid and the p*K*a value has variously been reported as 3.49,[8] 3.5,[5,14] and 3.6.[15] Therefore, the solubility of ASA is pH dependent, increasing with increasing pH above the p*K*a.[10]

Solubility

Values for solubility of ASA were obtained from several standard references. The European Pharmacopeia (Ph. Eur.)[16] states that ASA is slightly soluble in water (according to Ph. Eur. General Notices, 100–1000 mL of solvent per gram of solute). The USP[3] specifies its solubility as being one part solute in 300 parts solvent (water). A summary of literature values for the aqueous solubility of ASA is given in Table 1.

Review of the data published on the solubility of ASA reveals that the solubility data vary from study to study, that the sample preparation method was not always appropriate or described clearly, or that the

Table 1. Aqueous Solubility Values for Acetylsalicylic Acid Taken from the Literature

Solubility of Acetylsalicylic Acid in Water	References
Slightly soluble (15°C–25°C) (100–1000 mL of solvent/g of solute)	16
Slightly soluble[a]	14
1 in 300[a]	17
1 in 300[a]	14
1 in 300 (25°C)	18
1 in 100 (37°C)	18
4.6 mg/mL[a]	8
3.33 mg/mL[a]	14
3 mg/mL (25°C)	19
10 mg/mL (37°C)	19

[a]Temperature not specified.

values are irrelevant to BCS because of the study parameters. The hydrolytic degradation of ASA, combined with long and variable incubation times to attain equilibrium, is most likely the reason for the inconsistencies among the solubility data (see also section *Relevance of Conversion to SA for the Biowaiver Decision*).

To clarify the solubility characteristics of ASA in terms of the biowaiver approach, the solubility of ASA in 250 mL of buffer solutions at different pH values at 37°C was further investigated. The solubility data for ASA at 37°C in 250 mL of buffer solutions at different pH levels ($n = 3$) are summarized in Table 2.

Dosage Form Strengths

The WHO Essential Medicines List (EML) gives dosage strengths of 100, 300, and 500 mg for ASA.[20] Dosage strengths of 50, 75, 100, 250, 300, and 500 mg ASA are available in Germany,[21] whereas in the United States, dosage strengths of 81, 162, 325, and 500 mg ASA are available (URL: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm) in oral immediate-release (IR) formulations.

PHARMACOKINETIC PROPERTIES

Permeability and Absorption

In Vitro Data

LogP and ClogP. Kasim et al.[14] attempted to classify the permeability of drugs according to their partition coefficients. They used metoprolol as the reference compound, since 95% of this drug is known to be absorbed from the GI tract ($\log P_{\text{metoprolol}} = 1.72$). Drugs with estimated log*P* values of at least 1.72 were classified as high permeability drugs on this basis. As $\log P_{\text{ASA}}$ is considerably lower than $\log P_{\text{metoprolol}}$, ASA would not be classified as a high-permeability drug according to this method. In addition, drug having a greater Clog*P* than metoprolol ($\text{Clog}P_{\text{metoprolol}} = 1.35$) were classified by Kasim et al.[14] as being high-permeability drugs. As $\text{Clog}P_{\text{ASA}}$ is lower than $\text{Clog}P_{\text{metoprolol}}$, ASA would not be classified as a high-permeability drug according to this method, either.

It is noteworthy that the calculation of partition coefficients is a purely theoretical approach disregarding active transport mechanisms and yielding considerable numbers of false negatives (substrates for carrier-mediated mechanisms)/false positives (substrates for efflux transporters). The ability to correctly classify BCS permeability for estimated log*P* and Clog*P* when compared with experimentally determined human jejunal permeability was found to be only 70% and 66%, respectively.[22] The lack of reliability of these methods is one reason why they are not considered adequate evidence of permeability classification by any of the various regulatory authorities.

Caco-2 Experiments. The *in vitro* determination of permeability through cultured Caco-2 cells is the most commonly used *in vitro* model for drug absorption.

Table 2. Concentrations of Acetylsalicylic Acid Achieved in Buffers at Starting pH Values from 1.0 to 6.8 at 37°C

			Amount Dissolved (mg) in 250 mL[a]			
Medium	Initial pH	Final pH	a	b	c	Average Amount Dissolved (mg) in 250 mL[a]
0.1 N HCl	1.0	1.1	1221	1163	1143	1176
50 mM sodium phosphate buffer	3.5	3.0	1258	1237	1257	1251
50 mM sodium acetate buffer	4.5	3.5	1654	1675	1635	1655
50 mM sodium phosphate buffer	6.8	3.6	1930	1896	1904	1910

[a]At pH 6.8, the concentration achieved does not represent the solubility, but rather ASA was added until it was ensured that at least 1000 mg could dissolve in 250 mL buffer.

Table 3. Apparent Permeability Coefficient (P_{app}) Values for Acetylsalicylic Acid Applied to Caco-2 Monolayers at an Initial Concentration of 1 mM[28]

	$P_{app} \times 10^{-6}$(cm/s) ± SD			$P_{app} \times 10^{-6}$(cm/s) ± SD		
Quality	a − b (absorptive)		Mass Balance	b − a (secretive)		Mass Balance
Sigma (A5376)	2.50	±0.30	98	0.56	±0.02	102

Caco-2 cells are well-differentiated intestinal cells derived from human colorectal carcinoma, which retain many morphological and functional properties of the *in vivo* intestinal epithelial cell barrier.[23] A good correlation between the extent of oral drug absorption in humans and rates of transport across Caco-2 cell monolayers was obtained by Artursson and Karlsson.[24] Drugs that were completely absorbed in humans had permeability coefficients greater than 1×10^{-6} cm/s. Drugs that were absorbed to greater than 1% but less than 100% had permeability coefficients of 0.1–1.0 $\times 10^{-6}$ cm/s, whereas drugs and peptides that were absorbed to less than 1% had permeability coefficients of less than or equal to 1×10^{-7} cm/s. Absorption rate constants, expressed as apparent permeability coefficients (P_{app}), were determined for 20 drugs and peptides with different structural properties. ASA had a P_{app} of 2.4×10^{-6} cm/s, corresponding to complete absorption of an orally administered dose.[24]

Yee[25] also assessed Caco-2 cell monolayers as an *in vitro* tool to predict absorption in man. Excellent correlation was observed between *in vivo* absorption and *in vitro* P_{app} for a variety of compounds encompassing transcellular, paracellular, and carrier-mediated mechanisms. For ASA, Yee[25] reported a P_{app} of 30.67×10^{-6} cm/s and 68% *in vivo* human absorption[26] of nonhydrolyzed ASA.[27] Yee[25] suggested that compounds with P_{app} of less than 1×10^{-6} cm/s can be classified as poorly absorbed drugs (0%–20%), those with coefficients of 1–10 $\times 10^{-6}$ cm/s as moderately absorbed (20%–70%) and those with coefficients of 10×10^{-6} cm/s or more as well (70%–100%) absorbed drugs. According to this classification and the P_{app} value obtained by Yee,[25] ASA would also be classified as a well-absorbed drug.

Supportive information for the high permeability of ASA is given in Table 3 and in Figure 2.

P_{app} values for ASA fall within a range of 2–30 $\times 10^{-6}$ cm/s.[23] This is commensurate with the classification of ASA in the literature as a highly permeable compound.[1,10]

It should be noted that ASA is rapidly hydrolyzed enzymatically to the active metabolite SA. Such hydrolysis occurs almost exclusively after absorption into the enterocytes. Nevertheless, it is worthwhile to mention that SA is also a highly permeable API with a P_{app} of 11.9×10^{-6} cm/s,[24] and therefore the classification of SA as highly permeable is also justified.[10]

In Vivo Data

There is a general consensus that absorption of ASA following oral administration is rapid and complete, based on SA levels in the general

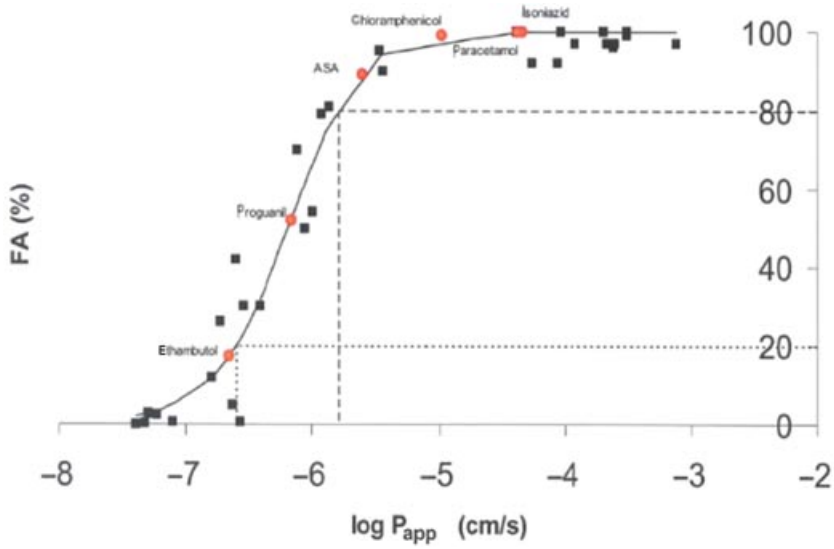

Figure 2. Absorption of APIs in humans after oral administration as a function of apparent absorption coefficients P_{app} plotted logarithmically.[28]

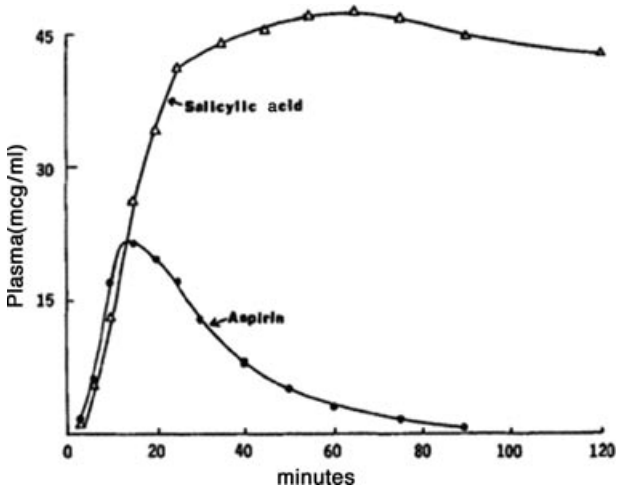

Figure 3. Acetylsalicylic acid (•) and salicylic acid (Δ) plasma levels following ingestion of an oral solution of 650 mg of acetylsalicylic acid (from Rowland 1972).[27]

circulation.[5–6,10,29–33] Ritschel[33] listed the FA of ASA as 1.0; others reported essentially complete absorption of ASA based on salicylate.[27]

After oral administration, only about 10% of a predissolved 250 mg dose of ASA was absorbed from an acidic solution in the stomach.[34] This can be attributed at least partly to the limited absorption surface of the stomach mucosa (0.2–0.3 m^2). In the small intestine, where the surface area is much larger (~200 m^2), ASA absorption is more extensive.[35] Needs[31] denoted the absorption process of ASA from the small intestine as a passive diffusion process.

The absolute bioavailability of ASA following oral administration is far from complete because of the high extent of metabolism in the gut wall by unspecific esterases and in the liver,[9] with the liver being the major site of first-pass metabolism.[35–36] Catalyzed hydrolysis is also possible after reaching the systemic circulation via esterases in plasma, erythrocytes, and synovial fluid.[36] As a result, most studies reported to date have expressed the bioavailability of ASA in terms of SA.[36]

There are relatively few studies on the bioavailability of ASA as such. Plasma concentration versus time profiles recorded for ASA reveal that peak concentrations of the parent drug ASA occur approximately 20–25 min after ingestion as an oral solution and that plasma concentrations decline rapidly after achieving peak values as plasma SA concentrations increase (see Fig. 3).[27]

Following oral administration of an aqueous solution of ASA, the absorption kinetics of ASA in man was found to follow a first-order process. In this study,[27] in which ASA and SA were simultaneously detected, 68% of the ASA dose reached the systemic circulation unhydrolyzed. The remainder of the dose was considered to have been metabolized during passage to the systemic circulation by esterases within the gut wall, plasma, or liver.[27]

In another study, in which ASA was administered intravenously and orally as capsules, about 50% of the oral dose reached the systemic circulation as unhydrolyzed ASA.[9,36]

Completeness of the absorption of ASA following oral administration can also be deduced, for example, from results obtained administering [carboxyl-^{14}C] ASA[30] and assaying salicylates in the urine over 48 h. The results published reveal that ^{14}C radioactivity was almost completely (94%–98%) and rapidly eliminated in the urine within the first 24 h (only about 1% 24–48 h postdosing) and that after administration of 1000 mg ASA, more than 900 mg salicylate (expressed as mg ASA) was excreted in urine over 48 h. These results are supportive of a high permeability of ASA.

Distribution

Acetylsalicylic acid is rapidly distributed into most body tissues and fluids. The volume of distribution of ASA is approximately the same as that of SA and is generally 0.15–0.2 L/kg in adults.[5] ASA is bound to plasma proteins to an extent of only 33% at a serum SA concentration of 120 μg/mL.

Metabolism and Excretion

The pharmacokinetics of ASA has been found to be linear over the dose range 30–400 mg. In contrast, the pharmacokinetics of SA is nonlinear owing to saturable metabolic and renal elimination routes.[37]

The elimination half-life of ASA in plasma is approximately 15–20 min. Only about 1% of an oral dose of ASA is excreted unchanged in urine. The remainder is excreted in urine as SA and its metabolites.[36] The enzymatic hydrolysis of ASA to SA has been discussed in detail in the section covering absorption. As SA like ASA inhibits COX, and thus exerts analgesic and antipyretic effects, SA is not only the primary metabolite of ASA but also contributes to the analgesic and antipyretic effects. Therefore, some discussion of the metabolism of SA is also warranted.

Salicylic acid is mainly eliminated by hepatic metabolism; the metabolites include salicyluric acid, salicyl phenolic glucuronide, SA glucuronide, gentisic acid, and gentisuric acid (Fig. 4). Although most of these metabolic routes are first-order processes, the formation of the major SA metabolites, salicyluric acid and salicyl phenolic glucuronide, is easily saturated and follows Michaelis–Menten kinetics. As a result, steady-state plasma salicylate concentrations increase disproportionately with dose.[5,37] After a 325 mg ASA dose, elimination is a first-order process and the plasma SA elimination half-life is about 2–3 h. But at high ASA doses, the half-life of SA can

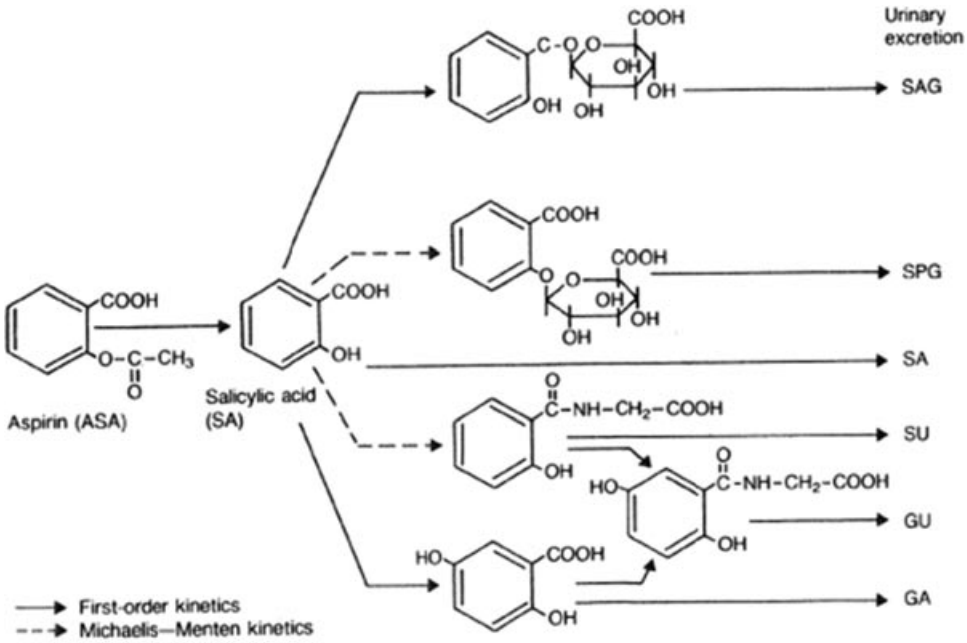

Figure 4. Metabolism of acetylsalicylic acid/salicylic acid metabolism.[31] SAG, salicyl acyl glucuronide; SPG, salicyl phenolic glucuronide; SU, salicyluric acid; GA, gentisic acid; GU, gentisuric acid.

increase to 15–30 h because of the capacity-limited metabolism of SA.

Besides metabolic elimination, SA is also excreted unchanged in the urine; the amount excreted by this route increases with increasing dose and also depends on urinary pH, about 30% of a dose being excreted in alkaline urine compared with 2% of a dose in acidic urine. Renal excretion involves glomerular filtration, active renal tubular secretion, and passive tubular reabsorption.[5]

DOSAGE FORM PERFORMANCE

Bioequivalence

Only three publications in the open literature reporting results from a comparison of IR ASA formulations have been identified.

Levy[38] investigated the relationship between *in vitro* dissolution rate and GI absorption rate of five commercially available brands of aspirin tablets.[38] The products included one brand of buffered aspirin, one brand containing calcium acetylsalicylate, and three brands of plain ASA tablets. The dissolution rates of these brands were determined in 0.1 N HCl at 37°C under standardized agitation conditions. *in vivo* urinary excretion data from 24 healthy adult volunteers obtained via a Latin square cross-over type study design were evaluated. Although the products were not evaluated according to the current BE criteria, the three plain ASA tablets were found to be statistically comparable. In addition, the study also demonstrated the dependence of absorption rate on the dissolution rate of ASA.[38]

Single-dose pharmacokinetics of four commercially available low dose (100 mg) oral ASA formulations were studied in six healthy men and six healthy women.[39] Two formulations were rapid release formulations (Cardiprin 100, Platelin) and the other two were enteric-coated formulations (Astrix 100, Cartia). Only the results for the two rapid release formulations will be considered in the following discussion, since enteric-coated products cannot be approved by the biowaiver approach.

The study used a randomized single-dose cross-over design with a washout period of 4–14 days between each dose. There were no significant differences in the mean time to maximum ASA concentrations between the rapid release formulations (0.48 h Cardiprin 100, 0.35 h Platelin). Likewise, the areas under the plasma ASA concentration–time curves were similar for the two rapid release products: Cardiprin 100 [1.60 mg/(h L)] and Platelin [1.54 mg/(h L)].[39] Cardiprin contains as excipients glycine, microcrystalline cellulose, sterilized talc, saccharin, saccharin sodium, corn starch, flavor. The excipients in Platelin were not reported.

Biovailability of ASA and SA from rapid- and slow-release formulations was also studied by Brantmark et al.[40] Single-dose concentration profiles of ASA and SA were studied in six healthy volunteers following intake of rapid release (1 G MgO-buffered ASA; sodium bicarbonate–citrate-buffered ASA). Within the rapid-release formulations, the area under the ASA plasma concentration–time curve

Table 4. Excipients* Present in Acetylsalicylic Acid IR Solid Oral Drug Products** with a Marketing Authorization (MA) in Belgium (BE), Canada (CA), Czech Republic (CZ), Germany (DE), Denmark (DK), Spain (ES), Finland (FI), France (FR), Greece (GR), Hungary (HU), Ireland (IE), the Netherlands (NL), Norway (NO), Portugal (PT), Romania (RO), Sweden (SE), Slovakia (SK), United Kingdom (UK), and the United States (US)***, and the Minimal and Maximal Amount of That Excipient Present Pro Dosage Unit in Solid Oral Drug Products with a MA in the US****

Excipient	Drug Products Containing That Excipient with a MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms with a MA in the US (mg)
Acacia	ES[1]	5–156
Ascorbic acid	PT[2]	7–28
Aluminum glycinate	FI[3,4]	–
Calcium carbonate	CZ[5–7], RO[8,9], SE[10], SK[11–13]	8.6–350
Calcium gluconate	HU[14]	–
Calcium hydrogen phosphate	US[15–24]	104–850
Carnauba wax	CA[25], US[26]	0.1–58
Cellulose, microcrystalline	BE[27], CA[28], CZ[6], DE[29–40], DK[41–45], ES[46], FI[3,4,47], NL[48–51], PT[2,52–54], RO[55–57], SE[58,59], SK[13], US[60–64]	4.6–1385
Cellulose, powdered	BE[65], CA[25,66,67], CZ[68], DE[31,33,34,36,37,39,69–71], DK[72], ES[73–76], FI[47,77], FR[78], HU[79,80], NL[48,81,82], NO[83], PT[54,84], RO[85], SK[86]	44–170
Citric acid	SE[10]	1–78
Cottonseed oil, hydrogenated	FI[3]	0.6–34
Croscarmellose sodium	CA[28], FI[3,4], RO[57], US[19,60–64]	2–180
Ethylcellulose	ES[87]	1.0–121
Gelatin	DE[29], SE[59,88,89]	1–756
Glycerol dibehenate	FR[90]	2.5–14
Glyceryl palmitostearate	NL[51]	18
Glycine	CZ[5–7], DE[71], SK[12,13]	3.6–163
Hypromellose	CA[25,66,67], DK[43,44], US[15–18,20–24,26,60,63,64,91–94]	0.8–537
Lactose	DE[29], FR[90], NL[49,50], NO[95]	23–1020
Macrogols	HU[14], US[19,91–94]	0.12–961
Magnesium hydroxide	DK[41–45], SE[89]	40–43
Magnesium oxide	FI[3,4], SE[59,88]	10–40
Magnesium stearate	DK[41–45], HU[14], NL[51], RO[8,9]	0.15–401
Mannitol	ES[1], PT[2,52]	33–992
Poly(vinylalcohol)	US[19]	0.7–20
Povidone	RO[96–98], SE[10]	0.17–80
Propylene glycol	CA[25], DK[43,44], US[91–94]	1.5–148
Shellac	CA[25]	4.4–25
Silica	BE[27], FI[3,4], FR[99], GR[109], HU[14], NL[51,100], RO[8,9,56,98], SE[10,59,88], US[19,26]	0.5–100
Sodium carbonate	US[26]	4.9–25
Sodium laurilsulfate	FI[3,4], FR[99], GR[109], NL[100]	0.65–52
Sodium starch glycolate	DE[29], PT[101]	2–876
Starch	BE[65], CA[25,66,67], CZ[5–7,68,102], DE[29–40,69–71], DK[41–45,72], ES[1,73–76,87], FI[47,77], FR[78,99,103], HU[14,79,80], GR[109], NL[48–51,81,82,100,104], NO[83,95], PT[2,53,54,84,101], RO[8,9,55,85,96–98], SE[10,58,59,88,89], SK[11–13,86,105], UK[106,107], US[15–22,60–64,91–94,108]	0.44–1135
Starch, pregelatinized	BE[27], ES[46]	5.0–600
Stearic acid	BE[27], DE[30,35,40], FI[3,4], PT[2]	0.9–72
Talc	CZ[5–7,102], DK[43,44], ES[46], FI[3,4], FR[103], HU[14], NL[104], NO[95], RO[8,9,55–57,98], SE[59,88,89], SK[11–13,105], US[15–24]	0.10–220
Triacetin	CA[25,66,67], US[15–18,20–24]	0.72–15
Vegetable oil, hydrogenated	FI[4]	2–261
Zinc stearate	US[26]	2–10

*Colorants, flavors, water, and ingredients present in the coating are not included.

**Excluded are: soft gelatin capsules filled with a solution, soluble tablets, effervescent tablets, dispersible tablets, chewable tablets, enteric-coated tablets, oral powders, oral granulates, oral suspension, powder for oral solution.

***Sources of data: BE, http://www.bcfi.be/(accessed 30 November, 2011); CA, www.hc-sc.gc.ca (accessed 28 November, 2011); CZ, www.sukl.cz/(accessed 09 November, 2011; DE, www.rote-liste.de (accessed 28 November, 2011); DK, www.dkma.dk (accessed 09 November, 2011); ES, www.aemps.es (accessed 29 November, 2011); FI, www.nam.fi (accessed 29 November, 2011); FR, www.vidal.fr/(accessed 29 November, 2011); HU, www.ogyi.hu (accessed 29 November, 2011); IE, www.imb.ie/(accessed 29 November, 2011); NL, www.cbg-meb.nl (accessed 29 November, 2011); NO, www.legemiddelverket.no/(accessed 29 November, 2011); PT, http://www.infarmed.pt/infomed/(accessed 09 November, 2011); RO, http://www.anm.ro/(accessed 29 November, 2011); SE, www.lakemedelsverket.se (accessed 29 November, 2011); SK, http://www.sukl.sk (accessed 29 November, 2011); UK, www.medicines.org.uk/emc/(accessed 30 November, 2011); US, www.dailymed.nlm.nih.gov (accessed 30 November, 2011); GR, http://www.eof.gr/web/guest/search (accessed 18 April, 2012).

****US: FDA's Inactive Ingredient Database, http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm (version date October 12, 2011)

(Continued)

Table 4. Continued

[1]AAS 100/-500 mg comprimidos.
[2]A-A-S 150, 150 mg, comprimidos.
[3]Disperin 500 mg tabletti.
[4]Disperin 50/-100 mg tabletti.
[5]Anopyrin 100 mg.
[6]Anopyrin 30 mg.
[7]Anopyrin 400 mg.
[8]Acid Acetilsalicilic T Sanosan 100 mg, comprimate.
[9]Acid Acetilsalicilic T Biofarm 500 mg, comprimate.
[10]Acetylsalicylsyra Ellem 500 mg tabletter.
[11]Anopyrin®.
[12]ANOPYRIN 100 mg.
[13]ANOPYRIN 30 mg.
[14]Kalmopyrin 500 mg tabletta.
[15]ASPIRIN tablet (CVS Pharmacy).
[16]ASPIRIN tablet (Hannaford Brothers Company).
[17]ASPIRIN tablet (Kroger Company).
[18]ASPIRIN tablet (Meijer Distribution Inc.).
[19]ASPIRIN tablet, film coated (Himprit Pharmachem Pvt. Ltd.).
[20]Berkley and Jensen aspirin (aspirin) tablet (BJWC).
[21]Care one aspirin (aspirin) tablet (American Sales Company).
[22]Equaline aspirin (aspirin) tablet (Supervalu Inc.).
[23]Equate aspirin (aspirin) tablet (Wal-Mart Stores Inc.).
[24]Good sense aspirin (aspirin) tablet (L Perrigo Company).
[25]Aspirin® Tablets Extra Strength (acetylsalicylic acid tablets, USP) 500 mg.
[26]Bayer (aspirin) tablet (Bayer Corporation Consumer Care Division).
[27]Asa Sandoz 100 mg tabletten.
[28]pms-ASA 325 mg tablets (acetylsalicylic acid tablets, USP).
[29]Acesal® tabletten.
[30]Acesal® 250 mg tabletten.
[31]ASS AL 100 TAH tabletten.
[32]ASS AL 500 tabletten.
[33]ASS-CT 50 mg/-100/-500 mg TAH tabletten.
[34]ASS-CT 50 mg/-100 mg TAH tabletten.
[35]ASS gamma® 75 mg Infarktschutz Tabletten.
[36]ASS-ratiopharm 100 TAH Tabletten.
[37]ASS-ratiopharm® 300 mg/-500 mg Tabletten.
[38]ASS STADA® 500 mg Tabletten.
[39]HerzASS-ratiopharm® 50 mg/-100 mg Tabletten.
[40]Togal® ASS 400 mg Tabletten
[41]Hjerdyl, tabletter 75/-150 mg.
[42]Hjertemin, tabletter 75/-150 mg.
[43]Magnyl Svage "DAK," filmovertrukne tabletter.
[44]Hjertemagnyl, filmovertrukne tabletter 75/-150 mg.
[45]Magnyl "DAK", tabletter.
[46]BIOPLAK 125/-250 mg.
[47]ASA-ratiopharm 500 mg tabletti.
[48]Acetylsalicylzuur-ratiopharm 500 mg, tabletten [1].
[49]Acetylsalicylzuur Apotex cardio 80 mg, tabletten.
[50]Acetylsalicylzuur Apotex neuro 30 mg, tabletten.
[51]Acetylsalicylzuur 500 PCH.
[52]ASP 100 mg comprimidos.
[53]ACTIPIRIL 500 mg comprimidos.
[54]Ácido Acetilsalicílico ratiopharm, 100/-500 mg comprimidos.
[55]Acid Acetilsalicilic 500 mg, comprimate (S.C. MAGISTRA C & C SRL).
[56] Acid acetilsalicilic 500 mg (S.C. SICOMED S.A.).
[57]ASAprin 500 mg.
[58]Aspirin 500 mg tabletter.
[59]Trombyl 75/-160 mg tablett.
[60]Aspirin tablet (Cardinal Health).
[61]Aspirin tablet (PureTek Corporation).
[62]Aspirin tablet (Winder Laboratories, LLC).
[63]ASPIRIN tablet, film coated (Moore Medical LLC)[1].
[64]Conney aspirin (aspirin) tablet, film coated (Conney Safety Products, LLC).
[65]Aspirine 100/-300/-500, 100/-300/-500 mg, tabletten
[66]Aspirin® (acetylsalicylic acid tablets, USP) 325 mg.
[67]Aspirin® Caplets (acetylsalicylic acid 325 mg).
[68]Aspirin /-100.
[69]Aspirin® Tabletten.
[70]Aspirin® N 100 mg/-300 mg Tabletten.
[71]Godamed® 50 mg/-100/-300 mg ASS TAH Tabletten.
[72]Aspirin, tabletter.
[73]Acido acetilsalicilico bayfarma 100/-300 mg comprimidos recubiertos
[74]ADIRO 100/-300.
[75]ASPIRINA 500 mg Comprimidos (Bayer Hellas ABEE).
[76]ASPIRINA 500 mg comprimidos (Bayer Hispania, S.L.).
[77]ASPIRIN 500 mg tabletti.
[78]Aspirine du rhone 500 mg cp.

(Continued)

Table 4. Continued

[79]Aspirin 100/-500 mg tabletta (Bayer Hungária Kft.).
[80]Aspirin 500 tabletta (EU Pharma Kft.).
[81]Aspirine 500 mg, tabletten 500 mg.
[82]Aspirine 100, tabletten 100 mg.
[83]Aspirin 500 mg tabletter.
[84]Aspirina 500 mg comprimidos.
[85]Acid acetilsalicilic 500 mg, comprimate (Ozone Laboratories BV).
[86]Aspirin /-100.
[87]Rhonal 500 mg comprimidos.
[88]Albyl minor 250 mg tabletter.
[89]Magnecyl 500 mg tabletter.
[90]Aspirine UPSA 325 mg gél.
[91]Aspirin (aspirin) tablet (Genuine First Aid LLC).
[92]ASPIRIN tablet, film coated (Moore Medical LLC)[2].
[93]Conney aspirin (aspirin) tablet, film coated (Unifirst First Aid Corporation).
[94]Genuine aspirin (aspirin) tablet (Woonsocket Prescription Center, Incorporated].
[95]Globoid.
[96]Acid Acetilsalicilic.
[97]Acid Acetilsalicilic Biofarm 500 mg, comprimate.
[98]ASPIMAX.
[99]ASPRO 320/-500 mg cp.
[100]ASPRO 320, tabletten 320 mg.
[101]A-A-S 500 mg comprimidos.
[102]ACYLPYRIN [1].
[103]ASPIRINE RICHARD 500 mg cp.
[104]Acetylsalicylzuur ratiopharm 500 mg, tabletten [2].
[105]ACYLPYRIN [2].
[106]Aspirin Caplets/Tablets 300 mg (Boots Company Plc.).
[107]Aspirin tablets BP 300mg (Actavis UK Ltd.).
[108]Aspirin tablet (A&Z Pharmaceutical, Inc.).
[109]Salospir 500mg (Uni Pharma Pharmaceutical Laboratories).

and the peak ASA concentrations were essentially equal.[40]

The limited reports on comparative bioavailability studies in the open literature show that plasma concentration versus time profiles of ASA for solid IR ASA formulations are very similar irrespective of the formulation administered. However, the study design and statistical methods used in these studies may not meet all current requirements for proof of BE.

Excipients

General Aspects

Review of the ASA formulations in the German Red List reveals that these formulations contain microcrystalline cellulose, sodium carboxymethylcellulose, lactose monohydrate, potato starch, corn starch, crospovidone, gelatine, stearic acid, aluminum hydroxide distearate, aerosil (colloidal anhydrous silica), citric aroma, glycine, and sodium saccharin.[21]

A complete list of excipients used in solid oral dosage forms of ASA in Europe, United States, and Canada is compiled in Table 4. Only products that contain ASA as the only active ingredient have been included in the list. Excluded from the list were some IR formulations in which ASA is coformulated with ascorbic acid or calcium gluconate and these are also regarded by the manufacturer as active ingredients. In addition, formulations containing aspirin in "buffered form" were excluded. For the products listed in Table 4, potato starch, maize starch, and wheat starch were all included under the general excipient description of "starch" and no distinction was made between them. However, pregelatinized starch and starch were considered as separate entities.

In Ireland and Japan, no IR product containing ASA as the single active ingredient was found.

There are no indications in the literature that microcrystalline cellulose, cellulose powder, sodium carboxymethylcellulose, crospovidone, potato starch, or corn starch has any impact on either motility or permeability. These are also the most often used excipients in IR oral solid drug products containing ASA.

It is conceivable that high levels of stearic acid, aluminum hydroxide distearate, or Aerosil could prolong dissolution of ASA from the tablet; however, such an effect would be detectable with the biowaiver dissolution test procedure.

Lactose in high amounts could be problematic for patients with lactose intolerance. However, since ASA is a high dose API, the content of lactose in the dosage form (maximum possible is $\sim$ 200 mg/unit in the products listed in Table 4) is too low to have any significant adverse effect in the patients.

DISCUSSION

Solubility

The solubility of the API has to fulfill the requirement that the highest single dose administered (EMA) or highest dose strength (WHO and FDA) dissolves in 250 mL over the pH range 1–7.5 (FDA) or over the pH range 1.2–6.8 at 37 $\pm$ 1°C (WHO, EMA) in order to qualify for a biowaiver. In the European Union (EU), the highest single dose administered as IR

formulation(s) has to meet all requirements for a BCS-based biowaiver as laid down in Appendix III of the EMA Guidance.[2] In the United States, however, the relevant guideline[3] notes that the solubility class boundary is based on the highest dose strength (i.e., 500 mg) of an IR product instead of the highest single dose approach as considered in the EU. Comparing the current provisions in force in the EU and the United States, it is evident that classification as highly soluble in line with the EU requirements will be accepted by the FDA as well (unless there is a pronounced drop in solubility of the API between pH 6.8 and 7.5), but not necessarily vice versa.

As the maximal single dose used in the indications claimed for ASA formulations is 1000 mg (usually given as two 500 mg tablets), the following assessment will focus on the question whether ASA can be classified as highly soluble, given that a single dose of up to 1000 mg is recommended for administration.

Kasim et al.[14] compiled solubility data for WHO Essential Drugs, including ASA. Solubility classification was based on the calculated dimensionless dose number (Do), the ratio of drug concentration in the administered volume to the saturation solubility of the drug in unbuffered water. Drugs were categorized as "highly soluble" if they had a Do $\leq$ 1. At a dose strength of 500 mg of ASA (dose given in the WHO EML) the Do is 0.601 (for $C_S = 3.33$ mg/mL),[20] whereas the highest therapeutically used single dose of 1000 mg of ASA gives a Do of 1.2, slightly exceeding the criterion for designation as a highly soluble drug. However, it is not clear that the solubilities were determined at 37°C and in any case they do not cover the entire biowaiver range of pH 1.2–6.8.

A further problem with determining the true equilibrium aqueous solubility of ASA lies in its hydrolysis to SA over the course of the experiment (often 24 h or more at 37°C). Although ASA ionizes and thus has a much larger solubility in basic solutions, the reaction kinetics of ASA hydrolysis is also pH dependent, with basic conditions generally accelerating the hydrolysis more than acidic conditions (Fig. 5). Thus, hydrolytic degradation over the timeframe of the solubility determination is the probable reason behind the inconsistency among the solubility data reported in literature. So, instead of seeking to establish the true thermodynamic equilibrium solubility, the goal of the experimental work reported here was to demonstrate that more than 1000 mg of ASA could be dissolved in 250 mL of buffer solutions with different pH values at 37°C.

The data in Table 2 indicate that the highest dose of ASA (1000 mg) is easily dissolved in 250 mL of buffers having an initial pH in the range of pH 1–6.8, including at values close to the p*K*a. As according to the Henderson–Hasselbalch equation, the solubility of a weak acid will increase exponentially with pH until

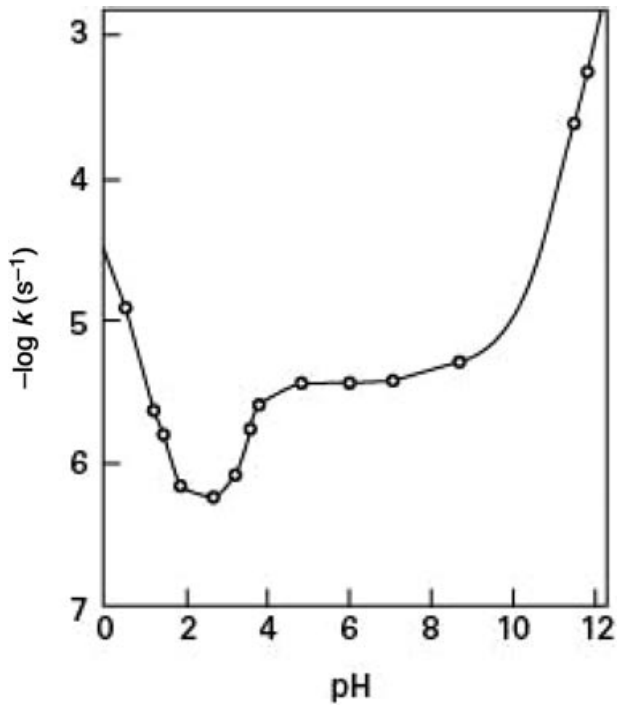

Figure 5. pH-rate profile for hydrolysis of acetylsalicylic acid at 25°C (rate constants in s^{-1}).[15]

the solubility of the ionized form is reached, one can assume with confidence that at a final pH of 6.8 the solubility at 37°C would also meet the criterion for "highly soluble." Owing to the high solubility of ASA in buffers around this pH and its subsequent influence on the pH, it is impractical to maintain the pH of the medium at pH 6.8 because of the excessively high concentration of buffer that would have to be employed.[41]

Thus, the solubility criteria of the WHO, FDA, and EMA are all fulfilled by ASA and it can be classified as "highly soluble."

Permeability

An API is considered highly permeable/completely absorbed when the extent of absorption in humans is at least 85% in the EU,[1–2] whereas 90% or more is required in the United States.[3] Acceptable test methods for permeability determination of APIs are mass balance determination, absolute bioavailability, or *in vivo* intestinal perfusion in humans.[1–3] As recovery of a C^{14} marker was 94%–98% in mass balance studies, and since ASA is converted to SA largely by first-pass metabolism, it can be assumed that ASA is highly permeable.

Supportive data can be provided by additional test methods, including *in vivo* or *in situ* intestinal perfusion in animal models, *in vitro* permeation studies using excised human or animal intestinal tissues,[3] or *in vitro* permeation across a monolayer of cultured epithelial cells (e.g., Caco-2) using a validated method.

Here too, results suggest that ASA can be regarded as highly permeable.

Although no regulatory authority currently permits estimation of permeability based on physicochemical parameters, the logP data are generally in line with the Caco-2 cell and mass balance study results.

As the absolute bioavailability of ASA is close to 100% (in terms of SA), the urinary recovery of ASA and SA after administration of radiolabeled ASA is over 90% and validated Caco-2 experiments indicate that ASA is a highly permeable substance, it can be concluded that ASA is "highly permeable."

Relevance of Conversion to SA for the Biowaiver Decision

The US regulations also address the issue of instability of the drug substance in the GI tract, which needs to be adequately considered during the collection and assessment of the permeability data. Significant degradation (>5%) in (simulated) gastric fluid within 1 h at 37°C and/or (simulated) intestinal fluid within 3 h at 37°C suggests potential instability of the drug substance.

Although ASA is also hydrolyzed to SA without enzymatic assistance, this process is rather slow.[15,27] Therefore, there is little or no conversion to its active metabolite SA in the intestinal lumen and ASA is absorbed unaltered rather than as SA.[32] To elaborate on this point further, we considered published data for the hydrolysis of ASA (Fig. 5). Connors[15] indicates that the hydrolysis rate of ASA is lowest at pH 2–3, suggesting that there is little conversion of ASA to SA in the fasting stomach, where the average pH value is 1.5–1.9.[42] ASA has a pH-dependent stability profile (see Fig. 5). The $-\log k$ (s^{-1}) for the reaction is 6.25 at 25°C and pH 2.5; over the pH range 1–7, the maximum rate constant is $-\log k$ (s^{-1}) = 5.5. Applying the energy of activation for ASA hydrolysis, 14,000 cal/mol, the intrinsic stability of ASA (t_{90}) at 39°C can be calculated from the above reaction rate constants. These calculations result in a t_{90} of 3.17 h under worst case pH conditions (pH 5–6) and 23.4 h at pH 2.5 (best case).

To verify these calculations, dissolution studies were run using ASA tablets at both pH 1.2 (SGFsp) and 6.8 (SIFsp), analyzing the samples with a stability-indicating assay. The data are shown in Table 5.

The data are consistent with the calculations from Connors.[15] Considering these data and the rapid absorption of ASA, it is evident that there is unlikely to be significant conversion to free SA in the stomach or intestinal lumen and that the hydrolysis occurs primarily through presystemic enzymatic-mediated mechanisms in the gut wall and liver.

Against this background, it is expected that only dramatic differences in the ASA release rate from the IR dosage form could exert a significant effect on the preabsorptive conversion. Thus, SA contributions to solubility and dissolution from ASA formulations in the framework of the biowaiver can be considered negligible.

BCS Classification of ASA

According to Potthast et al.[10] and Kasim et al.,[14] ASA would be assigned BCS Class II and BCS Class III status, respectively, when the single highest dose exceeds 100 mg. In the former case, this conclusion was justified by its pH-dependent solubility[10]; in the latter case, the decision was based on calculated partition coefficients indicative of moderate permeability.[14] On the basis of the additional data collected from the literature and generated for this Monograph, it is necessary to revisit these classifications.

Classification In Line with WHO Requirements

In former WHO reports, ASA had been classified as a BCS Class III substance, since it was not considered to meet the FDA criterion of 90% or more absorption.[3] In the meantime, however, the absorption criterion has been relaxed by the WHO to "at least 85%." In the most recent WHO report, ASA is assigned to BCS Class I.[1] This is based on the highest listed dosage strength of ASA on the EML of 500 mg.[20] The experimental solubility data for ASA at 37°C presented in Table 2 show that it easily meets the criterion for "highly soluble," that is, more than 500 mg is dissolved in 250 mL in buffers of initial pH within the range 1.2–6.8 and the solubility is expected to be even higher at an end pH of 6.8 due to the weak acid nature of ASA. Furthermore, the absorption and permeability data are commensurate with a WHO classification of "highly permeable."

Classification In Line with EU Requirements

According to the recent EMA Guideline on BE testing,[2] the highest single dose recommended in the

Table 5. Dissolution of Acetylsalicylic Acid Tablets Using a Stability-Indicating Assay

	SGFsp, pH 1.2		SIFsp, pH 6.8		
% Dissolved of Label Claim	15 min	30 min	15 min	30 min	60 min
ASA	75.83 ± 9.79	92.78 ± 6.47	97.82 ± 11.81	100.7 ± 1.54	96.73 ± 3.35
SA	0.279 ± 0.038	0.734 ± 0.0734	0.819 ± 0.121	1.72 ± 0.137	3.54 ± 0.168

Summary of Product Characteristics rather than the highest dose strength is to be used when determining the dose–solubility ratio. Following the arguments in *Classification In Line with WHO Requirements* above and using 1000 mg as the highest single dose, ASA meets the EMA criteria for both highly soluble and highly permeable API.

Classification In Line with FDA Requirements

According to the FDA guidance document, the highest dose strength (i.e., 500 mg) needs to be soluble in less than 250 mL aqueous media over a pH range of 1–7.5 to be classified as "highly soluble." On the basis of the data provided in Table 2, this requirement is fulfilled for ASA. Regarding permeability, the guidance stipulates that in the absence of evidence suggesting instability in the GI tract, a drug is considered to be "highly permeable" when the extent of absorption in humans is determined to be ≥90% of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose. Studies addressing the instability of ASA under conditions representing the upper GI tract (see section on *Relevance of Conversion to SA for the Biowaiver Decision*) indicate that less than 5% of ASA would decompose to SA before being absorbed.

Considering both the stability of ASA in the gastric fluid and the intestinal fluid, as well as the fact that all the ^{14}C radioactivity (94%–98%) administered is recovered within 24 h following oral administration[31] and that the total amount of salicylates determined in urine over 48 h corresponds to 94%–96% of the amount of ASA administered, it can be concluded that ASA absorption is virtually complete (>90%) and that it is therefore "highly permeable" according to the FDA standards.

Therefore, together with the available *in vitro* permeability data obtained by Caco-2 experiments, the absorption, permeability, and solubility data currently available for ASA fulfill FDA requirements for BCS Class I.

In summary, the available literature and new experimental data for both permeability and solubility demonstrate that ASA can be assigned to BCS Class I in accordance with the EU, FDA, and WHO requirements.

Patient Risks Associated with Bioinequivalence

In this section, we consider three types of risk:

- the risk that a product that is not, in fact, bioequivalent will be deemed bioequivalent by the biowaiver procedure
- the risks to the patient associated with subequivalent levels of ASA, and
- the risks to the patient associated with supraequivalent levels of ASA.

Restricting the use of excipients to those which are already used in ASA products (see Table 4) will reduce the risk of a nonequivalent product being deemed bioequivalent via the biowaiver procedure. Excipients that may affect GI transit (e.g., sorbitol, mannitol, etc.) and those that could affect permeability (e.g., surfactants) should be avoided unless also present in the comparator product.

Risks to the patient associated with subequivalent or supraequivalent levels of ASA and/or SA are in general very low, since this API (and its major metabolite) has a wide therapeutic index and a wide dosing range. Slight changes in plasma protein binding of the ASA due to subequivalent or supraequivalent formulations appear to place the patient at a minimal risk for changes in drug interactions based on this phenomenon. Idiosyncratic events are not addressed here, as they are not dose related and therefore not relevant to a BE discussion.

On the subequivalent side, there is the risk that prevention effects (low dose ASA) or relief of pain and fever (high dose ASA) will be reduced. This appears most relevant for very low doses, as ASA acts as an irreversible inhibitor of platelet aggregation, with SA having no role in this effect. However, the wide dosing range practiced for both indications would suggest that modest subequivalence poses little risk to therapeutic outcome.

On the supraequivalent side, there is a question about reduced effects for low dose ASA due to the competing effects on blood physiology, whereas for high dose ASA, there is some concern that dose-related side-effects, for example, GI problems might occur more frequently. Here too, because of the wide dosing ranges practiced, a modest supraequivalence is not expected to result in a different therapeutic outcome for the patient.

In summary, an analysis of the risks and benefits of biowaiving concludes in favor of applying the biowaiver procedure to IR solid oral products containing ASA as the only API.

CONCLUSION

On the basis of available literature and experimental data, ASA can be unequivocally assigned to BCS Class I, according to the criteria of the WHO, FDA, and EMA. Furthermore, risks to the patients associated with bioinequivalence due to approval based upon an errant biowaiver decision can be considered minimal.

Thus, two solid IR ASA formulations (test and comparator products) containing usual amounts of common excipients used in ASA products (e.g., those listed in Table 4) which meet the dissolution criteria of "rapidly dissolving" (≥85% release in 30 min) or "very rapidly dissolving" (≥85% release in 15 min) and, in

case of rapidly dissolving products, additionally, are shown to exhibit similar dissolution profiles according to the f_2 test ($f_2 \geq 50$), can be considered interchangeable without the necessity for a human pharmacokinetic BE.

REFERENCES

1. World Health Organization (WHO). Proposal to waive *in vivo* bioequivalence requirements for WHO model list of essential medicines immediate-release, solid oral dosage forms. Accessed August 10, 2010, 2006, at: http://whqlibdoc.who.int/trs/WHO_TRS_937_eng.pdf#page = 403.
2. European Medical Agency. Committee for medicinal products for human use: Guideline on the Investigation of Bioequivalence. Accessed February 1, 2010, at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf.
3. US Department of Health and Human Services:Food and Drug Administration. Guidance for industry: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. Accessed December 20, 2010, at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070246.pdf.
4. Kees F, Jehnich D, Grobecker H. 1996. Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Appl 677(1):172–177.
5. Sweetman SC, Ed. 2007. Martindale: The complete drug reference.,36th edt., London: Pharmaceutical Press. UK.
6. Lüllmann H, Mohr K, Hein L. 2006. Pharmakologie und toxicologie. 16th ed. Thieme, Sttutgart, Germany; pp 290–292.
7. Bayer Healthcare-Consumer Care. 2008. Acetylsalicylic acid for pain and fever. Company Core Data Sheet.
8. Drugbank. Acetylsalicylic acid. Accessed August 9, 2010, at: http://www.drugbank.ca/drugs/DB00945.
9. Hohlfeld T, Kojda G. 2002. Kopfschmerz zur Resistenz. Bekanntes und Neues zu Aspirin. Accessed August 9, 2010, at: http://www.uni-duesseldorf.de/kojda-pharmalehrbuch/apothekenmagazin/Fortbildungsartikel/2003-01-02.pdf.
10. Potthast H, Haufte S, Glaab V. 2003. Pharmazeutische Zeitung: Acetylsalicylsäure-Präparate im Vergleich. Accessed August 9, 2010, at: http://www.pharmazeutische-zeitung.de/index.php?id = 25029.
11. Tawashi R. 1968. Aspirin: Dissolution rates of two polymorphic forms. Science 160(823):76.
12. Summers MP, Carless JE, Enever RP. 1970. The polymorphism of aspirin. J Pharm Pharmacol 22(8):615–616.
13. Watanabe A, Yamaoka Y, Takada K. 1982. Crystal habits and dissolution behavior of aspirin. Chem Pharma Bull 30(8):2958–2963.
14. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernas H, Hussain AS, Junginger HE, Stavchansky SA, Midha KK, Shah VP, Amidon GL. 2004. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol Pharm 1(1):85–96.
15. Connors K. 1986. Chemical stability of pharmaceuticals. 2nd ed. Wiley Interscience, New York, U S A.
16. European Pharmacopoeia. 2010. Acetylsalicylic Acid. 7th ed, pp 1317–1318.
17. United States Pharmacopoeia. The National Formulary, (USP33/NF28) ed, Rockville: Maryland, The United States Pharmacopoeial Inc., 2010.
18. ABDA Pharma-Daten-Service. 2002. Pharmazeutische Stoffliste: Acetylsalicylsäure. 13. Auflage ed.
19. Sigma–Aldrich. 2010 Acetylsalicylic Acid A5376. Accessed August 2, 2010, at: http://www.sigmaaldrich.com/catalog/ProductDetail.do?D7=0&N5=SEARCH_CONCAT_PNO|BRAND_KEY&N4=A5376|SIGMA&N25=0&QS=ON&F=SPEC.
20. World Health Organization. 2010. WHO Model List of Essential Medicines. Accessed 16 August 10, 2010, at: http://www.who.int/medicines/publications/essentialmedicines/Updated_sixteenth_adult_list_en.pdf.
21. Rote L. 2010 Aspirin Tabletten. Accessed August 10, 2010, at: http://www.rote-liste.de/Online/jumpsearch.
22. Benet LZ, Amidon GL, Barends DM, Lennernas H, Polli JE, Shah VP, Stavchansky SA, Yu LX 2008. The use of BDDCS in classifying the permeability of marketed drugs. Pharm Res 25(3):483–488.
23. Castillo-Garit JA Marrero-Ponce Y, Torrens F, Garcia-Domenech R. 2007. Estimation of ADME properties in drug discovery: Predicting Caco-2 cell permeability using atom-based stochastic and non-stochastic linear indices. 11th International Electronic Conference on Synthetic Organic Chemistry- ECSOC.
24. Artursson P, Karlsson J. 1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun 175(3):880–885.
25. Yee S. 1997. *in vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man—Fact or myth. Pharm Res 14(6):763–766.
26. Insel PA. 1990.Analgesic-Antipyretics and Antiinflammatory agents;Drugs employed in treatment of Rhematoid Arthritis and Gout, In Goodman and Gilman'sThe Pharmacological basis of therapeutics; Gilman AG, Rall Tw, Nies AS, Taylor P, Eds. 8th ed. Elmsford, New York:Pergamon Press.
27. Rowland M, Riegelman S, Harris PA, Sholkoff SD. 1972. Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J Pharm Sci 61(3):379–385.
28. Bergström C, Dressman J, Artursson P. 2011. Permeability determination of six essential medicines. FIP International Symposium on BA/BE of Oral Drug Products. Tokyo, Japan.
29. Bogentoft C, Carlsson I, Ekenved G, Magnusson A. 1978. Influence of food on the absorption of acetylsalicylic acid from enteric-coated dosage forms. Eur J Clin Pharmacol 14(5):351–355.
30. Hutt AJ, Caldwell J, Smith RL. 1982. The metabolism of [carboxyl-14C]aspirin in man. Xenobiotica 12(10):601–610.
31. Needs CJ, Brooks PM. 1985. Clinical pharmacokinetics of the salicylates. Clin Pharmacokinet 10(2):164–177.
32. Hutt AJ, Caldwell J, Smith RL. 1986. The metabolism of aspirin in man: A population study. Xenobiotica 16(3):239–249.
33. Ritschel W. 1992. Handbook of Basic Pharmacokinetics, 4th ed. Drug Intelligence Publications, Bethesda, U S A, pp 530–535.
34. Cooke AR, Hunt JN. 1970. Absorption of acetylsalicylic acid from unbuffered and buffered gastric contents. Am J Dig Dis 15(2):95–102.
35. George CF. 1981. Drug metabolism by the gastrointestinal mucosa. Clin Pharmacokinet 6(4):259–274.
36. Medscape AHFS. 2009 Monograph: Enteric coated aspirin oral. Accessed August 5, 2010, at: http://www.medscape.com/druginfo/monograph?cid=med&drugid=211&drugname=Enteric+Coated+Aspirin+Oral&monotype=monograph&secid=5.
37. Dubovska D, Piotrovskij VK, Gajdos M, Krivosikova Z, Spustova V, Trnovec T. 1995. Pharmacokinetics of acetylsalicylic acid and its metabolites at low doses: a compartmental modeling. Methods Find Exp Clin Pharmacol 17(1):67–77.
38. Levy G. 1961. Comparison of dissolution and absorption rates of different commercial aspirin tablets. J Pharm Sci 50:388–392.

39. Bochner F, Somogyi AA, Wilson KM. 1991. Bioinequivalence of four 100 mg oral aspirin formulations in healthy volunteers. Clin Pharmacokinet 21(5):394–399.
40. Brantmark B, Wahlin-Boll E, Melander A. 1982. Bioavailability of acetylsalicylic acid and salicylic acid from rapid-and slow-release formulations, and in combination with dipyridamol. Eur J Clin Pharmacol 22(4):309–314.
41. Ozturk SS, Palsson BO, Dressman JB. 1988. Dissolution of ionizable drugs in buffered and unbuffered solutions. Pharm Res 5(5):272–282.
42. Vertzoni M, Dressman J, Butler J, Hempenstall J, Reppas C. 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. Eur J Pharm Biopharm 60(3):413–417.