Effect of Temperature and Fat Content on the Solubility of Hydrochlorothiazide and Chlorothiazide in Milk

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Abstract □ The solubility of hydrochlorothiazide and chlorothiazide in milk has been studied. Experiments were carried out at 5, 15, 25, and 37 °C on a buffer solution of pH 6.5, a 2.6% solution of casein, bovine skim milk samples, and bovine milk samples with fat contents of 0.75, 1.70, and 3.50%. The "total" solubility of both drugs in the media studied was higher than the buffer solubility. The highest "total" solubility for both drugs was observed in skim milk. Based on binding data of thiazides to milk, the "total" solubility was split into "free" and "bound" solubility. The increases of solubility noted cannot be explained on the basis of drug–milk binding data. The enhancement of solubility was attributed to the increase of intrinsic solubility of drugs in milk. Results of the thermodynamic analysis of solubility data showed that a different solubilization process of hydrochlorothiazide may be responsible for the high solubility values found in skim milk for this drug. In contrast, the thermodynamic parameters of chlorothiazide in all types of milk are similar, indicating a common solubilization mechanism. The biopharmaceutical significance of the findings is discussed in light of the freeze-dried drug–milk formulations and coadministration of drugs with milk in general.

Drug–milk freeze-dried formulations[1,2] are promising for the enhancement of the bioavailability of sparingly water soluble drugs. However, the proper design and development of drug–milk formulations require the evaluation of the factors involved in the preparation and in vivo performance of the drug–milk system.

Two of the most important factors which may affect the bioavailability of a drug from such a formulation are the binding of drug to milk and the solubility of drug in milk. When specific binding is operating, these two factors are interrelated since solubility is the highest point experimentally obtainable on the binding isotherm. However, we have previously shown,[3] using hydrochlorothiazide and chlorothiazide as model drugs, that the binding of these two thiazides to milk is not dependent on the drug concentration in the range used for formulation purposes. The question then arises as to how the solubility of drugs in milk is related to the estimates of binding of drugs to milk. An equally important issue relevant to this question is the overall effect of milk binding on the solubility of drugs in milk at various temperatures.

To the best of our knowledge there are no other reports in the literature dealing with the solubility of drugs in milk. The present work is aimed at evaluating for the first time the solubility of two model drugs (i.e., hydrochlorothiazide and chlorothiazide) in milk as a function of temperature and milk fat content, with regard to the milk binding studies with these two thiazides recently reported.[3]

Theoretical Section

It is well known that milk is a complex medium containing various components (casein micelles, fat globules, proteins) in various degrees of dispersion. For our purposes, however, milk can be considered to consist of two phases, namely, the aqueous phase (milk serum) and the organic phase (proteins and lipids). Binding of hydrochlorothiazide and chlorothiazide to milk reported previously[3] represents the partitioning of drugs between the aqueous and the organic phase of milk. In the previous work,[3] an attempt was also made to calculate how much of milk binding of thiazides is due to protein binding versus lipid distribution.

In the present investigation we attempt to determine the saturation solubility of two thiazides in milk. Since this work is an extension of the previous study[3] in the region of the saturation solubility of the two drugs in milk, we associate the estimates of thiazides binding to milk with the present findings. Accordingly, a number of definitions concerning the solubility of drugs in milk are introduced and used hereafter. *Total solubility of drug in milk* or *saturation solubility of drug in milk* represents the total amount of dissolved drug in both the aqueous and the organic phase of milk per unit volume of milk in equilibrium with the solid drug. *Free solubility of drug in milk* represents the amount of dissolved drug in the aqueous phase of milk (milk serum) per unit volume of milk in equilibrium with the solid drug and organic phase. *Bound solubility of drug in milk* represents the amount of dissolved drug in the organic phase of milk per unit volume of milk in equilibrium with solid drug and aqueous phase. Obviously, the sum of "free" and "bound" solubility is equal to the "total" solubility of drug in milk. *Intrinsic solubility of drug in milk* represents the amount of dissolved drug per unit volume of protein- and fat-free milk water (milk serum). It's value is identical to the "free" concentration of drug in milk at the equilibrium of saturation solubility. When the milk components do not influence the solubility of drug in the aqueous phase of milk, then the intrinsic solubility of drug in milk should be equal to its solubility in an aqueous buffer with a pH the same as that of milk.

Experimental Section

Materials—Hydrochlorothiazide was supplied by Ciba-Geigy, Athens, Greece. Chlorothiazide was obtained from Chropi, Piraeus, Greece. Both drugs were found to be 99% pure by high-performance liquid chromatography (HPLC). Soluble casein, light white, was obtained from BDH Chemicals, Poole, UK. Milk was provided by Landgenossenschaft, Ennstal, Stainach, Steiermark, Austria. The milk fat content was 0.75, 1.50, and 3.50%. Skim milk was from Evga, Athens, Greece, with a fat content of 0.1%. Inter- and intrabatch variability of fat, protein, and lactose content of the various milk types utilized were estimated directly in undiluted samples using an infrared milk analyzer (Multispec-M, York, UK). Fat contents were found to be in accord with the values declared by the manufacturing company. Protein and lactose contents of all types of milk examined were identical to those reported previously.[3]

Solubility Experiments in Milk—The solubility of the two thiazides in milk was studied using an equilibrium dialysis apparatus with Teflon cells (type Macro 2, Diachema AG Rüshlikon, Zürich, Switzerland) thermostated at 5, 15, 25, and 37 °C in a water bath (Julabo SW1). Solid drug in excess was placed in one (donor) of the two compartments of the dialysis cell. The cell was filled with milk,[4]

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which was then dialyzed against the same type of milk in the other compartment (receiver) for 6 h, using cellulose dialysis membranes (Diachema, type 10-14) with a declared molecular weight cutoff of 5000. The dialysis cell was rotated at 20 rpm. At the end of each dialysis run, the content of the receiver compartment was sampled and analyzed as described below. At least four dialysis runs were carried out for each solubility experiment.

Solubility Experiments in Casein—The solubility of both drugs in the presence of casein was also examined at the same temperature range. A solution of soluble casein 2.6% (w/w) in phosphate buffer (0.2 M, pH 6.5) was used. The procedure was identical to that described above for milk solubility experiments.

Solubility Experiments in Buffer—The aqueous solubility of the two drugs was also examined at 5, 15, 25, and 37 °C for comparative purposes. This study was carried out using 0.2 M phosphate buffer (pH 6.5). Solid drug in excess was dispersed in 10 mL of buffer and the solution was allowed to become saturated while being shaken at 140 rpm in an incubator (Julabo SW1). After equilibration (14 h), an aliquot was filtered through a Millipore 0.45-μm filter, and the drug concentration in the filtrates was determined by UV spectrophotometry using a Perkin Elmer model lambda 7 spectrophotometer. Absorbance values were measured at 272 and 280 nm for hydrochlorothiazide and chlorothiazide, respectively.

Determination of Drugs in Milk and Casein Samples—The determination of the drugs in the milk and casein samples taken from the receiver compartment cannot be performed directly without any sample pretreatment. Various precipitation and extraction procedures have been proposed[5–7] in order to avoid interferences caused by the complex matrix of milk. These time-consuming procedures have been developed and applied to milk samples with very low drug concentrations. Due to the high drug concentration in the milk samples of the present study, the applicability of those procedures is questionable at least from the point of view of drug recovery.

For the analysis of milk and casein samples in the present study, a novel flow-injection dynamic dialysis technique developed in this laboratory[8,9] was effectively utilized. Details of the procedure are as follows. Aliquots (1 mL) of each milk and casein sample were diluted with 9 mL of phosphate buffer (0.2 M, pH 6.5) and dialyzed as previously described,[9] using a dialysis time of 5.00 min and water as carrier solution. The obtained milk and casein dialysates (1.5 mL) were then analyzed by HPLC methods[10] using the mobile phase described previously,[3] while the internal standards were those recommended by Barbhaiya et al.[10]. Standard solutions of each drug in identical media of the solubility experiments, with final concentrations in the range 10–200 μg/mL, were treated in a similar way for the construction of the calibration curve.

Results and Discussion

Although the equilibration time (6 h) utilized for the solubility experiments in milk was relatively short, it was sufficient for the achievement of saturation solubility. This was verified by the fact that longer experiments (10 h) resulted in statistically equivalent estimates of solubility for both drugs examined. This observation is indicative of good wettability of drug powders in milk.

Clear chromatograms were obtained as a result of the pretreating dialysis step of milk and casein samples (Figure 1). The dialyzable species of milk were eluted first and did not interfere with either drug. Working calibration curves were linear in all cases in the concentration range 10–200 μg/mL, with correlation coefficients ranging from 0.997 to 0.999. The precision of the analytical method used (dynamic dialysis, measurement) was very good, with percent of RSD 1–2 (n = 5). Overall, the good reproducibility of the analytical methodology employed was also reflected by the low variability of solubility values (Tables I and II).

As can be seen in Tables I and II, the solubility of both drugs in all types of milk studied is higher than the solubility of drugs in the buffer. Obviously, the solubility of drugs in milk represent "total" solubility in this medium. Using the binding results reported previously,[3] which clearly show a binding phenomenon not dependent on drug concentration, it was then possible to partition the "total" solubility into "free" and "bound" solubility. This required the assumption that the constant binding observed[3] over a wide range of drug concentrations could be extrapolated to concentrations near the solubility of the drug. Thus, the estimation of "free" solubility was based on eq 1:

$$\text{"free" solubility} = \text{"total" solubility} - \text{"bound" solubility} = \text{"total" solubility} - (\text{fraction bound})(\text{"total" solubility}) = (\text{"total" solubility})(1 - \text{fraction bound}) \quad (1)$$

Estimates of the fraction bound of the two thiazides to the various types of milk and the casein solution were taken from the previous study.[3] The values of "free" solubility so obtained were further compared with the aqueous solubility of drugs in the buffer quoted in Tables I and II. This comparison revealed that the increase in milk solubility cannot be explained, in the majority of cases, on the basis of drug–milk binding data (Tables III and IV).

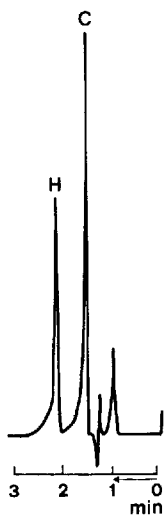

Figure 1—Typical chromatogram of a standard chlorothiazide (C) solution in milk of 80 μg/mL (before dialysis). Hydrochlorothiazide (H) was used as internal standard.

Table I—Solubility of Hydrochlorothiazide in Various Media at Different Temperatures[a]

Temperature, °C	Solubility, μg/mL					
	Buffer (pH 6.5)	Casein Solution (2.6% w/w)	Skim Milk	Milk (0.75%)[b]	Milk (1.70%)[b]	Milk (3.50%)[b]
5	232.7 (4.7)	256.9 (5.1)	398 (13)	339.9 (8.2)	331.1 (3.5)	339.4 (4.7)
15	380.1 (7.5)	391.2 (4.4)	635 (24)	515.8 (1.7)	518.6 (7.7)	504.1 (8.5)
25	568 (14)	661.4 (7.9)	955 (30)	855.8 (6.7)	815 (33)	729.7 (1.1)
37	1017 (12)	1268 (25)	1727.4 (0.58)	1626 (20)	1619 (66)	1657 (11)

[a] Mean of four experiments with standard deviations in parentheses. [b] Milk fat content.

Table II—Solubility of Chlorothiazide in Various Media at Different Temperatures[a]

Temperature, °C	Solubility μg/mL					
	Buffer (pH 6.5)	Casein solution (2.6% w/w)	Skim Milk	Milk (0.75%)[b]	Milk (1.70%)[b]	Milk (3.50%)[b]
5	115.7 (0.48)	138.8 (4.4)	208.8 (4.2)	182.8 (1.6)	161.9 (2.8)	158.2 (6.5)
15	160.5 (6.3)	265.6 (8.0)	286 (36)	310.8 (5.5)	292.0 (3.9)	282.2 (3.8)
25	355.5 (1.4)	485.5 (5.7)	699.2 (2.3)	545.5 (9.3)	551.4 (4.3)	491.8 (2.8)
37	785.9 (9.5)	1001.6 (8.2)	1135 (58)	1065 (23)	1033 (54)	950 (19)

[a] Mean of four experiments with standard deviation in parentheses. [b] Milk fat content.

An inspection of the results quoted in Tables III and IV indicates that the greatest enhancement of "free" solubility for both drugs was observed in skim milk at all temperatures studied. For hydrochlorothiazide, unaltered or slightly lower aqueous solubility in milk with the various fat contents than in buffer was noted at 5 and 15 °C. However, hydrochlorothiazide exhibits (at 37 °C) remarkably higher "free" solubility in all types of milk examined than in buffer. By contrast, the "free" solubility of hydrochlorothiazide in the casein solution is considerably lower than its solubility in buffer at 5, 15, and 25 °C. This could be attributed either to lower hydrochlorothiazide binding to casein at the solubility region than that observed in the binding experiments,[3] or to a decrease of its intrinsic solubility into the casein solution. The former hypothesis can be rationalized on the grounds of the saturable binding phenomenon; however, it is not in accord with the nonspecific character of the binding observed.[3] It is more likely that the changes occurring on the casein micelles over the lower temperature range[11] cause the reduction of the intrinsic solubility of hydrochlorothiazide. It is known that the deformation of micelles at lower temperatures reduces the hydrophobic character of the medium, since β-casein dissociates from the micellar "core"[11,12] and the voluminosity of micelles (i.e., the weight of water incorporated in the micellar structure per unit weight of protein) is less.[13–15] However, the reduction of intrinsic solubility of hydrochlorothiazide does not counterbalance the binding effect since the "total" solubility of hydrochlorothiazide in the casein solution is still higher than its buffer solubility over this temperature range (5–25 °C).

Table III—Comparison of "Free" Solubility of Hydrochlorothiazide in Various Media with Its Solubility in Buffer at pH 6.5

Temperature, °C	"Free" Solubility − Aqueous Solubility in pH 6.5 Buffer, μg/mL[a]				
	Casein Solution (2.6% w/w)	Skim Milk	Milk (0.75%)[b]	Milk (1.70%)[b]	Milk (3.50%)[b]
5	−33.6 (7.6)	47 (23)	NS[c]	NS[c]	NS[c]
15	−73.0 (9.8)	79 (27)	NS[c]	NS[c]	−25 (13)
25	−48 (28)	182 (36)	75 (17)	44 (38)	NS[c]
37	NS[c]	351 (20)	243 (36)	220 (73)	282 (29)

[a] SD in parentheses. [b] Milk fat content. [c] Nonsignificant difference at p = 0.05 level using paired *t* test.

Table IV—Comparison of "Free" Solubility of Chlorothiazide in Various Media with Its Solubility in Buffer at pH 6.5

Temperature, °C	"Free" Solubility − Aqueous Solubility in pH 6.5 Buffer, μg/mL[a]				
	Casein Solution (2.6% w/w)	Skim Milk	Milk (0.75%)[b]	Milk (1.70%)[b]	Milk (3.50%)[b]
5	NS[c]	47.8 (6.0)	15.4 (3.3)	NS[c]	NS[c]
15	52 (11)	65 (38)	80 (11)	63.5 (8.2)	55.7 (8.0)
25	74.2 (8.0)	254 (22)	81 (13)	91.7 (8.2)	29.6 (8.1)
37	87 (16)	194 (64)	113 (29)	85 (59)	NS[c]

[a] SD in parentheses. [b] Milk fat content. [c] Nonsignificant difference at p = 0.05 level using paired *t* test.

The "free" solubility of chlorothiazide in the casein solution appears to be enhanced at 15, 25, and 37 °C, while it remains unaltered at 5 °C. The dissimilarity of the influence of temperature on the "free" solubilities of two thiazides in the casein solution is apparently linked with the differences in one or more of the physicochemical properties[16] such as solubility, ionization, and lipophilicity.

Overall, the solubility of two thiazides in the casein solution is much too small to account for the increased total solubility in milk. This implies that the noted increases in solubility are linked to the more complex nature of milk and therefore the casein solution cannot be used as a predictor of the solubility of drug in milk. The effects of temperature and fat content on the solubility of drugs in milk can be related to the numerous dynamic equilibria[11] between the various components of milk (i.e., salts, proteins, lipids). In particular, the lipid–protein interactions,[11] which are dramatically influenced by the milk fat content, can be responsible for the greatest enhancement of thiazides in skim milk which has the lower fat content (0.1%), and for the progressive decrease of enhanced "free" solubility in the milk types with the higher fat contents. Nevertheless, the exact mechanism(s) of the change in the intrinsic solubility of the two thiazides in milk is unknown. It can be postulated that phenomena like the formation or deformation of micelles and/or the salting in and the salting out effects can be also involved.

Thermodynamic Analysis—A thermodynamic analysis of the solubility data was performed while keeping in mind that the possible failure in the van't Hoff relationships might be attributed to the changes in the system with temperature. It is well known[17] that the use of van't Hoff operators to obtain enthalpy (ΔH) data becomes less valid when systems become more complicated, and milk is, in fact, a complex system.[18,19]

The thermodynamic elements were generated from the experimental values of solubility as a function of temperature in all the media studied. Thus, the enthalpy and entropy of solution (ΔH_{sol} and ΔS_{sol}) were derived in all cases from the intercepts and slopes of the van't Hoff plots, respectively. The relative solubility values were used for this purpose, receiving the solubility of each drug in buffer at 5 °C as unity.[20,21]

In all cases the van't Hoff plots were linear (typical plots are seen in Figure 2), with correlation coefficients ranging from 0.982 to 0.999 for both drugs. This indicates that the enthalpy of solution is independent of temperature in the specific temperature range studied or, in other words, that the solution process is isoenthalpic. The values of ΔH_{sol} and ΔS_{sol} for both thiazides in all media examined are seen in Table V. For both drugs the highest value for the heat of solution occurs in the casein solution, in keeping with the lowest solubilities determined. For hydrochlorothiazide, the heat of solution in skim milk is much lower than the values in other milk types studied, indicating that a different solubilization

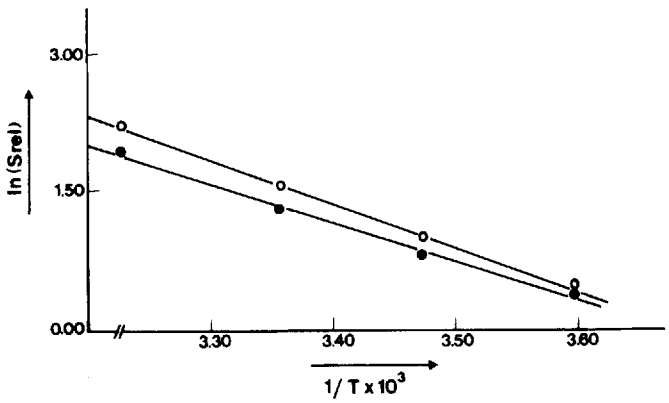

Figure 2—Typical van't Hoff plot of the relative solubilities of hydrochlorothiazide (●) and chlorothiazide (○) in milk with 0.75% fat content.

Table V—Thermodynamic Parameters of Thiazides in Various Media

Drug	Parameter	Buffer pH 6.5	Casein Solution 2.6% w/w	Skim milk	Milk (0.75%)[a]	Milk (1.70%)[a]	Milk (3.50%)[a]
H[b]	ΔH_{sol}[d]	32.63	39.92	32.52	35.16	35.26	34.73
	ΔS_{sol}[e]	11.73	14.28	12.12	12.91	12.93	12.73
C[c]	ΔH_{sol}[d]	44.18	44.13	40.23	39.51	41.82	40.09
	ΔS_{sol}[e]	15.78	16.01	14.48	14.57	15.32	14.67

[a] Milk fat content. [b] Hydrochlorothiazide. [c] Chlorothiazide. [d] Expressed $\times 10^{-3}$, J/mol. [e] Expressed $\times 10^{-1}$, J/mol · K.

process may be responsible for the higher solubility values found in skim milk. The orderliness of the hydrochlorothiazide–skim milk system is also higher as derived from the significantly lower ΔS_{sol} value. In contrast, the values of the thermodynamic parameters of chlorothiazide in all types of milk utilized are similar (Table V). This is in agreement with the smaller differences calculated between the solubilities in these media at most temperatures studied (i.e., 15, 25, and 37 °C).

The results of this study indicate that the solubility of the two model drugs, hydrochlorothiazide and chlorothiazide, is enhanced in milk. The percent increase of total solubility of two thiazides in the various types of milk utilized versus the solubility in buffer ranged from 28.4 to 71.2% and from 20.9 to 96.7% for hydrochlorothiazide and chlorothiazide, respectively. These results constitute indications that the enhanced bioavailability of freeze-dried drug–milk formulations[1,2] is attributed to the increased solubility of drugs in milk. This work and relevant investigations in this topic can be considered as a basis for rationalizing the effect of milk on drug absorption. Further studies are required to derive general guidelines for the behavior of solubility of drugs in milk in relation to physicochemical properties like lipophilicity, aqueous solubility, pK_a, and degree of milk binding.

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