Factors Influencing the Selection of Medium for Evaluating Drug Solubility and Dissolution in Bovine Milk

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ABSTRACT

Milk or milk-containing beverages can be used as vehicles for drug product administration and as a component of human fed-state simulated gastric fluids. Unprocessed bovine milk is also the matrix within which drugs must be solubilized or released when formulations are administered into the bovine mammary gland. Therefore, an appreciation of factors impacting the effect of milk on drug solubility and product dissolution is necessary. Although an off-the-shelf container of cow milk may be adequate for evaluating drug solubility, the composition of milk varies as a function of fat content (e.g., differences in fat content). Differences can occur between bovine breeds, diet, environment, and suppliers. Importantly, it is unclear how to quantify differences in drug solubility across types of milk products versus when the drug is infused directly into the bovine udder. To address these concerns, a two-tiered approach was employed. The first tier involved comparing drug solubility across a range of milk products, including raw (unprocessed) bovine milk obtained from healthy cattle and an aqueous buffer. The second tier, which is the subject of this review, explores publicly available information on the composition of bovine milk and the potential variability of its constituents. The goal of this work is to provide the basis for inclusion of milk as one of the biologically relevant media described in the *United States Pharmacopeia* general chapter on solubility testing.

KEYWORDS: Solubility, complex matrices, bovine milk, bioavailability, dissolution

INTRODUCTION

The *United States Pharmacopeia* (*USP*) general chapter <1236> Solubility Measurements describes the measurement of drug solubility across a range of biorelevant media relevant both to human and veterinary medicine. (*1*). Recently, the issue of cow milk as a medium for testing drug solubility and for evaluating product dissolution has been raised as a point of interest for several reasons (*2–8*).

- **Veterinary drug delivery**: When administered into the bovine mammary gland, the drug acts within the udder (typically, minimal systemic absorption) for the purpose of treating bovine mastitis. Bovine mastitis is a major health problem encountered within the US and around the world (*2*). Its importance is reflected in the incidence of clinical and subclinical

mastitis within the US: approximately 20–25 cases per 100 cows per year. Clinical mastitis occurs in all dairy herds, even those that are well-managed (*3*). Therefore, there is a tremendous need for safe and effective antimicrobials for treating bovine mastitis.

- **Human drug delivery**: Cow milk is a potential vehicle for delivering drugs to pediatric and geriatric patients (*4–6*). Therefore, the issue of drug solubility in milk, or its adsorption to milk proteins and fats, is relevant for both human and animal health. This led the USP to initiate an effort to define the composition of milk in normal and mastitic cattle and the variability that may exist across cow nutritional and health status.

- Milk has been suggested as a component of fed-state simulated gastric fluids (SGFs) (*7*).

- **Pharmacokinetic considerations**: The partitioning and solubilization of environmental contaminants in milk is an important consideration from the perspective of their presence and persistence in milk ingested by human consumers (*8*).

Whether evaluating drug solubility or product dissolution, the challenges associated with evaluations conducted in milk are due to the variability and highly complex nature of this medium. It contains more than 20 proteins along with fats, and there is the potential for preferential binding to casein milk proteins, whey, or fat (*9*). For example, looking at three hydrophobic drug molecules (flunixin, meloxicam [weak acids charged at the pH of milk], and thiabendazole [weak base that is unionized in milk]) confirmed that compound hydrophobicity alone could not explain the disparities in drug solubilization. Rather, solubilization appeared to relate to whether the drug would bind preferentially to either casein (meloxicam and thiabendazole), whey (flunixin), or milk fat (*10*) (Table 1).

Table 1. In Vitro Drug Association with Components of Raw Whole Milk (10)[a]

Drug	Log P	pKa	Log D (pH 6.8) (native pH of milk)	In vitro (measured in raw whole milk)		
				Casein	Whey[a]	Fats
Meloxicam	3.43	4.08 (acidic)	0.71	66%[b]	21%	11%
Flunixin	4.0	5.82 (acidic)	3.08	29%	54%	13%
Thiabendazole	2.92	4.64 (basic)	2.92	50%	29%	18%

[a]*Note that the study did not distinguish between drug concentration in the aqueous versus whey components of the milk serum. Therefore, no definitive statement can be made regarding the proportion of drug in serum that is associated with whey proteins.*
[b]*Fraction of active pharmaceutical ingredients (API) concentration in the specific component tested versus the total concentration present in milk.*

With the intent of exploring milk as a drug delivery vehicle for humans, Macheras et al. evaluated the solubility of nine drugs representing a range of aqueous solubilities and extent of binding to milk proteins (equilibrium dialysis with a molecular cutoff of 5000) (*11*). Drug solubility was markedly higher in milk than in buffer (pH 6.5) at all temperatures, and the extent of protein binding tended to correlate with drug lipophilicity. For most drugs, this binding tended to be higher at 15 °C vs 37 °C (especially in the low milk-fat samples). While the magnitude of protein binding was similar in 0.75% vs 3.5% fat content, for most drugs studied by these authors (exception being dicumarol and nitrofurantoin), the observed solubility in whole milk tended to be greater than that in skim milk.

These results were interpreted to imply that drug binding to milk proteins is only one of the reasons for higher drug solubility in milk and that the other aqueous phase components may have an important influence on the solubilization of active pharmaceutical ingredients (APIs).

Milk has been used to simulate biorelevant SGFs. For example, the in vitro dissolution of acetaminophen (Biopharmaceutics Classification System [BCS] class I compound) and BCS II compounds danazol and mefenamic acid were previously studied in milk. The 3.5% fat bovine milk was purchased from an Austrian supplier (pH 6.5, buffer capacity of 14 mEq/L/pH). Although the various aqueous media used in the dissolution study did not influence the dissolution of acetaminophen, milk markedly slowed the tablet release rate. In contrast, as compared to that seen using aqueous buffers containing surfactants, milk markedly enhanced the dissolution rate of poorly soluble drugs such as danazol and mefenamic acid (*12*). The same group also studied in vitro dissolution of poorly soluble drugs such as troglitazone, atovaquone, sanfetrinem cilexetil, and an experimental drug (GV150013X) in whole milk (3.5% fat) versus traditional aqueous buffers (USP 23 fasted-state simulated intestinal fluid [FaSSIF] with pancreatin (*13*). In some cases, faster and more complete dissolution was observed in fed-state simulated small intestinal fluid (FeSSIF) versus milk (troglitazone and GV150013X). Conversely, milk appeared to provide a more formulation-dependent dissolution profile for sanfetrinem cilexetil and a markedly faster dissolution of atovaquone as compared to that seen with the other media, including FeSSIF. However, the milk used in these studies cannot be considered standardized media, as would be the case for the other aqueous buffers. The authors raised the issue of potential batch-to batch variability in milk and its potential effects on in vitro dissolution study results (*13*).

Given the drug-specific influence of the various milk constituents and large variability that can occur in milk, depending on the source or commercial processing, there is a need to establish some level of standardization in the milk used for solubility and dissolution testing. To that end, the USP <1236> provides an overview of the concepts and equations relevant to solubility measurements, including a description of experimental methods for assessing drug solubility and species-specific biorelevant media for generating the drug solubility assessments (*1*). To date, the media and methods described in <1236> have pertained to conditions associated with the gastrointestinal (GI) tract. The formulations provided include biorelevant media for humans, dogs, and cattle,

with the aim of expanding to include other veterinary species (e.g., poultry, cats, swine, and horses).

With these points in mind, it is important to define the protein, lipid, and aqueous composition of milk when trying to understand the factors that can influence drug solubility assessment or in vitro dissolution characteristics in this medium. Ultimately, the question is whether the complexity of bovine milk will preclude establishing a particular "recipe" for drug solubility testing, and, if so, what alternative can be used to provide a standardized-like medium. Doing so will impact assessments of drug product performance when administered in milk to human patients, the evaluation of the solubility of drugs intended for bovine intramammary infusion, and the inclusion of milk as a component of fed-state SGF.

The aim of this work is to review milk composition in cows and humans with a goal of expanding *USP* <1236> to include a proposal for a "standardized" bovine milk medium.

The composition of bovine milk was studied from a range of publicly available sources, including published articles and government publications.

Proteins
Cow milk contains more than 20 proteins, the main ones being casein (about 80% of milk proteins) and whey (about 20% of milk proteins). Casein is fractionated into α_{s1}, α_{s2}, β, and κ-casein. The proportion of the various caseins in bovine milk can differ across dairy breeds. However, they all are amphiphilic and present in several conformations when in solution. Their amphiphilic nature renders them relatively insensitive to denaturation. Unlike whey proteins, caseins are insoluble in aqueous media and therefore form micelles. They are characterized by a high capacity for binding phosphorus and calcium. Casein micelles typically have an open structure with serum-filled cavities accessible to small molecules, but the micelle structure itself exhibits pH-dependent behavior. It becomes more compact as the pH drops and swells (becoming less compact) with an increase in pH. Therefore, caseins are being explored as a potential candidate for controlled-release drug delivery (*14*).

Although fat-soluble compounds appearing in milk are believed to associate with the fat fraction, it has been hypothesized that the open structure of native casein micelles provides a better environment for the binding and transport of lipophilic substances. To explore the influence of casein on drug adsorption, Cheema et al. examined three hydrophobic APIs (meloxicam, flunixin,

and thiabendazole) (*10*). Interestingly, the outcome showed differences between when the drug enters milk via secretion from plasma (i.e., administered to dairy cattle as per the approved product label) versus when the drug is introduced in vitro by addition to milk samples maintained at room temperature (~25 °C). Although in vivo and in vitro binding to casein was similar for flunixin, OH-flunixin, and OH-thiabendazole (the parent thiabendazole molecule could not be quantified in the in vivo milk samples), statistically significant in vivo/in vitro differences were observed with meloxicam. More than twice the percentage of casein-associated meloxicam was observed in vitro (61% of the amount added to the whole raw milk) vs in vivo (31% of the recovered drug). Conversely, twice the percentage was associated with whey protein in the in vivo versus the in vitro samples (21% in vitro vs 52% in vivo).

In contrast to the insoluble caseins, the major whey proteins are water soluble (*14*). Whey is predominantly made up of proteins β-lactoglobulin (β-LG), which comprises about 50% (g/L relative to total whey proteins), α-lactalbumin (α-LA), which comprises about 26% (g/L relative to total whey proteins), bovine serum albumin (BSA) 8% (g/L relative to total whey proteins), immunoglobulins A, M and C (total = 14% g/L relative to total whey proteins), lactoferrin 2% (g/L relative to total whey proteins), and lactoperoxidase 0.6% (g/L relative to total whey proteins). The primary three proteins in whey are considered to be the two lactoglobulins and BSA.

The challenge facing efforts to define the whey fraction is that this complex mixture is difficult to standardize. Multiple variants of β-LG exist, with the A and B variants being the most common (*15*). The relative amounts of these all-whey proteins can vary per breed and diet, and the stability of the major proteins is variable. There is also a noted difference in the whey protein composition between milk produced by healthy cows and that produced by mastitic cows, with mastitic milk having an increase in albumin and serotransferrin and a decrease in β-LA and α-LA (*16*).

Whey protein isolate (WPI) is a commercially available protein raw material that could be used to represent this protein component in a standardized milk formulation. However, modern milk processing techniques such as ultra-high temperature (UHT) treatment have been demonstrated to reduce the amount of β-LG and alter the tertiary structure of whey protein (*17*). Depending on the methods used to isolate the WPI, there could be problems with assuming that a WPI is representative of

the whey composition in raw milk. Furthermore, other than BSA, none of the other ingredients are available as USP-grade material. The proteins β-LG, α-LA, and BSA share an ability to interact and bind to the milk fatty acids, and the binding affinity of BSA to some fatty acids tends to exceed that of β-LG (*18*). Whether the physicochemical differences seen across the three primary whey proteins will impact drug solubilization and binding has not been adequately evaluated.

The United States Department of Agriculture (USDA) data for whole milk indicates that the total protein content of milk is 3.15% w/w (*19*). Given that the composition of milk proteins is 80% casein and 20% whey, it is proposed that a standardized milk formulation would be targeted to contain 2.52% technical-grade casein and 0.63% BSA. The problem is that there are many factors (breed, diet, stage of lactation, seasonal variation, ruminal fermentation) that can potentially influence milk composition (*20*). This raises the question of whether such variations may affect solubility test results, and if so, how to adjust test conditions to accommodate these variations.

When caseins are isolated from milk, they are typically acidified during the isolation process. As a result, the use of caseins typically requires the addition of a base to adjust the pH back to neutral to swell and rehydrate the caseins. In contrast, sodium caseinate is a readily available casein material that has already been neutralized with sodium hydroxide to convert to sodium salt. Although either form of casein could be used in a standardized milk formulation, the use of sodium caseinate would minimize the need to adjust the pH of the formulation. Again, there are uncertainties that arise when striving to develop some standardized milk medium.

Milk Fat
Milk fat contains approximately 400 different fatty acids. Its relative proportion to the total milk constituents is 3.3–4.4%, depending upon breed, stage of lactation, diet, presence of mastitis, and the ruminal flora (*21*, *22*). Trace fatty acids will likely have little influence on drug solubility owing to the small amount present in milk.

The fat is present in milk as an oil-in-water emulsion formed by the endoplasmic reticulum in the epithelial cells of the mammary gland. When secreted, they are enveloped with the plasma membrane of the epithelial cell. Therefore, membrane-associated materials comprise approximately 2–6% of the globule mass. The milk fat globule membranes contain about 70% membrane proteins, 25% phospholipids, 3% cerebrosides, and 2% cholesterol (all units based upon w/w). This is in contrast with the composition of the milk fat itself, which contains about 98% triglycerides and 2% diacylglycerol. The amount of cholesterol is less than 0.5%, about 0.5–1% phospholipids and 0.1% free fatty acids (*23*).

The remainder (~0.2%) consists of trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, and other constituents that may be secreted into the milk from the feed. Although cholesterol is a minor component in milk fat membranes, the phospholipid content (25% w/w) may be important for formation of the fat globule. The number of milk fat globules (MFG) is 10^{10} per mL of milk, with a total area of 700 cm^2 per mL of milk, with that total area estimate being a function of the MFG fat content (*21*). Although diacylglycerol, which is a component of both triglyceride biosynthesis and lipolysis, can constitute up to 2% of the lipid portion, diacylglycerol is typically part of the milk fat globule membrane and therefore not anticipated to directly affect the solubility of drugs in milk (*21*).

For a comparison of documented fatty acid composition in cow milk, four data sources from 2007–2020 were used: Månsson (Swedish cows), Zou et al. (Danish cows), and two USDA data sources including Haug et al (*19*, *21*, *24*, *25*) (Table 2). Note that values are reported as %w/w by Månsson, Zou et al., and in the 2007 USDA database (*19*, *21*, *24*), whereas %w/v values are reported by Haug et al (*25*). To facilitate comparison, the 2007 USDA data were converted to %w/w using a specific gravity of 1.030 g/cm^3. The numbers from the Månsson and Zou et al. were calculated to indicate the %w/w in milk using a total fat content of 3.25% per the 2020 USDA data (i.e., the standardization of values to w/w is based on the USDA description of 3.25% milk fat content) (*19*).

As can be seen in Table 2, the data gleaned from the various sources confirms the variability of milk lipid composition. This is not surprising when considering that milk fatty acids are derived both from feed and ruminal microbial activity. Variations may be attributed to breed, diet, stage of lactation, mastitis, and ruminal flora (*22*). According to Table 2, total fatty acids are lower than that described by the USDA (2.9% vs 3.25%). This shortfall is due to the elimination of various trace fatty acids.

Aqueous Phase
Linzell provides a high-level overview of the composition of the aqueous phase of milk (*26*). The concentrations of ions more closely resemble that of the cell than it does plasma, including K+, Ca++, Mg++, citrate--5, and HPO$_4$--, and low concentrations of Na+, Cl-, and HCO$_3$. Lactose is the main osmole in milk at around 5% w/w, and

Table 2. Estimates of Milk Fatty Acid Content

	USDA (*19*)	Månsson (*21*)	Zou et al. (*24*)	Haug et al. (*25*)
	% w/w	% w/w	% w/w	% w/w*
Saturated Fatty Acids				
4:0 Butyric acid	0.075	0.143	0.135	-
6:0 Caproic acid	0.075	0.078	0.101	-
8:0 Caprylic acid	0.075	0.046	0.076	-
10:0 Capric acid	0.075	0.088	0.159	-
12:0 Lauric acid	0.077	0.107	0.212	0.077
14:0 Myristic acid	0.297	0.354	0.675	0.291
16:0 Palmitic acid	0.829	0.995	1.041	0.777
18:0 Stearic acid	0.365	0.397	0.133	-
Monounsaturated Fatty Acids, cis				
16:1 Palmitoleic acid	-	0.033	-	-
18:1 Oleic acid	0.812	0.741	0.429	0.777
Polyunsaturated Fatty Acids				
18:2 Linoleic acid	0.12	0.052	0.067	0.117
18:3 alpha linoleic acid	0.075	-	-	0.073
Trans Fatty Acids				
18.1t Vaccenic acid	-	0.068	0.036	-

Values converted from %w/v to %w/w by assuming a density of 1.03 g/cm³.

once secreted cannot ordinarily pass back through the secretory or duct epithelia.

Similar findings were published by Gaucheron (*27*). The mineral fraction, which is a small fraction of milk (about 8–9 g×L⁻¹), contains cations (calcium, magnesium, sodium, and potassium) and anions (inorganic phosphate, citrate, and chloride). In milk, these ions play an important role in the structure and stability of casein micelles (*27*). The mineral content undergoes some variation as a function of the lactation phase. For most ions, fluctuations amount to no more than 20%, but relatively larger differences (40–50%) can occur in terms of sodium, potassium, chloride, and soluble calcium. Nevertheless, given the relatively small fraction of ions versus total bulk volume of milk, for the purpose of assessing the rate and extent of drug solubility in milk, it should be adequate for defining ionic composition in terms of what has been reported in bulk skim milk (Table 3). For the sake of completeness, Table 3 is based on information from Gaucheron in terms of what is found in subclinical mastitis (*27*).

In milk, all macro-elements are distributed differently into diffusible and non-diffusible fractions (essentially casein micelles). Potassium, sodium, and chloride ions are essentially diffusible although calcium, and inorganic phosphate and magnesium are partly bound to the casein micelles. About one-third of calcium, half the inorganic phosphate, two-thirds of magnesium, and over 90% of citrate are in the aqueous phase of milk. A small proportion of calcium is also bound to α-LA (there is one atom of calcium per protein). There is little to no binding of these elements to either lactose or fat. Furthermore, Gaucheron lists nine forms of calcium and magnesium salts that can be seen in a typical milk ultrafiltrate while noting that 1) the addition of one ion can impact the number of other ions in the diffusible phase; and 2) the addition of NaCl to milk leads to a slight decrease in pH and increases in Ca^{2+} concentrations in the diffusible phase.

The majority of calcium and magnesium appear to be in the form of calcium citrate and magnesium citrate. Phosphate and chloride are in the form of sodium and potassium salts, which can be well represented by a mixture of sodium phosphate and potassium chloride (*26*). The only significant differences between the soluble and total ions are for calcium, magnesium, and phosphate and can be represented by addition of these insoluble salts. A buffer composition that approximates these concentrations is shown in Table 4.

DISCUSSION

Optimally, a medium would be standardized to allow for reproducible assessments of in vitro dissolution and drug solubility. However, given the diversity of milk

Table 3. Milk Mineral Composition (mmol/L) and pH

	Healthy (Bulk)	Subclinical Mastitis	Aqueous Buffer
Total Calcium	30.1	29.4	-
Total Calcium	9.5	9.1	9.5
Total Magnesium	5.1	4.9	-
Soluble Magnesium	3.3	3.2	3.3
Total Inorganic Phosphate	20.9	19	-
Soluble Inorganic Phosphate	11.2	9.2	11.25
Total Citrate	9.8	8.8	-
Soluble Citrate	9.2	8.3	9.2
Total Sodium	25.5	34.5	18.1
Total Potassium	36.8	36.1	36.1
Total Chloride	30.3	40.5	36.1
pH	6.72	6.87	6.8

Based on Gaucheron [27].

Table 4. Buffer Composition of Milk (% w/w and mmol/L)

Soluble Buffer Ingredients	w/w (%)	mmol/L
Potassium chloride	0.27%	36.1 mmol/L K, 36.1 mmol/L Cl
Calcium citrate, 4 H$_2$O[a]	0.18%	3.16 mmol/L = 9.5 mmol/L Ca, 6.3 mmol/L citrate
Phosphoric acid, 85%	0.130%	11.25 mmol/L Phosphate
Magnesium citrate, 9 H$_2$O[b]	0.067%	1.1 mmol/L = 3.3 mmol/L Mg, 2.2 mmol/L citrate
Citric acid, anhydrous	0.013%	0.7 mmol/L citrate
Sodium hydroxide, 2N (q.s. to pH 6.8)	~1.11%	~22.2 mmol/L Na
Insoluble Buffer Ingredients		
Calcium phosphate	0.23%	7.5 mmol/L = 22.5 mmol/L Ca, 15 mmol/L P
Magnesium phosphate	0.02%	0.7 mmol/L = 2.7 mmol/L Mg, 1.8 mmol/L P

Based on Gaucheron [27].
[a]*Estimates based upon molecular weight values for calcium citrate tetrahydrate.*
[b]*Estimates based upon molecular weight values magnesium citrate tribasic nonahydrate.*

composition, such a standardization is not feasible, and any effort to establish a singular definitive recipe would reflect only a single set of conditions (breed, diet, lactation stage, etc.). Moreover, the complexity of the milk matrix renders the genesis of a synthetic milk extremely challenging and subject to variation due to manufacturing procedures and difficulty in obtaining many of the ingredients.

Is Standardization Possible?

Among the many potential concerns that would need to be addressed in the development of a standardized medium is how closely the casein micellar structure mimics that which is found in cow milk. While technically, one could generate a 3D structure analysis on these proteins in the two media, the most critical issue for our purposes would be to compare the corresponding estimates of solubility of a range of compounds. Another issue is that milk processing can alter the amount of β-LA. This may impact the relative solubility drug estimates in raw milk (relevant to bovine mastitis), processed milk (relevant to the use of milk to facility drug administration to humans or to drug solubility in mastitic milk) or the proposed synthetic milk medium. This can be assessed by testing drug solubility in all three media during the initial validation of the proposed medium.

Therefore, it is likely that the best possible solution will be to obtain a commercially available milk source. However, the question remains how the various sources may compare to raw milk obtained from a lactating cow. To that end, we engaged in studies to evaluate that possibility. Our first set of collaborative studies, conducted by Dr. Fang Zhao and colleagues at St. John Fisher University, Rochester, New York, involved such an evaluation (*28*). That study involved an assessment of the solubility of a

range of APIs in buffer solution, pH 6.8, raw bovine milk, and commercially obtained skim milk, whole milk, and reconstituted dehydrated whole milk. The second set of comparisons employed the same media but focused specifically on intramammary infusion, examining the solubility of two related compounds that are approved for use in the treatment of bovine mastitis: cephapirin sodium (highly soluble) and cephapirin benzathine (low solubility) (29). These data suggest that an off-the-shelf milk product can be used to assess drug solubility across a range of commodities containing bovine milk and as well as for assessing/comparing the solubility of compounds intended for intramammary infusion (28, 29).

Using Milk as a Dissolution Medium

Of particular interest has been the use of milk as a vehicle for drug administration when evaluating the dissolution of pediatric formulations. This may be particularly important when the medicine is formulated as granules or crushed tablets (30). In these situations, it has been of value to use milk as a component of the dosage form that is added to the biorelevant in vitro dissolution medium. The inclusion of milk in the sample of drug plus vehicle added to the dissolution vessel successfully reflected the higher oral bioavailability of the low solubility drug, montelukast, and provided a higher correlation with the in vivo oral bioavailability reported in fed infants as compared to in vitro dissolution testing conducted when infant (pediatric milk-based) formula or applesauce was used as the administering vehicle.

In some studies, the milk is gradually digested over time to reflect what occurs in vivo (31). In fact, it has been suggested that the lack of attention to the use of milk as a drug delivery vehicle may be a function of the lack of attention given to the importance of post-ingestion milk digestion on the solubility and dissolution of highly lipophilic drugs (32). Along those lines, to identify effective ways to administer this antiparasitic drug to infants in tropical countries, Eason et al. examined the dissolution of the poorly soluble drug, praziquantel (BCS class 2 drug) in milk or infant formula and the consequence of adding pancreatic lipase to these dissolution on drug dissolution (33). These results were compared to the solubility and in vitro dissolution of praziquantel in SGF and in simulated intestinal fed and fasted fluids (FeSSIF and FaSSIF, respectively). Despite a positive food effect on oral bioavailability, the solubility of praziquantel in undigested milk was slightly lower than that in either FeSSIF or in 0.1 M HCl + 2 mg/mL sodium lauryl sulfate (SLS). Drug solubility in infant formula was similar to that in the aqueous media containing SLS or bile salts

(FeSSIF). However, upon digestion with pancreatic lipase, praziquantel solubility in milk and formula increased to more than 3-fold that of the aqueous media. The impact of milk digestion on drug solubility was reflected in the marked rise in the percent drug released into the dissolution medium (100% dissolved), with the profound impact of digestion occurring in milk (33). This work showed that the amount of bile salt needed to effectively solubilize and dissolve praziquantel is markedly greater than the bile salt concentration known to be present in infants. Accordingly, given the digestion of milk (or infant formula) as it moves down the gastrointestinal tract, milk can serve as an effective vehicle to dose this drug to infants.

When used for evaluating highly soluble drugs, the use of milk can delay drug release as compared to that seen using aqueous media (34). This was shown for several acetaminophen formulations where the milk medium contained 3.5% fat (without digestion). Although three acetaminophen (paracetamol) formulations (two immediate-release tablets, one uncoated and one film-coated tablet, and one suspension) exhibited rapid release in simulated gastric fluid, FaSSIF, and FeSSIF, dissolution was markedly slower in milk. Moreover, only minor differences in product dissolution rates were seen in the aqueous buffers, but substantial formulation-associated differences in dissolution rates were seen when tested in milk. In terms of blood level data, when administered to fasted dogs with 200 mL water, the two tablet formulations were found to have differences in T_{max}. However, they were equivalent when administered to dogs with 200 mL milk despite observed differences in gastric tablet disintegration rates seen in a subset of fistulated canine subjects. The authors concluded that the bioequivalence in milk was a result of the delayed gastric emptying in these dogs, which then camouflaged the effects on differences in in vivo dissolution. Importantly, it was only in milk that the formulation effects were seen when the paracetamol tablets administered to dogs with water (34).

The influence of gastric contents in the fed state has led to an examination of how dietary constituents may influence drug dissolution in the fed human stomach. The effect of various food constituents (e.g., casein, egg albumin, and gelatin) on the intrinsic in vitro dissolution of two BCS II drugs (itraconazole and ketoconazole) was examined in SGF media containing milk (3.6%, 1.7% and 0.1% milk fat) or SGF plus other food ingredients such as egg albumin, wheat gluten, glucose, starch, bovine gelatin, glycine, leucine, and aspartic acid (35, 36). The drug solubility

in each dissolution medium was determined using a modified shaker-flask method. The intrinsic dissolution rate (15–240 min) for ketoconazole was similar in SGF plus whole milk, SGF plus partly skimmed milk, and SGF-plus skim milk, whereas dissolution for itraconazole in part SGF-part skim milk was significantly higher than in the other milk media. A markedly lower intrinsic dissolution rate for ketoconazole occurred in the presence of SGF plus other food ingredients, but unlike ketoconazole, itraconazole solubility and dissolution was substantially higher in SGF plus high concentrations of albumin or gelatin. For these two compounds, although casein has excellent emulsifying properties, casein-containing media only slightly increased the solubility or the in vitro dissolution rate as compared to that seen with the milk-media, indicating that the effect of milk was not attributable to its casein content. The positive influence of albumin on itraconazole solubility exceeded that observed for ketoconazole (when ketoconazole is measured between 15-240 min) whereas that of gelatin was greater for ketoconazole as compared to itraconazole. The addition of amino acids had a greater effect on the solubilizing of ketoconazole than of itraconazole. Observed drug-related differences in the relationships between milk-containing media or food ingredients on solubility and in vitro dissolution appear to be related to drug lipophilicity (itraconazole greater than ketoconazole) and charge (itraconazole is mono-ionic at pH 3 while ketoconazole is positively charged at pH 3).

The interest in bovine milk as a vehicle for drug delivery (especially in pediatric and geriatric patients) and its use in predicting drug solubility and formulation-associated product dissolution in the fed stomach points to the need to: 1) understand the composition of milk; and 2) to explore the possibility of defining a way to minimize the variability in milk composition that can occur across sources of the milk. This review can facilitate inter- and intra-laboratory consistency in milk-associated study results.

CONCLUSION

Understanding the complexity of the milk medium, including the magnitude to which solubility and in vitro dissolution evaluations can differ as a function of the inherent variability in milk composition, provides valuable information with respect to the potential challenges that need to be considered when milk is the matrix within which a drug must be solubilized. Efforts to develop a single standardized milk matrix for testing drug solubility would need to address many potential sources of variability; however, this review provides the necessary

information for adding milk as a matrix for testing drug solubility within *USP* <1236>. Furthermore, recent drug solubilization collaborative studies suggest that a close estimate of drug solubility can be obtained by using off-the-shelf whole milk as the surrogate matrix.

FUNDING

CONFLICT OF INTEREST

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