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Evaluating the solubility of compounds intended for intramammary infusion based upon tests conducted across a range of milk matrices

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Abstract

The ability to evaluate drug solubility in milk and milk-related products has relevance both to human and veterinary medicine. Model compounds explored in a previous investigation focused on drug solubility assessments when delivered in milk-associated vehicles for administration to human patients. In the current investigation, we focus on the solubility of drugs intended for delivery via intramammary infusion to cattle. Because there are logistic challenges typically associated with obtaining raw milk samples for these tests, there is a need to determine potential alternative media as a substitute for raw bovine milk. Given the complexity of the milk matrix, aqueous media do not reflect the range of factors that could impact these solubility assessments. This led to the current effort to explore the magnitude of differences that might occur when substituting raw bovine milk with off-the-shelf milk products such as whole milk, skim milk, or reconstituted whole milk powder. We considered conclusions based upon the solubility assessments derived from the use of the model compounds studied in our previous report and compared them to conclusions obtained when testing two drugs with differing physicochemical characteristics that are approved for administration via bovine intramammary infusion: cephapirin benzathine and cephapirin sodium. Based upon these results, we recommend that whole milk or reconstituted whole milk can substitute for bovine raw milk for the solubility assessment of compounds intended for administration via intramammary infusion. However, unlike the human drug situation, these tests should be conducted at 38˚C.

Introduction

Bovine mastitis is a major health problem encountered both within the US and around the world [1]. 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

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herds, even those that are well-managed [2]. Therefore, there is a tremendous need for safe and effective antimicrobials for treating this disease.

Insights into the solubility of a drug within the matrix of interest is a first step towards an appreciation of potential formulation effects based upon an understanding of drug solubility. The ability to predict drug solubility in milk is important both for human and veterinary medicine. In terms of its veterinary application, a number of compounds are approved for the treatment of bovine mastitis via intramammary infusion (IMM). This method of drug administration typically involves the insertion of a syringe tip into the teat canal whereupon the contents of the syringe are expelled into the infected quarter. Subsequently, the quarter is gently massaged to facilitate distribution of the medication (Administration Technique of Intramammary Treatment in Dairy Cows—Trousse Tactic (reseaumammite.org). When administered into the bovine mammary gland, the drug acts locally within the udder and by using an IMM infusion, the ratio of target site drug exposure versus systemic drug exposure is far greater than that which can be achieved by other routes of administration.

Given its unique characteristics in terms of proteins, fats, and ionic composition, it is not appropriate to evaluate the solubility of these products using typical aqueous buffer systems. Milk contains more than 20 proteins, along with a variety of fats and a wide range of ions [3]. Moreover, there is the potential for preferential binding to casein milk proteins, whey, or fat. In addition, we need to consider the structure of the fat globules as well as the structure and stability of casein micelles (which can be impacted by the mineral content of the milk). Therefore, we considered the possibility of developing a standardized milk matrix to support these assessments. However, given the problems we encountered during efforts to produce a synthetic milk medium (both in terms of the lack of availability of some of the necessary components and difficulty in replicating the physicochemical quality of milk from available components of the milk matrix), the possibility of developing some standardizable approach was not considered feasible. It was also unclear as to whether some of the differences that might be encountered across herds could alter conclusions pertaining to drug solubility [4,5]. For this reason, we explore the possibility of variations in milk composition by comparing readily available milk matrices (i.e., off-the-shelf milk products such as whole milk, skim milk, or reconstituted whole milk powder) versus that obtained from freshly obtained raw (unprocessed) bovine milk. Logistic challenges associated with obtaining the raw milk samples necessitated that only a single local farm was evaluated.

In our previous study, the aqueous solubility estimates of six drug compounds (amitriptyline, acetaminophen, dexamethasone, nifedipine, piroxicam and prednisone.) were similar when tested in whole milk, reconstituted whole milk, or raw bovine milk [6]. However, in that paper, the data were not evaluated from the perspective of its relevance to what might occur when drugs are administered by IMM. Therefore, in this manuscript we re-examine those data from the perspective of the implications of those findings to what may be relevant regarding drug versus matrix solubility evaluations for those compounds intended for administration via IMM infusions. The results from the previous study [6] are compared to those associated with the solubility assessment of two approved salt forms of the beta-lactam antimicrobial, cephapirin, which is approved for IMM infusion: [ToMORROW (NADA 109–114, ToMORROW® cephapirin benzathine cefapirina benzatínica FOR INTRAMAMMARY INFUSION INTO THE DRY COWPARA INFUSIÓN INTRAMAMARIA EN VACAS SECAS (nih.gov), which contains cephapirin benzathine and ToDAY (NADA 97–222, DailyMed—TODAY INTRAMAMMARY INFUSION- cephapirin sodium gel (nih.gov)], which contains cephapirin sodium].

Cephapirin sodium is very soluble in water, insoluble in most organic solvents. The pH of a solution in water containing the equivalent of cephapirin 1% is between 6.5 and 8.5. In

Table 1. Basic information of cephapirin and the salt forms [8].

Form	Formula	MW	CAS #	% Active (based upon MW)
Cephapirin (free form)	C17H17N3O6S2	423.5	21593-23-7	100%
Cephapirin sodium (1:1 salt)	C17H16N3NaO6S2	445.4	24356-60-3	95.1%
Cephapirin benzathine (2:1 salt)	C50H54N8O12S4	1087.3	97468-37-6	77.9%

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contrast, cephapirin benzathine is practically insoluble in water, in ether, and in toluene; freely soluble in alcohol; soluble in 0.1 N HCl [7].

Information pertaining to the two API's are provided in Table 1.

For completeness, we also provide information on the model drugs used in our prior assessment of solubility of human drugs when administered in milk related vehicles [6] (Table 2).

The foundation for this evaluation is that by comparing the solubility of several compounds covering a range of physicochemical characteristics and by generating those assessments in milk media that likewise reflect a range of physicochemical characteristics, we can ascertain the robustness of our assessment of drug solubility in the bovine udder (and the corresponding variability that may be present due disease or to factors listed above) and to the milk-associated factors that can most greatly influence that solubility for certain compounds. Lastly, if for reasons of formulation development, an investigator is concerned about the potential influence of pH changes in the mastitic udder to alter the solubility of an ionizable compound (i.e., when the pKa of the compound is within the range of pH values that can occur in mastitic milk), milk pH adjustments can be considered.

Materials and methods

The materials and methodology used for the solubility assessment for the six API's other than cephapirin sodium and cephapirin benzathine are provided in the manuscript by Li et al., 2022 [6]. Source information for the cephapirin sodium and benzathine is provided in Table 3.

The source information for the vehicles used for the solubility testing are provided in Table 4. The milk powder was reconstituted with warm water (30–40˚C) at 13% w/v. All vehicles were stored under refrigeration (2–8˚C) until use. Raw milk and reconstituted whole milk were used within 24 hours. Skim and whole milk were used within expiration dates.

Table 2. Model drugs and their selected physicochemical properties [9–15]. Vendor information is provided in [6].

Drug	Solubility in Water at 25˚C[a]	pKa	Ionization state at pH 7	Log P	Plasma Protein Binding
Acetaminophen	Sparingly Soluble (10–33 mg/mL)	9.5 (acid)	Neutral	0.2	20–25%
Amitriptyline HCl [b]	Freely Soluble (100–1000 mg/mL)	9.45 (base)	Positively charged	3.0 (pH 7.4) 5.0 (free base)	~ 95%
Dexamethasone	Practically Insoluble (≤ 0.1 mg/mL)	Non-ionizable	Neutral	1.83	~77%
Nifedipine	Practically Insoluble (≤ 0.1 mg/mL)	-0.9 (base) 13 (acid)	Neutral	2–4	91–98%
Piroxicam	Practically Insoluble (≤ 0.1 mg/mL)[c]	1.86 (base) 5.46 (acid)	Negatively charged	-1.33 (pH 6.5) 0.72 (pH 6.6)	~ 99%
Prednisolone	Very Slightly Soluble (0.1–1 mg/mL)	Non-ionizable	Neutral	~ 1.6	60–90%

[a] The solubility descriptions and ranges are based on USP definitions [16].

[b] Amitriptyline was only available as the HCl salt commercially at the time of the study.

[c] vvv is pH dependent. At pH 6.8, 37˚C, it was reported as 0.47 mg/mL [14].

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Table 3. Cephapirin source information.

Drug	Vendor	Catalog #	Lot #
Cephapirin sodium USP RS	Sigma-Aldrich Inc St. Louise, vMO	1102500-500MG	R116A0
Cephapirin benzathine USP RS	Sigma-Aldrich Inc St. Louise, MO	1102408-100MG	F03082

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Two types of syringe filters were purchased from Phenomenex (Torrance, CA) and used for solubility sample treatment prior to HPLC analysis. These included Phenex-GF 1.2 μm filters (Cat# AF0-8515-12) and Phenex-RC 0.45 μm filters (Cat# AF0-3103-52).

A Milli-Q Direct 8 system from Millipore Sigma (Burlington, MA) was used to produce purified water. Acetonitrile and trifluoroacetic acid (TFA), both HPLC grades, were purchased from Thermo Fisher Scientific (Waltham, MA).

Physicochemical characterization of milk vehicles

The milk vehicles were characterized by pH, osmolality, viscosity, and globule size distribution. A 10 mM sodium phosphate buffer pH 6.8 was also included as a control.

pH. A Mettler-Toledo (Columbus, OH) Seven Easy model pH meter was used with a gel-filled pencil-thin pH electrode from Thermo Fisher Scientific (Waltham, MA). All vehicle samples were equilibrated to room temperature prior to the pH measurement.

Osmolality. A μOsmette Model 5004 osmometer from Precision Systems (Natick, MA) was used for osmolality measurement, which operates based on the freezing point depression method. For each measurement, 50 μL of the vehicle was placed in the sample tube and lowered into the freezing chamber. After the solenoid-induced pulse freezing, the liberated heat of fusion was related by the microprocessor to the freezing point of the sample, and the osmolality was automatically calculated and displayed.

Viscosity. A DHR-2 rheometer with a Peltier temperature-controlled cup and a double-gap concentric cylinder measurement system from TA Instrument (New Castle, DE) was used for viscosity measurements at 25˚C. For each measurement, 12 g of the vehicle was placed in the cup, and the shear rate was scanned from 0.001 to 1 sec^{-1} on a log-scale with 5 points/decade. The method was set to determine the steady-state viscosity result with a maximum equilibration time of 480 sec at each point.

Globule size distribution. A LA-960 laser scattering particle size analyzer from Horiba (Piscataway, NJ) was used to analyze the globule size distribution of the milk related vehicles at room temperature. A refractive index of 1.45 was used for the oil phase and 1.333 for the aqueous phase. The milk was added dropwise to deaerated water to achieve 90–95% transmission for the red laser. A circulation speed of 2 was used with no sonication, and a data acquisition of 10,000 per read.

Table 4. Vehicle information.

Vehicle	Vendor/Manufacturer
Skim Milk	Wegmans Food Markets, Rochester, NY
Whole Milk	Wegmans Food Markets, Rochester, NY
Whole Milk Powder (Nestle Nido® brand)	Walmart, Rochester, NY
NIST Whole Milk Powder (SRM 1549a)	National institute of standards and technology, Gaithersburg, MD
Raw Milk	Craft Dairy Farm, Williamson, NY

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Solubility assessment and sample treatment for HPLC analysis

Solubility assessment was performed in all milk vehicles listed above. A 10 mM sodium phosphate buffer pH 6.8 was also included as a control. For each solubility sample, an excess amount of drug powder was weighed in a 20-mL glass vial. A suitable volume of the vehicle, pre-equilibrated to the desired temperature, was added to the drug powder. The samples were stirred for 60 minutes at 38˚C or 20˚–25˚C [room temperature (RT)] prior to processing. The temperature of 38˚C reflects the rectal temperature the healthy adult cow, 38˚C [17].

Upon completion of this portion of the test procedure, approximately 2 mL of the solubility sample was passed through the Phenex-GF 1.2 μm filter to remove the excess drug solid. The filtration and solvent extraction steps were carried out immediately for each sample after the solubility testing was completed. All supplies used in the filtration were pre-equilibrated at the corresponding temperature. As noted in manuscript by Li et al., 2022 [6], that pore size does not filter out the milk proteins present as whey or casein micelles, or the drug entrapped within fat globules. An aliquot of 0.5 mL filtrate was accurately transferred to a microcentrifuge tube followed by the addition of 1 mL extraction solvent. The extraction solvent was 0.1% TFA in acetonitrile (mobile phase channel B). The mixture was then centrifuged at 10,000 rpm, and about 1 mL of the supernatant was passed through a Phenex-RC 0.45 μm filter. The filtrate was collected in an HPLC autosampler vial and analyzed by the HPLC method described below.

HPLC analysis

A gradient HPLC method, described in Table 5, was used to analyze the concentration of all drug solubility samples after treatment.

The HPLC output confirmed the separation of cephapirin from other impurities, including that of any potential metabolite.

Results

When the highly soluble cephapirin sodium was added to the various milk samples, the color of the sample changes from white to deep yellow, even when tested at RT (Fig 1). However, increasing the temperature enhanced solubilization. This is illustrated by the increase in sample clarity in raw and reconstituted whole milk when the temperature is elevated from RT to 38˚C (Fig 2).

Physicochemical properties of milk media (Table 6)

The pH values of all milk vehicles ranged from 6.6 to 6.8 (Table 6). With the except of the reconstituted whole milk powder, all other milk forms exhibited similar osmolality. The reason

Table 5. HPLC method parameters.

HPLC System	Shimadzu Model# LC-2010AHT
Column	Phenomenex Kinetex C18, 150×4.6 mm, 5 μ, 100 Å (Phenomenex, Cat# 00F-4601-E0)
Column Temperature	40˚C
Mobile Phase	Channel A: Water with 0.1% v/v TFA Channel B: Acetonitrile with 0.1% v/v TFA Linear gradient program: 5% to 60% Channel B in 10 minutes with 5 minutes of re-equilibration time. Flow rate: 1.0 mL/min
UV Detection	260 nm
Inject Volume	10 μL

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Fig 1. Photos of cephapirin sodium (CES) samples in various milk media at RT. Left to right: skim milk control, CES in skim milk, whole milk control, CES in whole milk.

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for the lower osmolality of the reconstituted Nestle and NIST whole milk powder is speculated to be attributable to an incomplete dissociation of the ingredients in water. All products except the reconstituted whole milk powder from NIST exhibited a viscosity of <5 mPas. This disparity is consistent with the larger globule diameter of the NIST product, being about 10-fold greater than that of raw milk. The larger globule size of the raw milk as compared to whole milk is not surprising given that the whole milk was homogenized. Similarly, the skim milk is effectively devoid of milk fat, rending the globule sizes about 5.3-fold less than that of the whole milk. While most of the data in this table was presented in the prior publication, it is listed again here for comparison with the new data generated with the NIST powder.

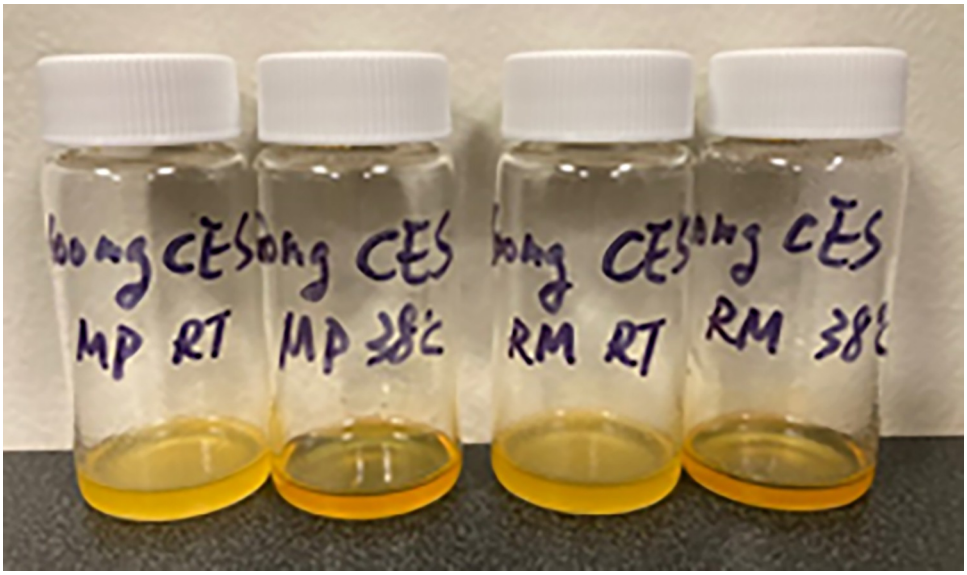

Fig 2. Photos of cephapirin sodium (CES) samples in various milk media at RT or 38˚C. Left to right: CES in recon milk powder RT, recon milk powder 38˚C, raw milk RT, and raw milk.

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Table 6. Pvvroperties of milk related vehicles [mean ± standard deviation (SD)].

Vehicle	pH	Osmolality (mOsm/kg)	Viscosity at 1 sec^{-1} (mPas)	Globule size D_{50} (μm)
Skim milk	6.65 ± 0.05	282 ± 1	< 5	0.136
Whole milk	6.68 ± 0.04	277 ± 1	< 5	0.726
Raw milk	6.70 ± 0.01	284 ± 2	< 5	3.61
Reconstituted whole milk powder (Nestle)	6.72 ± 0.03	234 ± 2	< 5	0.512
Reconstituted whole milk powder (NIST)	6.61±0.01	242±1	35	33.7

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Although the information contained in Table 5 was previously included in [6], this information on the properties of milk-related vehicles is included in the current manuscript because of its relevance to an interpretation of the results from the current investigation.

The NIST powder was included in this study by virtue of its standard chemical composition. Given the large globule size and high viscosity, it did not provide the physical properties of raw milk upon reconstitution and was therefore excluded from any further analysis. A potential reason for these differences may be the amount and composition of fats in the NIST product (as described in SRM 1549a) versus what has been reported in whole milk (e.g., that reported by the USDA [3]).

Solubility

With the exception of the pH 6.8 aqueous buffer, the solubility of cephapirin sodium exceeded 350 mg/mL in the tested media. This is consistent with its classification of freely soluble. In contrast, markedly lower solubility was observed for the benzathine salt. Interestingly, the solubility in raw milk was somewhat lower than that observed in whole milk or whole milk powder but rather was more closely aligned with that of the skim milk. Nevertheless, across all media, the USP solubility classification of very slightly soluble remained the same (Table 7).

Since this study was directed specifically for drugs used for IMM infusion, we repeated the assessment of solubility using raw or reconstituted whole milk maintained at 38˚C. Due to limited quantities of the API source, only two vehicles were tested at 38˚C. This temperature is within the range of normal body temperatures for an adult cow (37.8–39.2˚C) [9]. We also used two lots of milk powder to determine the potential for interlot variation. The results of these assessments are shown in Table 8.

In general, although its USP solubility classification [18] was not influenced by temperature, the use of higher temperatures increased the mg/mL of cephapirin dissolved from both salt forms, with the ratio of solubility results at 38˚C/RT ranging between 1.42–1.50. Therefore, the temperature-associated increase in cephapirin solubility was comparable in either

Table 7. Solubvility of cephapirin in selected milk vehicles at room temperature (n = 3) (mean ± SD).

Drug	Solubility (mg/mL) [a]				
	pH 6.8 buffer (control)	Skim Milk	Whole Milk	Reconstituted Whole Milk Powder (Nestle)[c]	Raw Milk [c]
Cephapirin Sodium [b]	> 200	> 350	> 350	> 350	> 350
Cephapirin Benzathine	0.352 ± 0.003	0.460 ± 0.007	0.509 ± 0.010	0.508 ± 0.006	0.476 ± 0.003

a. Solubility values are expressed as the free form of cephapirin, not the salt form.

b. Due to the high solubility of cephapirin sodium and limited availability of drug substance, each value was obtained from only one sample.

c. Raw and reconstituted whole milk samples, with or without drug, exhibited high back pressure during filtration (1.2 μm pore size) at RT.

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Table 8. Solubility of cephapirin in selected milk vehicles at 38˚C and nifedipine at RT or 38˚C (n = 3) (mean ± SD).

Drug	Solubility (mg/mL) [a]		
	Reconstituted Whole Milk Powder (Nestle)		Raw Milk
Nifedipine RT	0.0334 ± 0.0067		0.0120 ± 0.0014
Nifedipine 38˚C	0.0947 ± 0.0143		0.0396 ± 0.0043
Cephapirin Sodium [b]	> 500		> 500
Cephapirin Benzathine[c]	Lot 1	Lot 2	0.706 ± 0.021
	0.740 ± 0.005	0.762 ± 0.028	

a. Solubility values are expressed as the free form of cephapirin, not the salt form.

b. Due to the high solubility of cephapirin sodium and limited availability of drug substance, each value was obtained from only one sample.

cv per lot of reconstituted whole milk.

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reconstituted whole milk or raw milk. Similar outcomes were observed with nifedipine where the solubilized ratios at 38˚C/RT were 2.8 and 3.3 in whole and raw milk, respectively [6].

Nifedipine is included in Table 8 because it was the one compound where the solubility in the reconstituted versus raw milk differed, regardless of temperature (2.78-fold higher in reconstituted than raw milk at RT and 2.4-fold higher in reconstituted than raw milk at 38˚C). Moreover, within each matrix, there was approximately a 3-fold increase in nifedipine solubility when temperatures were increased from RT to 38˚C. This is in contrast to what was observed with the two cephapirin compounds where less than a 10% difference was seen across all milk media (RT). Only the aqueous buffer failed to provide quantitatively similar results. Since the cephapirin sodium had high solubility at (>500mg/mL) in reconstituted and raw milk, we only evaluated cephapirin benzathine to assess the impact of temperature at batch of reconstituted milk. The ratio of mean solubility values across all media tested RT or 38˚C) is provided in Fig 3.

The unusual behavior of nifedipine, even with relationship to the previously investigated human model compounds is evidenced further in Table 9 where the solubility of each compound in four media (aqueous buffer, pH 6.8, skim milk, whole milk and reconstituted whole milk) are compared to that observed in raw bovine milk. Other than nifedipine, all of the other four compounds (with differing physicochemical characteristics as described in Table 2) had a ratio of solubility in reconstituted whole milk versus raw milk ranging between 0.88–1.16.

Interestingly, as compared to that of raw milk, the greater relative solubility of piroxicam in aqueous buffer versus skim milk most probably the use of mean values. When considered in light of the standard deviation associated with the solubility estimates obtained in aqueous buffer (0.287 ± 0.019 mg/mL) versus skim milk (0.262 ± 0.005 mg/mL) [6], piroxicam solubility in these two media are effectively the same. Therefore, both skim milk and aqueous buffer matrices are considered equally poor in terms of reflecting drug solubility in raw bovine milk.

One important difference between observations at 38˚C versus RT was that the raw and reconstituted whole milk samples at RT, with or without drug, exhibited elevated back pressure during filtration (1.2 μm pore size). This problem was not encountered when the test was conducted at the bovine body temperature.

Discussion

The goal of this study was to identify readily available media that could be used to predict the solubility of compounds when administered via infusion into the bovine mammary gland. To meet this goal, both previously examined model drugs and two products approved by the FDA for IMM infusion (cephapirin sodium and cephapirin benzathine) were evaluated in aqueous

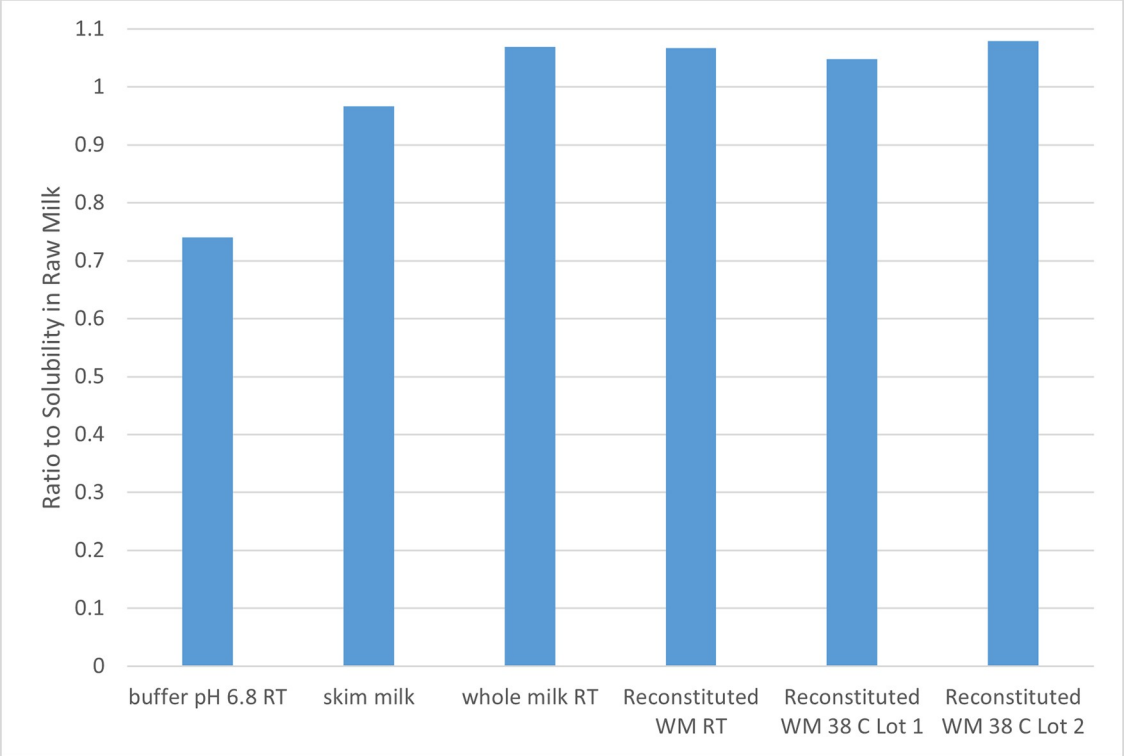

Fig 3. Ratio of cephapirin benzathine solubility across the 3-milk media and at RT versus 38˚C.

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buffer, skim milk, whole milk, reconstituted whole milk, and raw bovine milk. Along with studying the effects of media, we also examined potential experimental challenges that could arise as a function of this assessment.

Filtration problems encountered with reconstituted whole milk and raw milk at room temperature were not seen when testing was conducted at 38˚C. While this may have had some contribution to the temperature-associated differences in drug solubility in raw versus reconstituted whole milk, it is unlikely to have had a substantial impact since the solubilities in raw milk, reconstituted whole milk, and whole milk were similar under room temperature conditions (with whole milk not having the same filtration challenges).

Regarding the testing at RT versus 38˚C, we find that the higher temperature is associated with greater solubility estimates. An even greater difference was seen for nifedipine in the previous study [6] where the solubility in raw milk and reconstituted whole milk increased approximately 3-fold. Since the increase in temperature is more physiologically relevant for the cow, the demonstrated increase in the solubility of active pharmaceutical ingredients

Table 9. Ratio of solubility estimate across compounds in the various media versus raw milk in RT (data from Li et al., 2022 [6]).

	pH 6.8 buffer	Skim milk	Whole milk	Reconstituted whole milk
acetaminophen	1.05	1.08	1.05	1.01
dexamethasone	**0.64**	1.04	1.08	1.16
nifedipine RT	**0.43**	**1.53**	**2.53**	**2.78**
piroxicam	**0.74**	**0.67**	0.87	0.88
prednisolone	**0.33**	1.04	1.02	1.00

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across a range of physicochemical characteristics, along with the reduced problems associated with filtration pressure, leads us to conclude that when considering solubility testing of drugs intended for IMM infusion, testing should be conducted at 38˚C.

Due to limitations with drug quantities, the day-to-day variability associated with the analytical method was not quantified. Therefore, it is unclear the degree to which this may have contributed to small differences seen between whole milk, raw milk, and lots of the reconstituted product. Nevertheless, given that the solubility values were typically similar across these three media, any bias in results due to inter-day variability should be minor.

With the exception of nifedipine, the solubility estimates of the model compounds provided by Li et al., 2022 [6] resulted in the same USP solubility classification across all media except that of the aqueous buffer. However, some differences were observed in the magnitude of drug solubility across the milk media used in this investigation. When considered relative to the solubility results obtained with raw bovine milk, the results obtained in whole milk and reconstituted milk were similar, but one compound, piroxicam, had a markedly lower solubility in skim milk versus raw milk. All media failed to adequately reproduce the solubility of nifedipine, with whole milk and reconstituted whole milk having about 2–3-fold higher solubility than that in raw milk. In addition, skim milk failed to adequately reproduce the solubility piroxicam, a compound that for which similar solubility was observed in whole milk, reconstituted whole milk, and raw milk.

An additional uncertainty was whether drug solubility in homogenized whole milk would be comparable to that in raw milk. In particular, the raw milk contains milk fat globules, where the lipids are contained within a membrane consisting of proteins, cholesterol, and phospholipids. In contrast, the homogenization process alters the contents and geometry of the milk fat globules, with the nature of the change varying as a function of method pressure and temperature [19]. Whether the availability of lipids within the natural membrane structure impacts the solubilization of lipophilic compounds has yet to be determined. However, from the data generated in this investigation and that of the model compounds [6], it can be concluded that any difference in the solubility of drugs in whole versus raw bovine milk will be relatively small.

The results obtained with nifedipine are particularly interesting since it is a weak acid (pKa 13) that is insoluble in aqueous (~10 μg/mL in water at 37˚C), soluble in organic solvents, has a log K_{OW} of 2.2 and is known to present problems associated with in vitro dissolution [20–22]. Therefore, we expected that its solubility in whole milk would provide a far better reflection of raw milk as compared to that seen with skim milk. However, this was not the case, as shown in Table 8. In part this may be a function of the homogenization process allowing for greater dispersion of the nifedipine within the homogenized whole milk (i.e., enhancing its solubility) as compared to that associated with the raw milk where the fat is present as distinct globules or in skim milk. Nevertheless, this does not explain why the solubility in skim milk was still about 50% higher than that seen with raw milk. That this was not associated simply with the aqueous phase of the milk is supported by the lower solubility observed in buffer. Given the 92–98% plasma protein binding of nifedipine [23] the use of milk clearly provided an advantage over protein-free buffer systems in terms of solubilizing this API. Given that the protein contents of whole and skim milk are similar (15.4 g versus 16.5 g, respectively per 16 oz. glass) [24], there remains an uncertainty as to why it had a higher solubility as compared to that of raw milk. Clearly, further investigation is needed to understand these results more fully.

We know that the composition of raw and commercially available whole milk products can vary as a function of breed, diet, environment, and lactation stage [5]. Moreover, when administered into an inflamed and infected udder, further differences in milk composition are likely

to exist [25]. Therefore, we cannot expect any singular medium to provide an absolute solubility value for an IMM dosage form. Correspondingly, the solubility assessment should strive to provide an assessment sufficient to determine a range of drug solubilities such that it can be characterized in accordance with the descriptive terms provided in USP 29 of USP characteristics [18].

Conclusion

Since the model compounds, including the cephapirin benzathine, exhibited similar solubility in whole milk and in reconstituted whole milk, it appears that either store-bought whole milk or reconstituted whole milk could be used in lieu of raw milk to obtain an estimate of drug solubility within the bovine udder. Although we recognize that we did not evaluate the impact of factors that could influence the composition of bovine milk (as discussed in the introduction), the similarity in solubility estimates across these two sets of media suggest that normal variations that may occur in bovine milk under field conditions would not impact conclusions associated with the solubility of drugs intended for IMM. In that regard, because of its similarity to the solubility characteristics of raw milk, ready availability, and lesser challenges with respect to filtration, we conclude that testing solubility of API's intended for IMM injection can be performed using shelf-ready whole milk. Lastly, given the impact of temperature on the solubility results, we recommend that all tests be conducted at 38˚C.

Author Contributions

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